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Research Article

Quantitative Estimation of Phytoconstituents in Hydroalcoholic Extract of *Pancratium triflorum* Roxb. by RP-HPLC Method

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ABSTRACT

Pancratium triflorum Roxb (Amaryllidaceae) is a perennial bulbous herb traditionally used to treat wounds, fever, skin infections, and digestive issues. Despite its traditional use, a gap exists in the standardization of its bioactive markers. This study aimed to develop and validate a robust Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for quantifying Quercetin, a primary flavonoid marker, in the plant's hydroalcoholic bulb extract. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, and saponins. The optimized method utilized a C18 column with a mobile phase of methanol and O-phosphoric acid (60:40) at a flow rate of 1 ml/min and detection at 370 nm. The method demonstrated excellent linearity ($R^2 = 0.99945$) over 62.5–1000 µg/L. High sensitivity was confirmed with a Limit of Detection (LOD) of 0.113 µg/mL and a Limit of Quantification (LOQ) of 0.341 µg/mL. This validated method provides a reliable tool for the quality control and standardization of *P. triflorum* extracts.

INTRODUCTION

Quality is important in every product and services, but it is vital in medicine as it involves life. Quality of a drug is determined by using various analytical methods chemistry. Traditional medicine remains a primary source of healthcare for over 80% of the world's population. Approximately 40% of modern pharmaceuticals are derived from natural products or traditional leads. However, the global

demand for herbal remedies necessitates scientific evidence for quality control and standardization.



***Pancratium triflorum* Roxb**

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Pancratium triflorum Roxb. is traditionally valued for its therapeutic potential, including antioxidant, anti-inflammatory, and antimicrobial activities [1]. Quercetin, a prominent flavonoid found in various plants, is known for its potent biological activities, such as inhibiting pro-inflammatory enzymes like COX-2 and LOX [2].

High-Performance Liquid Chromatography (HPLC) is the gold standard for separating and quantifying components in complex herbal mixtures. Among its modes, Reverse-Phase HPLC (RP-HPLC) is the most widely used due to its versatility and reproducibility. RP-HPLC operates on the principle of hydrophobic interactions. It utilizes a non-polar stationary phase typically a C18 column and a polar mobile phase such as water and methanol. In this system, molecules are separated based on their polarity [3]. Polar compounds elute faster, while non-polar compounds like Quercetin are retained longer. This research focuses on establishing a measurable benchmark for *P. triflorum* by developing a validated RP-HPLC method for Quercetin.

MATERIALS AND METHODS

Materials

Quercetin reference substance was purchased from Kanton Laboratories (batch no: KL4239); Methanol and Phosphoric acid employed here were HPLC grade reagents. Distilled water and Ethanol were used in the experiment. The plant *Pancratium triflorum* is collected from Malappuram and identified as the same by Dr. Pramod C, University of Calicut. The plant is dried and coarse powdered.

METHODS

Preparation of stock and working standard solution

A stock solution of Quercetin was prepared in HPLC-grade methanol. 10 mg Quercetin was accurately weighed and put into 10ml volumetric flask containing 5ml of methanol and sonicated for 10 minutes. Then the volume was adjusted with methanol up to the mark. Standard solution of different concentration (62.5, 125, 250, 500 and 1000ppm) was prepared from above solution and diluted with methanol and filtered through 0.22µ millipore membrane filters and injected in HPLC system.

Extraction of plant sample

The plant is extracted by using water and alcohol in Soxhlet extractor. The extract is then concentrated by evaporation. The 10µL of *P. triflorum* extract was taken in 10 ml volumetric flask and was dissolved in methanol up to the mark, sonicated for 15 minutes, and filtered through a 0.22µ millipore membrane filter.

RP-HPLC Method Development

The analysis was performed on a Shimadzu HPLC system equipped with an SPD-M40 PDA detector and a C18 column (4.6 x 150 mm, 5 µm) with an autosampler and a LabSolutions software. The mobile phase consisted of Methanol: O-Phosphoric acid in a 60:40 ratio. The flow rate was maintained at 1 ml/min, with an injection volume of 20 µl and detection at 370 nm.

