

Phytochemical screening and HPLC identification of quercetin in *Annona reticulata* leaf extract with potential anti-epileptic relevance

Rajashekar S¹, Balasubramaniam M², Kathir Varman A², Mohamed Anas J², Raja Rajeshwari A P², Sadhana P²

¹ Associate Professor, Department of Pharmaceutical Chemistry, Edayathangudy G. S. Pillay College of Pharmacy, Nagore Road, Nagapattinam, Tamil Nadu, India

² Department of Pharmaceutical Chemistry, Edayathangudy G. S. Pillay College of Pharmacy, Nagore Road, Nagapattinam, Tamil Nadu, India

DOI: <https://doi.org/10.66856/ijrpps.2026.11.3.11086>

Abstract

Annona reticulata Linn., a medicinal plant belonging to the family Annonaceae, is recognized as a source of various biologically important phytoconstituents. The present study was conducted to evaluate the preliminary phytochemical profile of *A. reticulata* leaves and to obtain preliminary chromatographic evidence for the presence of quercetin in its ethanolic extract. Mature leaves were collected, authenticated, shade-dried, powdered, and extracted with ethanol using a Soxhlet apparatus. The obtained extract was subjected to preliminary phytochemical screening followed by High-Performance Liquid Chromatography (HPLC) analysis. Chromatographic separation was performed using a C18 column with an isocratic mobile phase comprising buffer, acetonitrile, and methanol in the ratio of 10:45:45 (v/v). The flow rate was maintained at 0.8 mL/min, with UV detection at 230 nm and a column temperature of 25°C. Five replicate injections of the quercetin reference standard showed retention times ranging from 4.982 to 5.002 min, whereas the ethanolic leaf extract exhibited a peak at 5.064 min. The close correspondence between the retention time of the sample peak and that of the reference standard provides preliminary evidence supporting the presence of quercetin in the ethanolic leaf extract. However, retention-time comparison alone is not sufficient for definitive structural confirmation. Further studies involving confirmatory analytical techniques, quantitative estimation, and biological evaluation are recommended to establish the identity and pharmacological significance of quercetin in *A. reticulata* leaves.

Keywords: *Annona reticulata*, phytochemical screening, quercetin, HPLC, ethanolic extract, flavonoid

Introduction

Histroy: *Annona reticulata* is a medicinal plant traditionally used for various therapeutic purposes. Its leaves contain several phytoconstituents, including flavonoids and phenolic compounds. Among these, quercetin is an important flavonoid reported to possess antioxidant and neuroprotective properties. Epilepsy is a neurological disorder characterized by recurrent seizures. Oxidative stress has been associated with neuronal damage and may contribute to seizure-related changes. Therefore, naturally occurring compounds such as quercetin have attracted interest for their potential neuroprotective and anti-epileptic relevance.

Based on this background, the present project was carried out to investigate the phytochemical constituents of *Annona reticulata* leaf extract and to perform preliminary HPLC identification of quercetin in the extract.

Introduction: *Annona reticulata* is a medicinal plant belonging to the family Annonaceae and is traditionally used for various therapeutic purposes. Different parts of the plant contain bioactive phytoconstituents such as flavonoids, phenolic compounds, alkaloids, tannins and terpenoids.

Among these phytoconstituents, quercetin is an important flavonoid known for its antioxidant and neuroprotective properties. Its presence in medicinal plants has attracted interest because oxidative stress is associated with neuronal damage and neurological disorders.

Epilepsy is a neurological disorder characterized by recurrent seizures resulting from abnormal electrical activity

in the brain. Oxidative stress and neuronal injury may contribute to the development and progression of seizure-related changes.

Therefore, the present study focuses on the phytochemical screening and preliminary HPLC identification of quercetin in *Annona reticulata* leaf extract, with potential relevance to its anti-epileptic activity.

Aim & Objectives

To collect and prepare *Annona reticulata* leaf samples for analysis and extract phytoconstituents using a suitable solvent.

To perform preliminary phytochemical screening and HPLC analysis for the identification of quercetin present in *Annona reticulata* leaves.

To discuss the potential biological significance of quercetin in relation to anti-epileptic activity based on reported literature.

Extraction Procedure

Soxhlet About 20 g of dried powdered *Annona reticulata* leaves was accurately weighed and placed in a extraction thimble. Approximately 200 mL of ethanol was taken in a round-bottom flask, and the Soxhlet apparatus was assembled with a condenser. The solvent was heated under controlled reflux conditions, allowing the condensed ethanol to repeatedly pass through the powdered leaf material and return to the flask through the siphoning process. The extraction was continued until the solvent became comparatively pale and successive cycles showed little

additional colour. After completion of extraction, the apparatus was allowed to cool and the ethanolic extract was collected and filtered. The solvent was removed under reduced pressure using a rotary evaporator at a controlled temperature, preferably not exceeding 40 °C, to obtain the concentrated crude ethanolic extract. The extract was weighed, and the percentage extraction yield was calculated using the weight of the dried extract relative to the initial weight of the powdered plant material. The concentrated extract was stored in a tightly closed container protected from light and moisture until further phytochemical screening and HPLC analysis.

