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Quantitative Estimation of Quercetin in *Mimusops elengi* L. (Bakul) Leaves by HPTLC

Sakshi Sehgal, Vineet Gupta, Rajiv Gupta*, Shubhini A. Saraf

Faculty of Pharmacy, Babu Banarasi Das National Institute of Technology & Management, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow, Uttar Pradesh, India

ABSTRACT

Mimusops elengi L. is a medicinally valuable herb in the Ayurvedic and traditional systems of medicine. Various activities have been reported in almost all parts of *Mimusops elengi*, some of which includes diuretic activity, antidiabetic, antibacterial etc. Quercetin, one of the most important flavanoid is active against various cardio vascular diseases, cancer, tuberculosis, neurological diseases, cataract etc. In the present study High Performance Thin Layer Chromatography has been developed for detection and quantification of quercetin in *Mimusops elengi* leaves. Increasing serial dilutions of reference standard quercetin (200 to 1000 µg/mL) were scanned at 366 nm to detect and quantify the concentrations of quercetin in the test sample. The estimated values obtained from the same was 19.191mg/gm quercetin in powdered leaf sample. The method provided a rapid and easy approach for detection and the quantitation of the bio-marker quercetin. The authors also aim to validate the present method in terms of ruggedness and accuracy and undertake the isolation of quercetin from the said plant.

Keywords- *Mimusops elengi*; Bakul; quercetin; HPTLC.

INTRODUCTION

Mimusops elengi, family- Sapotaceae called as Maulsiri. Other synonyms are Bakul (Bengal), Gokul (Assam), is large glabrous evergreen tree 12-15m high with compact leafy head, short erect trunk, dark grey fissured bark and dense spreading crown [1,2]. In various different systems of medicines, *Mimusops elengi* places an important role. Leaf is one of Sushruta's snake remedies. In practice about half teaspoonful of expressed juice of fresh leaves is poured in nostrils in stupor and coma [1]. Various activities have been reported in almost all parts of *Mimusops elengi*, some of which includes diuretic activity, antidiabetic, antibacterial, cognitive enhancing activity and *in vitro* antioxidant activity has also been recently

reported [3,4,5,6,7]. Quercetin (3,3_,4_,5,7-pentahydroxyflavone) is a flavonoid that forms the chemical backbone for other flavonoids. Quercetin offers several potential therapeutic uses in the prevention of CVD, cancer, cataract, schizophrenia and prostatitis [8].

Phytochemical evaluation is one of the tool for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques. In the last two decades high performance thin layer chromatography (HPTLC) method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations. The present study aimed at quantitative estimation of concentration of quercetin in *Mimusops elengi* L. leaves extract.

MATERIALS AND METHODS

Plant material:

The leaves of *M.elengi* were collected from local gardens of Lucknow, Uttar Pradesh and were authenticated by CSIR recognized institute, National Botanical Research Institute, (NBRI) Lucknow. A voucher specimen was submitted for future reference. (Ref no. NBRI/CIF/178/2010)

Reagents and other materials:

Toluene, ethyl acetate, formic acid, methanol (all Reagents of analytical grade, Sigma) and silica gel F254 precoated TLC aluminum plates (E-Merck).

Preparation of standard solution:

Quercetin 10 mg was accurately weighed into a 10 mL volumetric flask, dissolved in 5 mL methanol and the solution was made up to 10 mL with the same solvent (1 mg/mL). From this stock solution, further dilutions were made, of concentrations ranging from 200-1000 µg/ml [9].

Preparation of sample solution:

Dried powdered leaves was extracted in methanol in soxhlet apparatus. Extracts was dried and concentrated under vacuum. 10mg of the dried methanolic extract was dissolved in 10ml of solvent. (Concentration- 1000µg/ml)

Development of HPTLC technique:

The samples were spotted using Camag microlitre syringe (2µl) on a precoated silica gel plates F 254 (10 cm X 10 cm, E.Merck). The plates were developed in a solvent system in glass chamber, previously saturated with the solvent for 30 min. TLC plates were air dried and scanning was performed on a Camag TLC Scanner in absorbance at 366 nm and operated by Wincats software [10].