Instrumentation and Optimized chromatographic Conditions



Table 1. Instrumentation of HPLC

Sr. No.	Name of Equipment / Instrument	Model / Specification	Manufacturer
1	HPLC	Series 20	Shimadzu
2	Pump	LC- 20 AP	
3	System Controller	CBM- 20A	
4	PDA Detector	SPD-M40	
5	Software	Shimadzu LabSolutions	

Table 2. Optimized chromatographic condition

Sr. No.	Parameter	Specifications
1	Stationary phase	C-18 (4.6 x 150 mm id, 5µm particle size)
2	Elution mode	Isocratic elution mode (60:40)
3	Mobile phase	Methanol: 0.5% v/v O- phosphoric acid
4	Detector	Photodiode Array (PDA) detector
5	Absorption maxima	370 nm
6	Column temperature	40°C
7	Flow rate	1.0 mL/min
8	Injection volume	20µL
9	Diluent	Methanol
10	Run time	15 minutes

METHOD VALIDATION

1. Linearity:

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

A calibration curve was plotted over a concentration range of 62.5 to 1000 µg/L for Quercetin. Accurately measured working stock solution of drug were transferred to separate series of 10ml volumetric flask and diluted up to the mark with methanol. Filtered through 0.2 µm membrane filter and injected for HPLC analysis. The Linearity was constructed by plotting concentration against area from each reading.

2. Limit of Detection and Limit of Quantification

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve:

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ is the standard deviation of response and S is the slope of the calibration curve.

3. Precision

The term precision is defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values



obtained by replicate measurements of a quantity under specified conditions. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance or the coefficient of variation.

Repeatability is the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution of Quercetin (62.5 µg/L) was prepared and analysed three times as per the proposed method.

4. Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120%). At each level, two determinations were performed. The accepted limits of recovery are 90% - 120%.

RESULTS AND DISCUSSIONS

Identification of marker compound

The solution of plant extract sample was chromatographed and obtained the concentration of quercetin of plant extract by using the regression equation. In the research, the HPLC process is used to study and quantify the quercetin in the *Pancratium triflorum*.

Preparation of the calibration curve of the quercetin

For the preparation of calibration curve, taken standard solutions (quercetin) and injected into the column and calculate the mean of peak area of standard drug and plotted the graph against different concentration (62.5, 125, 250, 500 and 1000ppm) of the drug by using methanol as solvent system. The regression equations were figured out on the basis of the curve. The calibration curve of the standard solution was used for comparing the peak of plant extract.

Table 3: Preparation of Calibration curve

Sr No.	Concentration (µg/L)	Area
1	62.5	6207516
2	125	13259247
3	250	26516835
4	500	51563620
5	1000	99356508

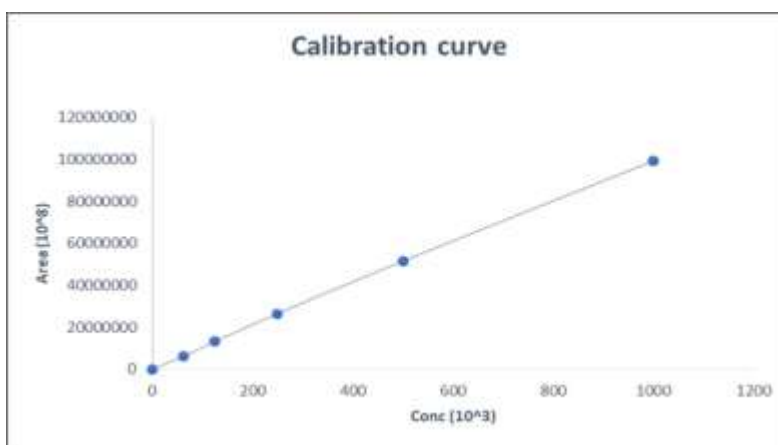


Figure 1: Calibration Curve of the Quercetin

The calibration curve of flavonoids was observed linear relationship over the range of 62.5, 125, 250,

500, 1000µg/L ($R^2=0.99$) concentration of standard. The linear regression equation for the

curve was $Y = 98946.6x + 1038940$, where y is the ratio of peak area of flavonoids and x is the flavonoid concentration ($\mu\text{g/L}$).

