IJPSR (2025), Volume 16, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 August 2024; received in revised form, 01 September 2024; accepted, 25 October 2024; published 01 January 2025

QUANTITATIVE ESTIMATION OF QUERCETIN FROM *PHOENIX SYLVESTRIS* USING VALIDATED HPTLC METHOD

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Keywords:

Quercetin, *Phoenix sylvestris*, HPTLC, Methodvalidation, Bioactive

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ABSTRACT: Introduction: *Phoenix Sylvestris* (L.) Roxb also known as date palm or Indian sugar palm, belongs to the Arecaceae family. In our present study, we used the fruit part (epicarp, endocarp). The fruit is very delicious in taste worldwide. It's flavonoid part is reported with quercetin but its quantification has not yet been reported. **Aim:** The study aimed to carried out the Quantitative estimation of quercetin from *Phoenix sylvestris* using a validated HPTLC method. **Method:** The chromatographic analysis was carried out with the solvent system toluene: ethyl acetate: formic acid (5:4:1, v/v/v) to confirm the presence of quercetin. The HPTLC method was validated by using aluminium-backed silica gel plates with the same solvent system. Quantitative estimation was done by AUC plot. **Results:** Total Flavonoid Content of the methanolic extract of *Phoenix sylvestris* is 0.79 mg QE/g. The HPTLC method was good, accurate and specific. The methanolic extract was found to possess 1.07mg/100g of quercetin. **Conclusion:** This study discussed the validated HPTLC method which is simple, accurate and sensitive tool for the analysis quercetin in different plant extracts. Quercetin content of *P. sylvestris* has been established using this method. Further estimation of Quercetin can be carried out from other palm trees in future studies.

INTRODUCTION: Aracaceae (palme) is called palm family from Areca [1]. This family is the largest group of family of flowering plants. The plants are economically used for their huge diversity of compounds which are found in the edible parts of the plant basically (fruits, seeds). These family trees commonly called "palm trees" [2].Vast amount of studies have been done for their activity. Mostly this plant is used in ayurvedic medication systems for health benefits in different regions [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.16(1).162-66

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(1).162-66

Different pharmacological activities including antidiabetic, antimicrobial, anticancer, anti-inflammatory, neuroprotective, antioxidant, antimicrobial, gastroprotective, antifungal activity of various parts of these plants are revealed in nowadays. *Phoenix sylvestris* widely distributed in India like Pakistan, Myanmar, Nepal, Bhutan, Bangladesh, China, and Sri Lanka [4].

The fruit contains much nutritional value. It contains rich sources of carbohydrates, phenols, amino acids, flavonoids, tannins, alkaloids, terpenoids, dietary fibres, essential vitamins and minerals. And also contains different bioactive compounds like flavonoids, carbohydrates, phenolic compounds, steroids, isoflavones, terpenoids [5]. *Phoenix sylvestris* fruits are used traditionally in different regions.

Fruits are effective for treating toothache, gonorrhoea, asthma, tuberculosis, cough dehydration, arthritis, piles, diabetes, and diuretic. And the others parts are like heart wood effected on increase lactation activity. Roots have effect on toothache, and dysentery activity, root juice also used as tonic. The sap also used to treat diarrhoea, stomach ache. In the ancient era used for tribals according to ayurvedic system for health benefits in different regions of India, Pakistan, Bangladesh [6].

MATERIALS AND METHODS:

Plant Material: *P. sylvestris* was collected from Kanchrapara, West Bengal. The plant part was authenticated from Central National Herbarium, Botanical Survey of India, Shibpur, West Bengal, India. Specimen no-GNIPST/2024/06.

Chemicals: Standard quercetin was purchased from Loba Chemie Pvt. Ltd. Other chemicals and solvent system used in analytical grade.

Extraction: Shed dried Plant part coarsely in powdered form was using methanol extracted by maceration process [7].

Qualitative Test of Flavonoids:

Phytochemical Tests: The methanolic fruit extract was carried out using standard method [8].

Quantitative Determination of Total Flavonoid Content:

Preparation of Standard Quercetin for Calibration Curve: Total flavonoid content was determined by Aluminium chloride method.100 milligrams quercetin was dissolved in 1ml methanol to prepare a stock solution and the standard solution was diluted to make like 200,400,600,800 and 1000µg/ml. Add 4 ml of distilled water and 0.3 ml of 5% $NaNO_2$ were added in a test tube. After 5 minute 0.3 ml of 10% $AlCl_3$ and after 6 minutes 1M NaOH in 2ml were added. And add distilled water to up to level 10ml. Spectrophotometer was used to measure the absorbance at 510nm[9].