Result & discussion

Preliminary Phytochemical Screening

The ethanolic extract of *Annona reticulata* leaves was subjected to preliminary phytochemical screening to

determine the presence of different classes of phytoconstituents. The observations, inference, and intensity of the tests are presented in Table 2.

HPLC Conditions

Parameter	Condition
Column	C18, 100 mm × 2 mm, 4 µm
Mobile phase	10% Buffer +45% Acetonitrile +45% methanol
Ratio	10:45:45 (v/v)
Elution mode	Isocratic
Flow rate	0.8 mL/min
Detection wavelength	230 nm
Column temperature	25 °C
Sample solvent	HPLC-grade methanol
Sonication	20 minutes

S. No.	Phytochemical Constituent	Test	Observation	Inference	Intensity
1	Flavonoids	Alkaline reagent test	Intense yellow colour was observed, which disappeared on addition of dilute HCl.	Flavonoids present	Positive
2	Flavonoids	Shinoda test	Pink to reddish colour was observed.	Flavonoids present	Positive
3	Flavonoids	Lead acetate test	Yellowish precipitate was observed.	Flavonoids present	Positive
4	Phenolic compounds	Ferric chloride test	Bluish-green colour was observed.	Phenolic compounds present	Positive
5	Tannins	Gelatin test	White precipitate was observed.	Tannins present	Positive
6	Alkaloids	Dragendorff's test	Orange to reddish-brown precipitate was observed.	Alkaloids present	Positive
7	Saponins	Foam test	Persistent froth was observed.	Saponins present	Positive
8	Terpenoids	Salkowski test	Reddish-brown colour was observed at the interface.	Terpenoids present	Positive
9	Steroids	Salkowski test	Red colour was observed in the chloroform layer/interface.	Steroids present	Positive
10	Carbohydrates	Molisch's test	Violet/reddish-violet ring was observed at the interface.	Carbohydrates present	Positive

Results

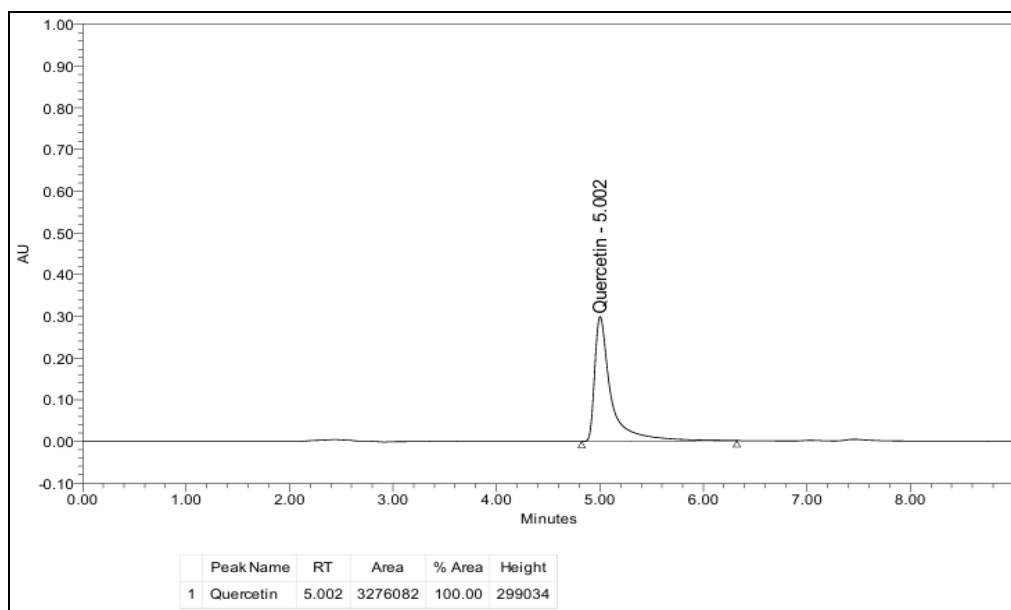
The HPLC analysis was carried out for the preliminary identification of quercetin in the leaf extract of *Annona reticulata*. Quercetin reference standard injections were compared with the chromatographic profile of the plant extract on the basis of retention time.