Identification of quercetin in plant extract

The qualitative and quantitative study of quercetin of selected Phytoextracts was performed by the HPLC method. The HPLC chromatogram of ethanol extracts of bulb of *Pancratium triflorum*

revealed their highest peaks area for the quercetin as secondary metabolites component. The resulting HPLC chromatogram of standard quercetin and plant extracts sample were recorded and presented in below (Table and Figures). The qualitative analysis of phytochemicals was supportive of the quantitative analysis of its. The ongoing study confirmed the presence of flavonoids (quercetin) in medicinal plant as secondary metabolites.

Table 4: Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range ($\mu\text{g/L}$)	Correlation coefficient	Slope	Intercept
	62.5-1000	0.99	98946.6	1038940

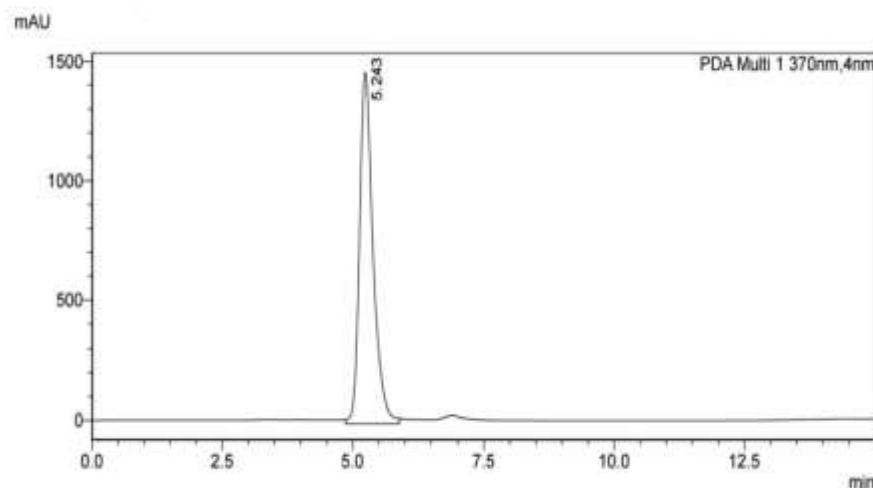


Figure 2: Chromatogram of standard Quercetin

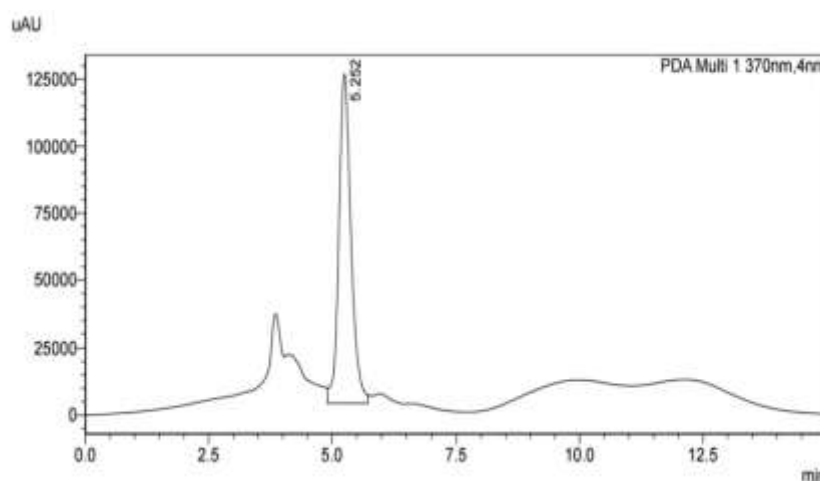


Figure 3: Chromatogram of ethanolic bulb extract of *Pancratium triflorum*

Chromatographic analysis of ethanolic bulb extracts of plant performed by using methanol and

ortho-phosphoric acid 60:40 (V/V) solutions as mobile phase and used a flow rate of 1ml per min

and absorbance at 370 nm. It gave good separation of quercetin at RT 2.25 min.