Preparation of Sample for Total Flavonoid Content: Stock solution was prepared with methanol 100mg/ml. dilute the solution and create different 0.3mg /ml concentrations of solutions. The extracts were prepared a similar method to describe for quercetin. Spectrophotometer set at 510nm used to detect the absorbance. Using linear equation based on the standard calibration curve calculate the flavonoid concentration as quercetin equivalent.

Qualitative and Quantitative Study of Quercetin Thin Layer Chromatography: Established solvent system of quercetin Toluene: ethyl acetate: formic acid (5:4:1, v/v/v) was usedfor study the plates TLC study[10].

Quantitative Study of Quercetin using High-Performance Thin-layer Chromatography:

Standard Preparation for HPTLC: A standard solution of quercetin (100mg/ml) was prepared using methanol.

Sample Preparation for HPTLC: Sample solution was prepared with methanol with methanolic extract of *P. Sylvestris* (100mg/ml).

Method Validation:

Linearity range: The linearity range was validated through the analysis of standard solutions (100 mg/ml) of quercetin by five different concentration levels (50-200 L). Linear least-squares regression was used to verify linearity and produce a calibration curve. The regression equation, including slope, intercept, and coefficient of correlation (R^2), was obtained **Table 1** [11].

Limit of Detection & Limit of Quantification: LOD (Limit of Detection) & LOQ (Limit of Quantification) values were determined as amounts signal to noise ratios were 3:1 and 10:1.

Accuracy: Recovery study method represents the percentage of average value for quercetin was calculated which analysed by HPTLC **Table 2.** The method was done in triplicate.

Specificity: The specificity method was done by analysing standard quercetin and the methanolic extract. Spot of quercetin was confirmed by comparing the R_f value with the spot of standard.

Intra and Inter-day Precession Analysis**:** Intraday and inter day method was evaluated for precision by analysing three replicates of the substance at three concentration levels of quercetin (100, 150, and 200 µl per band) on the same day **Table 3.**

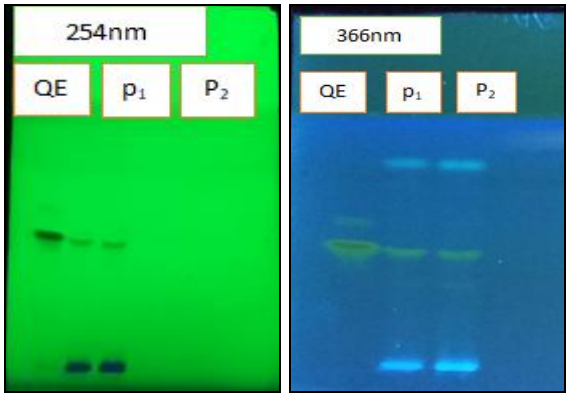

FIG. 1: HPTLC PLATE OF QUERCETIN (QE) AND SAMPLE (P1 AND P2) OF *P. SYLVESTRIS.* QE = Quercetin, P_1 & P_2= *Phoenix sylvestris*

Quantification of Quercetin of Methanolic Extract of *Phoenix sylvestris:* The methanolic extract (sample) was applied on HPTLC plates and peaks were obtained under the same condition for standard quercetin and the R_f value of quercetin was recorded and Area Under the Curve calculated.

RESULT:

Qualitative Test for Flavonoids:

Phytochemical Test: Flavonoid was found to be present in the standard of *P. sylvestris* methanolic extract.

Quantitative Determination of Total Flavonoid Content:

Total Flavonoid Content (TFC): Aluminium chloride method was used for TFC of *Phoenix sylvestris* methanolic extract. Quercetin equivalent of methanolic extract was calculated using the calibration curve of Quercetin that shows y=0.0005x + 0.029, R^2= 0.9994 **Fig. 2**. Total Flavonoid Content of the extract of *Phoenix sylvestris* (0.79 mg QE/g).

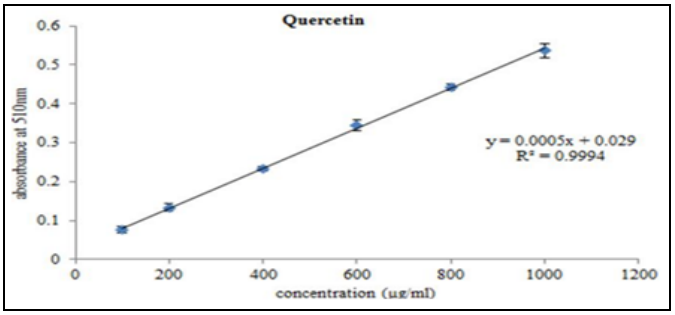

FIG. 2: CALIBRATION CURVE OF STANDARD QUERCETIN

Qualitative Study of Quercetin:

TLC (Thin Layer Chromatography): Analytical TLC profiling confirmed the quercetin presence of present in methanolic extract of *Phoenix sylvestris* where the R_f of the sample (0.63) was found to be same with that of the standard quercetin. Therefore, HPTLC was carried out using the solvent system same as use for TLC.