The HPLC analysis of *Annona reticulata* leaf extract was performed using an isocratic chromatographic method with

quercetin as the reference standard. The chromatographic conditions used for the analysis were as follows:

HPLC Analysis of Quercetin Reference Standard

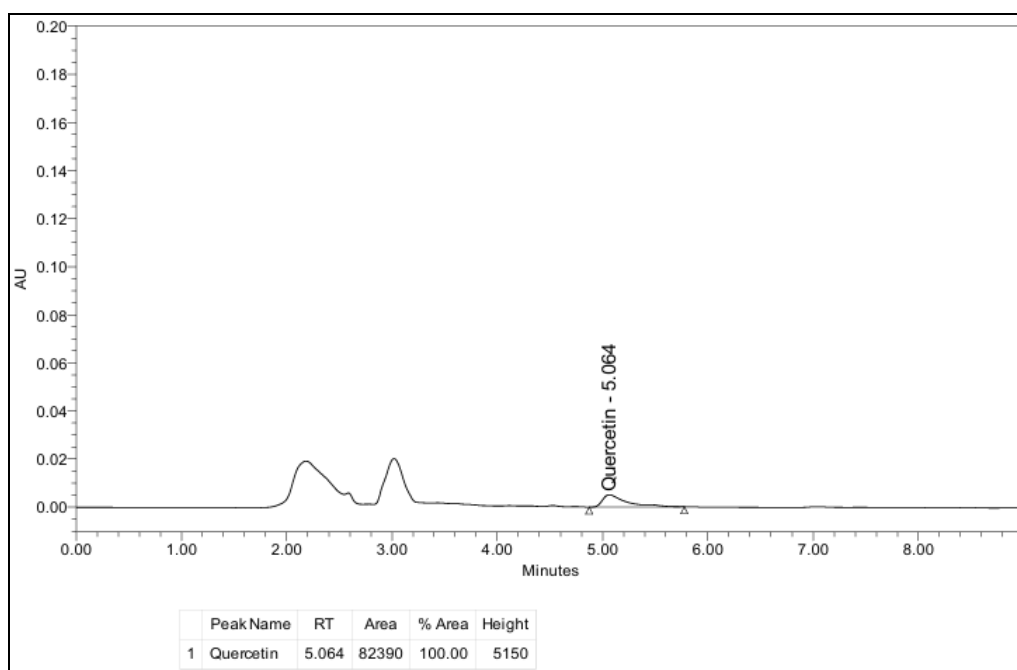
Five replicate injections of quercetin reference standard were analysed. A quercetin peak was reported at approximately 5 minutes in each standard injection. The chromatograms obtained for all five standard injections are presented below.



HPLC chromatogram of quercetin reference standard (Std-03).

HPLC Analysis of *Annona reticulata* Leaf Extract

The *Annona reticulata* leaf extract was analysed under the same chromatographic conditions. The sample chromatogram showed a peak reported as quercetin at a retention time of 5.064 min, with a peak area of 82,390 and a peak height of 5,150.



HPLC chromatogram of *Annona reticulata* leaf extract showing the reported quercetin peak.

Results

HPLC analysis of the *Annona reticulata* leaf extract produced a chromatographic peak at 5.064 min that was reported in the laboratory peak table as quercetin. The five quercetin standard injections showed retention times between 4.982 and 5.002 min. The sample retention time was therefore close to the retention time observed for the quercetin reference standard.

Based on retention-time comparison, the HPLC result supports the presence of quercetin in the *Annona reticulata* leaf extract.

References

1. Jamkhande PG, Wattamwar AS. *Annona reticulata* Linn. (Bullock's heart): *Plant profile*, phytochemistry and pharmacological properties. *Journal of Traditional and Complementary Medicine*. 2015;5(3):144-152.
2. Zaman K, Pathak K. Pharmacognostical and phytochemical studies on the leaf and stem bark of *Annona reticulata* Linn. *Journal of Pharmacognosy and Phytochemistry*. 2013;1(5):1-7.
3. Perez-Flores FA, Jiménez-Zurita JO, Bautista-Rosales PU, *et al.* Overview of custard apple (*Annona reticulata* L.), pharmacology and phytochemicals: A review. *Revista Bio Ciencias*. 2023;10:e1401.
4. Ngbolua KN, Inkoto CL, Bongo GN, *et al.* Phytochemistry and bioactivity of *Annona reticulata* L. (Annonaceae): A mini-review. *South Asian Research Journal of Natural Products*. 2018;1(1):28-38.
5. Kumar SM, Azamthulla M, Saravanan KS. Pharmacognostical evaluation and anticonvulsant property of *Annona reticulata* Linn. (Annonaceae) root. *Future Journal of Pharmaceutical Sciences*. 2021;7:173.