Quercetin estimation in *Mimusops elengi* :

Stationary Phase- Silica gel F 254 plates

Mobile phase- Toluene: Ethyl acetate: Formic acid (5:4:1)

Standard- Quercetin- 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml, 1000 µg/ml (2 µL)

Sample- *M. elengi* extract 1000 µg/ml (2 µL)

Migration distance- 80 mm

Scanning wavelength- 366 nm

RESULTS AND DISCUSSION

The Rf value of standard quercetin was found to be 0.58 in all tracks. Rf values of all tracks along with their areas are mentioned in table 1. Chromatograms of different concentrations of standard quercetin is given from Fig 1-5. Fig 6 depicts the chromatogram for the test sample. Calibration curve for the standard quercetin is given in Fig 7. On putting the values in the regression equation, $y = 2.250x + 838.9$, the concentration of quercetin in test sample was calculated to be 19.191mg/gm. Fig 8 depicts the spectral comparison of test sample track alongwith standard tracks.

Table 1. Rf values and Area under curves of standard quercetin varying dilutions

TRACKS (Std quercetin conc. in µg/ml)	Rf VALUES	AREA UNDER CURVE (AU)
Track 1- 200	0.58	1248.2
Track 2- 400	0.58	1802.7
Track 3- 600	0.58	2207.4
Track 4- 800	0.58	2968.3
Track 5- 1000	0.58	4115.9

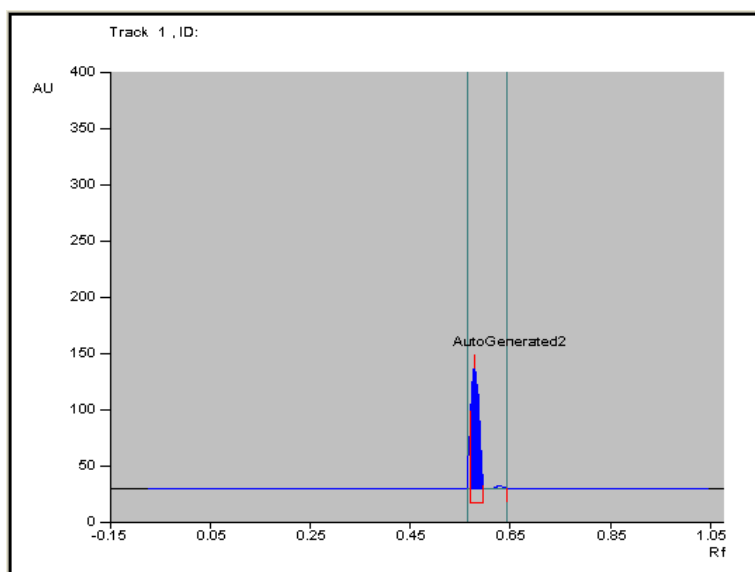


Fig 1. HPTLC chromatogram of standard quercetin (conc-200µg/ml)

Recent years have seen an exponential increase in research antioxidant properties of medicinal plants. Polyphenols like quercetin attributes to the antioxidant property in plants and has potential therapeutic uses in the prevention of Cardio vascular diseases (CVD), cancer, cataract etc. High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation and quantification of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous

samples can be run in a single analysis thereby dramatically reducing analytical time, the most important advantage of this method is accuracy, selectivity and simplicity.