The peak of quercetin in the chromatogram of plant extract was compared with the RT values with those obtained by the chromatogram of the standard quercetin under the similar conditions.

Standard quercetin gave a strong peak area at the RT 2.24 min and HPLC of extracts of the ethanolic bulb extract of plant gave different peaks area at the same retention times as shown in the above figures. Results of the peaks of plant extracts did show more differences with standard quercetin, which further confirms the occurrence of quercetin in the ethanolic bulb extracts of *Pancreatium triflorum*. Recently, this technique is achieving popularity within numerous analytical techniques as the primary choice for the study of

fingerprinting and for quality control method of herbal plants characterization of secondary metabolites of plant extracts.

Quantitative analysis of quercetin in plant extract

This research stated the presence of a large quantity of total flavonoids content in *Pancreatium triflorum*. Flavonoid is mainly occurred in various parts of the plant as glycosides and quercetin and is known to be imparting luxuriant colour to the leafy parts, fruits, flowers, etc. It is stated to reveal anti-cancer, anti-histamine, and anti-inflammatory activities which mostly followed its antioxidant features. Quantitative estimation of quercetin in hydroalcoholic extracts of *Pancreatium triflorum* were calculated in the given table.

Table 3: Quantitative estimation of Quercetin in extract of *Pancreatium triflorum*

Sr No.	Name of sample	Retention time (min)	Area	Concentration (µg/L)
1	<i>Pancreatium triflorum</i> bulb	5.252	2090781	10.630

Method validation

Linearity

Linearity regression data, summarized in Table, show a good linear relationship between concentration and peak areas over a concentration range of 62.5- 1000µg/L for quercetin (Figure 1).

Table 4. Linearity table of Quercetin

Concentration (µg/L)	Area	Statistical Analysis
62.5	6207516	Intercept = 1038940 Slope = 98946.6 Linear equation = 98946.6 x + 1038940 Regression coefficient R ² = 0.9994549
125	13259247	
250	26516835	
500	51563620	
1000	99356508	

LOD and LOQ

Table 5. LOD and LOQ

Drug	LOD (µg/mL)	LOQ (µg/mL)
Quercetin	0.113	0.341

The Limit of Detection and Limit of Quantification of the method was calculated based on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD was found to be 0.113µg/ml and LOQ was found to be 0.341µg/ml respectively which showed that sensitivity of the method was high. The results obtained were within the limit.

Precision

Table 6. precision results showing repeatability

Concentration (µg/L)	% Recovery
1000	99.364
1000	100.022
1000	99.85
1000	100.012



1000	98.92
1000	99.39
Mean	99.593
SD	0.446
% RSD	0.448

The mean, standard deviation and the percentage of relative standard deviation were calculated and are presented in above tables. The results reveal that a low relative standard deviation showing that the developed methods are precise.

Accuracy

Table 7. Accuracy reading of Quercetin

Level of addition	% Recovery	Statistical Analysis		
		Mean	SD	%RSD
80%	99.67	99.305	0.516	0.520
	98.94			
100%	99.36	99.655	0.417	0.419
	99.95			
120%	99.54	99.880	0.481	0.481
	100.22			

Observed data (Table 7) of Quercetin are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 8. Summary of the method developed

Parameter	Result
Mobile phase	Methanol: O-Phosphoric acid (60:40)
Injection volume	20µL
Flow rate	1mL/min
Absorption maxima	370 nm
Conc. range	62.5-1000 µg/L
Correlation coefficient	0.999
Regression equation	$Y = 98946.6x + 1038940$
Slope	Slope = 98946.6
Intercept	Intercept = 1038940
Accuracy (%RSD)	80% (0.520), 100% (0.419), 120% (0.481)
LOD (µg/L)	0.133
LOQ (µg/L)	0.341

The data from the validation of RP HPLC method developed can be summarised in table 8.