Quantitative Study of Quercetin Using High-Performance Thin-Layer Chromatography: HPTLC analysis- High performance thin layer chromatography analysis was carried out the mobile phase toluene- ethyl acetate- formic acid in a (5:4:1, v/v/v) which effectively response for the separation of quercetin and plates were visualised under the visible light 254 nm and 366 nm.

Method Validation: Linearity range-Calibration plot shows in figure and the concentration range is (100 mg mL^{-1}) of quercetin at five different concentration levels (50-200µl). Linear least-squares regression was used to verify linearity and produce a calibration curve **Table 1**.

Calibration Curve: In **Fig. 3** indicate the response linear concentration range 50-200µg/ml of quercetin. The linear equation represents a slope, intercept, correlation coefficient **Table 2.**

Accuracy: The recovery studies **Table 2** represent limit, which indicate the accuracy of the method. The value is 109.50%.

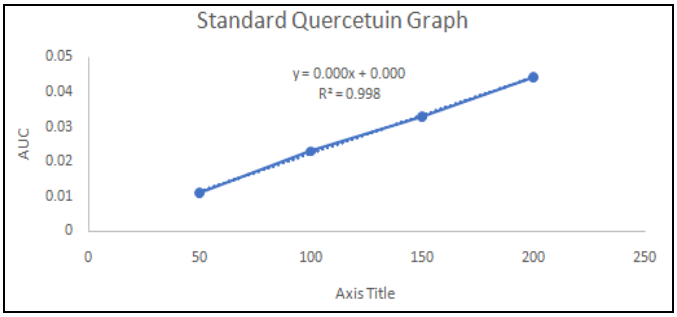

FIG. 3: STANDARD CURVE OF STANDARD QUERCETIN

TABLE 1: LINEAR REGRESSION DATA FOR STANDARD QUERCETIN

Linear Regression Parameter	Data
Linearity range (µg/spot)	50-200
Regression equation	0.0002x + 0.0003
Correlation equation (R^2)	0.9988
Slope	0.0002203
Intercept	0.0003
SE of Intercept	0.000598811
SD of Intercept	0.001338983
LOD	0.004418643
LOQ	0.013389828

Recovery Studies: The recovery studies presented in **Table 1** yielded results within acceptable limits, achieving a value of 109.50%. This indicates that the method demonstrates good accuracy in quantifying the target analyte.

TABLE 2: RECOVERY STUDY FOR PROPOSED METHOD (N=3)

Excess drug added to analyte (%)	Conc. found (µg±SD)	%Recovery	%RSD
50	54.03±0.53	90.98	0.88
100	97.96±0.79	92.66	0.84
150	165.4±0.68	109.50	0.58

Limit of Detection and Limit of Quantification: LOD and LOQ method was found to be 0.0044µg/spot and 0.0133 µg/spot which indicates the proposed method indicate the quantifying quercetin in methanolic extract.

Intra and Inter-day Precession: The findings for repeatability and intermediate precision, presented as standard deviation (SD) percentages, are detailed in **Table 3**.

The relative standard deviation (RSD) ranged from for 0.81- 1.30 repeatability and from for 0.71-2.30 inter-day precision. These low values demonstrate the method's high level of precision.

TABLE 3: PRECISION OF THE PROPOSED METHOD (N=3)

Concentration	Repeatability (Intra-day precision)			Repeatability (Intra-day precision)		
	Area±SD	Standard error	%RSD	Area±SD	Standard Error	%RSD
100	0.0112±0.59	0.00012	0.81	0.0122±0.4	0.00019	0.71
100	0.0117±0.56	0.00019	0.73	0.0145±0.8	0.00014	0.88
100	0.0210±0.73	0.00021	1.30	0.0220±0.7	0.00011	2.30