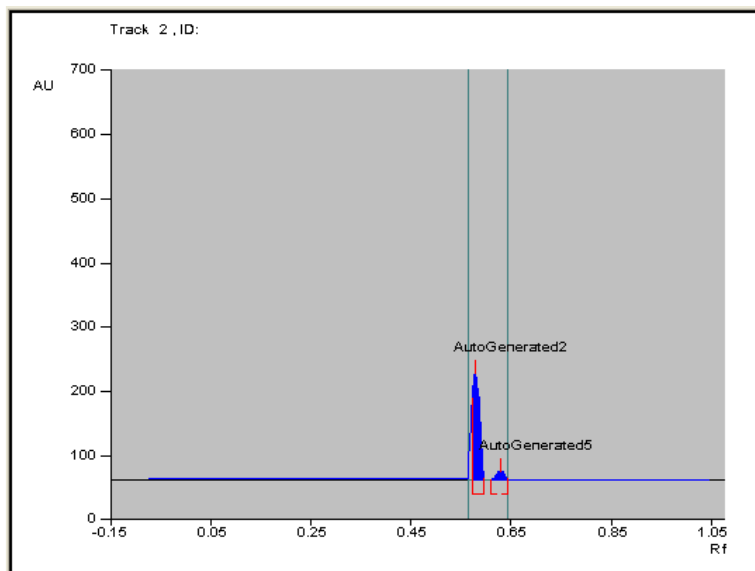


Fig 2. HPTLC chromatogram of standard quercetin (conc-400µg/ml)

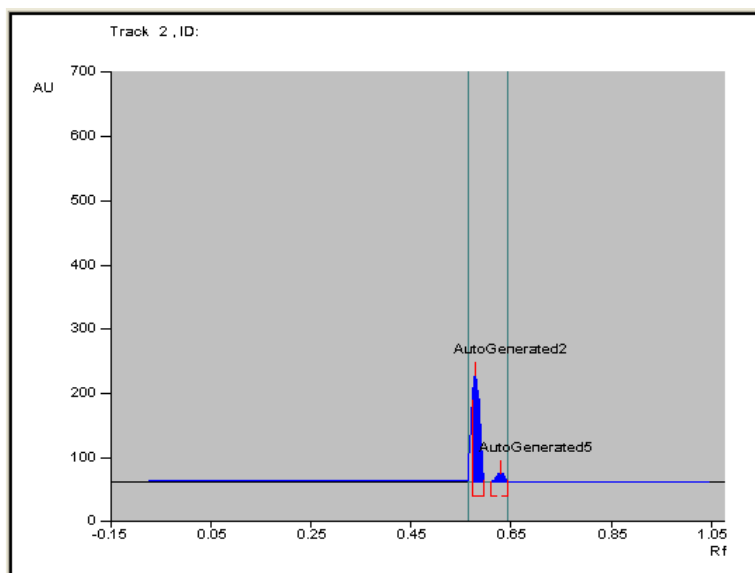


Fig 3. HPTLC chromatogram of standard quercetin (conc-600µg/ml)

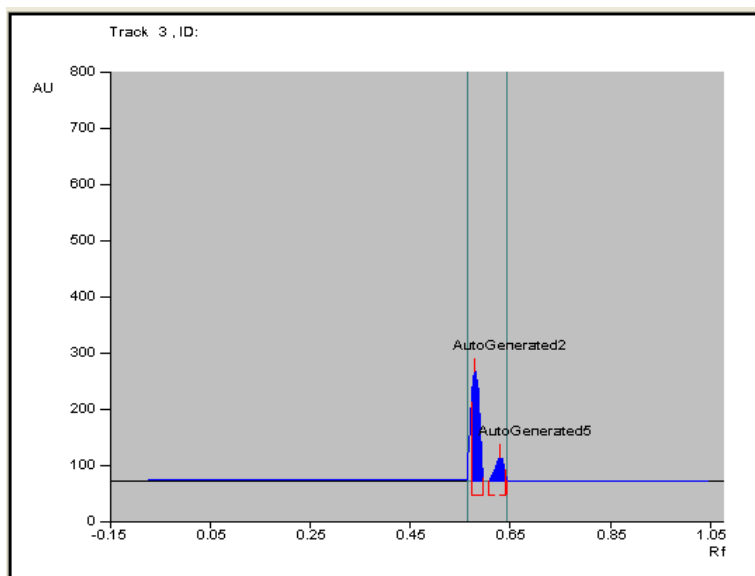


Fig 4. HPTLC chromatogram of standard quercetin (conc-800µg/ml)