CONCLUSION

The present study successfully developed and validated a simple, precise, accurate, and sensitive RP-HPLC method for the quantitative estimation of quercetin in the hydroalcoholic extract of *Pancratium triflorum* Roxb. The validated method demonstrated excellent linearity, low LOD and LOQ values, and satisfactory precision and accuracy in accordance with ICH guidelines. This method can be effectively applied for routine quality control, standardization, and phytochemical evaluation of *P. triflorum* and its herbal formulations.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest regarding the publication of this research work.

REFERENCES

1. Johnson M, Maharaja P, Janakiraman N, Adaikala Raj G, Menezes IRA, da Costa JGM, et al. *Pancratium triflorum* Roxb. (Amaryllidaceae) and *Molineria trichocarpa* (Wight) N.P. Balakr (Hypoxidaceae): Cytotoxic and antioxidant activities. Food Chem Toxicol. 2018;119:290–5.



2. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, et al. Quercetin, inflammation and immunity. *Nutrients*. 2016 Mar 15;8(3):167.
3. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. 3rd ed. Hoboken (NJ): Wiley-Blackwell; 2010.
4. Prameela R, Venkaiah M, Swamy J, Prakasarao J. Amaryllids of Andhra Pradesh, India. *Trop Plant Res*. 2020;7(3):594–603.
5. Mathew R, Varkey J. Development of a validated RP-HPLC method for estimation of Quercetin. *Asian J Pharm Hea Sci*. 2021 Jan-Mar;11(1):2415-2421.
6. Ang LF, Yam MF, Fung YTT, Kiang PK, Darwin Y. HPLC method for simultaneous quantitative detection of quercetin and curcuminoids in traditional Chinese medicines. *J Pharmacopuncture*. 2014;17(4):36-49.
7. Nikolova M, Gevrenova R. Determination of phenolic acids in Amaryllidaceae species by high performance liquid chromatography. *Pharm Biol*. 2005;43(3):289-91.
8. Yue-ling M, Yu-jie C, Ding-rong W, Ping C, Ran X. HPLC determination of quercetin in three plant drugs from genus *Sedum* and conjecture of the best harvest time. *Pharmacogn J*. 2017;9(6):725-728.
9. Al-Ansi W, Al-Homaidan AA, Al-Ghanayem AA, Al-Dbass AM, Al-Qattan SY, Al-Abbad MS, et al. HPLC analysis, antioxidant and antimicrobial activities of some *Sargassum* species from the Red Sea coast, Saudi Arabia. *Pharmacogn J*. 2021;13(5):1219-27.
10. D'Mello PM, Joshi UJ, Shetgiri PP, Dasgupta TK, Darji KK. A simple HPLC method for quantitation of quercetin in herbal extracts. *J AOAC Int*. 2011;94(1):100-5.
11. Burin VM, Arcari SG, Bordignon-Luiz AM, Costa LL. Determination of some phenolic compounds in red wine by RP-HPLC: method development and validation. *Journal of chromatographic science*. 2011 Sep 1;49(8):647-51.
12. Ivanov I, Berkov S, Pavlov A. Improved hplc metod for the determination of amaryllidaceae alkaloids. *Biotechnology & Biotechnological Equipment*. 2009 Jan 1;23(sup1):809-13.
13. Ahmed R. High-Performance Liquid Chromatography (HPLC): Principles, Applications, Versatility, Efficiency, Innovation and Comparative Analysis in Modern Analytical Chemistry and In Pharmaceutical Sciences.
14. Vidushi Y, Meenakshi B, Bharkatiya M. A review on HPLC method development and validation. *Res J Life Sci, Bioinform, Pharm Chem Sci*. 2017;2(6):178.

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