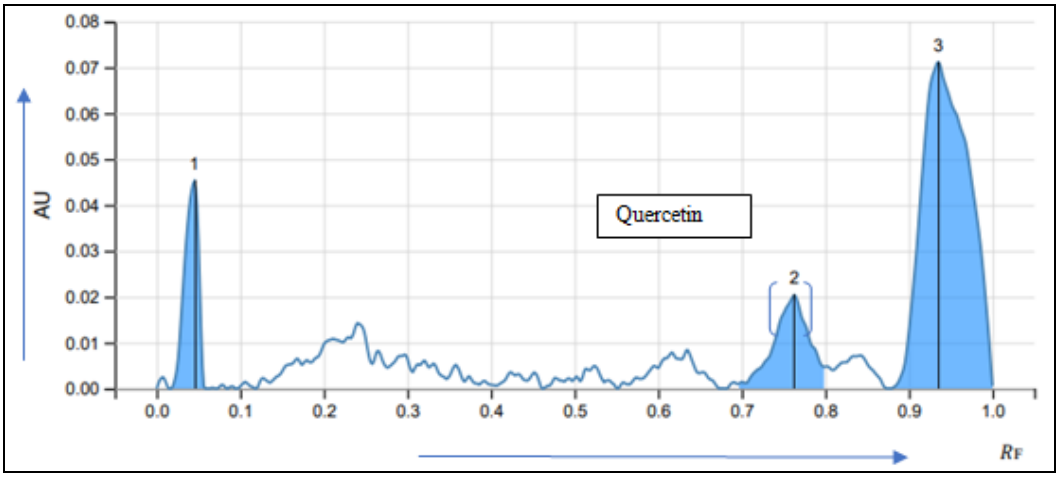

FIG. 4: HPTLC CHROMATOGRAM OF STANDARD QUERCETIN

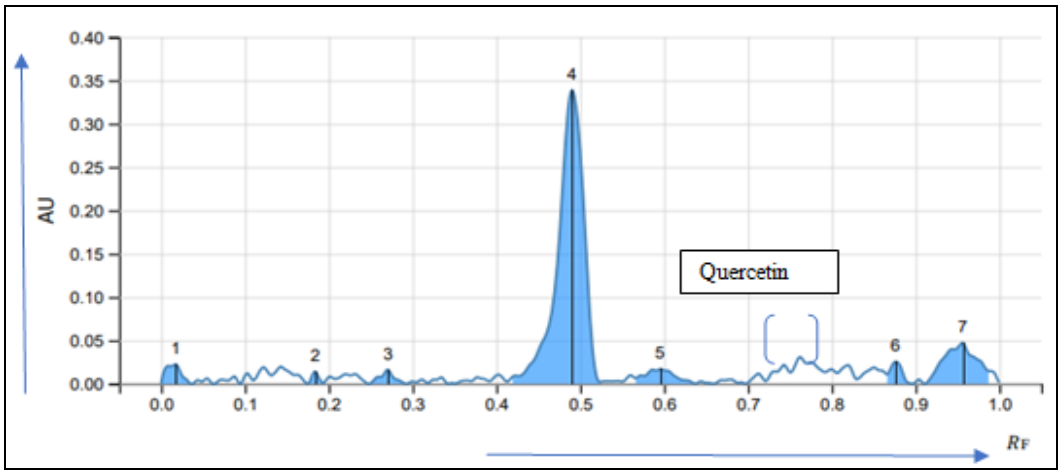

FIG. 5: HPTLC CHROMATOGRAM OF STANDARD METHANOLIC EXTRACT OF *PHOENIX SYLVESTRIS*

Quantitative Estimation of Quercetin the Methanolic Extract of *Phoenix sylvestris:* The quercetin content was quantified from the methanolic extract of *P. sylvestris* using HPTLC and quercetin content was found to be1.07mg/100g.

DISCUSSION: The methanolic extract of *Phoenix sylvestris* fruits has shown positive result of phytochemical test of flavonoids. Good amount of flavonoid was found to be in *P. sylvestris* extract. TLC study was done with an established solvent system toluene- ethyl acetate- formic acid in a (5:4:1, v/v/v) which shows similar R_f at 0.63 as standard quercetin. HPTLC analysis is a good tool for identifying of secondary metabolites from plant extract. It was used to identify and quantify the flavonoid quercetin in the fruit part of *Phoenix sylvestris*. Quercetin was identified with a single peak and estimated to be 1.07mg/100g in the methanolic extract.

CONCLUSION: *Phoenix sylvestris is* one of the available common fruits of Arecaceae family having different therapeutic activities. In our daily life it is a good choice to consume for their nutritional value. The preliminary study shows the flavonoid content in it the study and HPTLC study provided quantity of the Quercetin in the fruit extract. Future study can reveal the mechanism of therapeutic activities of Quercetin in the *P. sylvestris* extract.

ACKNOWLEDGEMENT: The authors would like to acknowledge the *Acharya prafulla* Chandra Ray Guru Nanak Institute of Pharmaceutical Science and Technology for providing the support for this research work.

Ethical Approval: None

Funding: None

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:
Majumder S and Saha P: Quantitative estimation of quercetin from *Phoenix sylvestris* using validated HPTLC method. Int J Pharm Sci & Res 2025; 16(1): 162-66. doi: 10.13040/IJPSR.0975-8232.16(1).162-66.