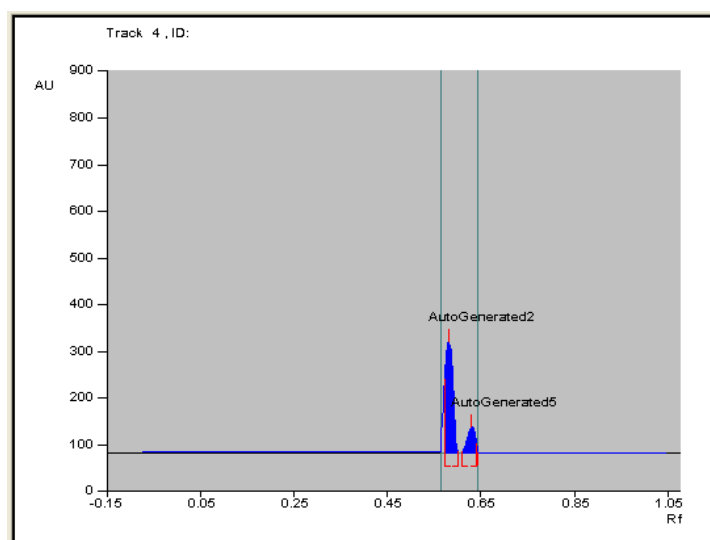
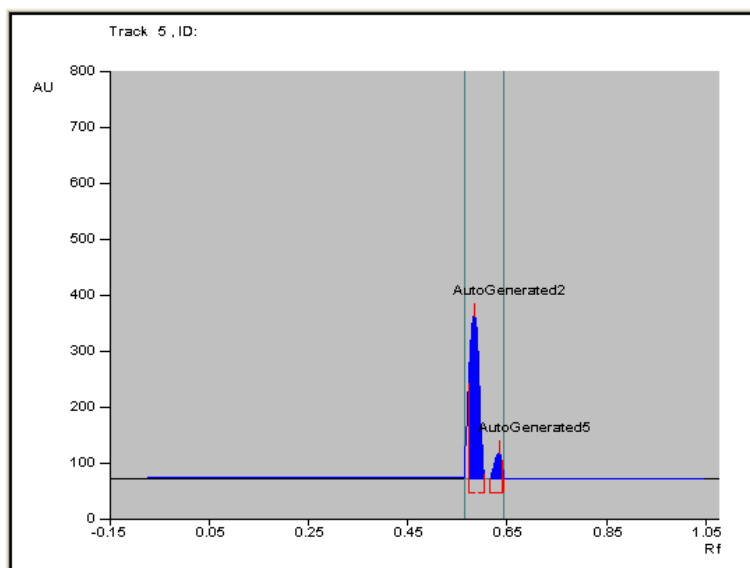
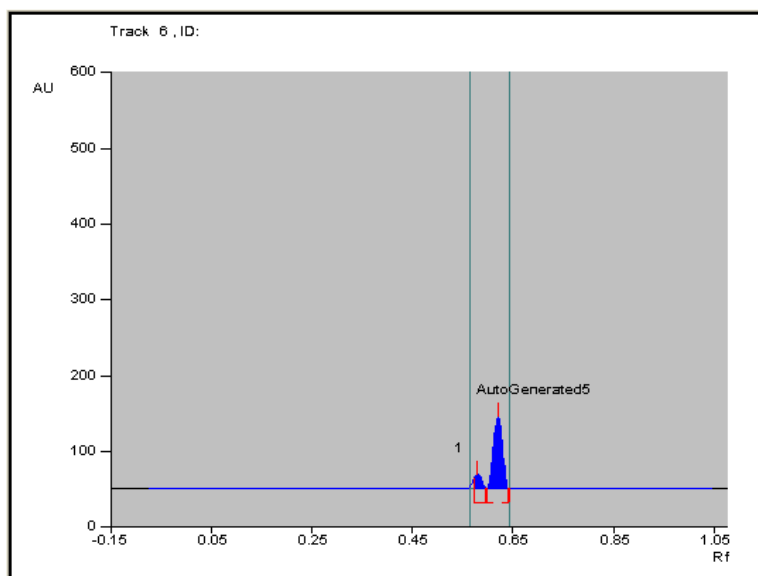


Fig 5. HPTLC chromatogram of standard quercetin (conc-1000µg/ml)

**Fig 6. HPTLC Chromatogram of test sample****Fig 7. Standard curve of quercetin**

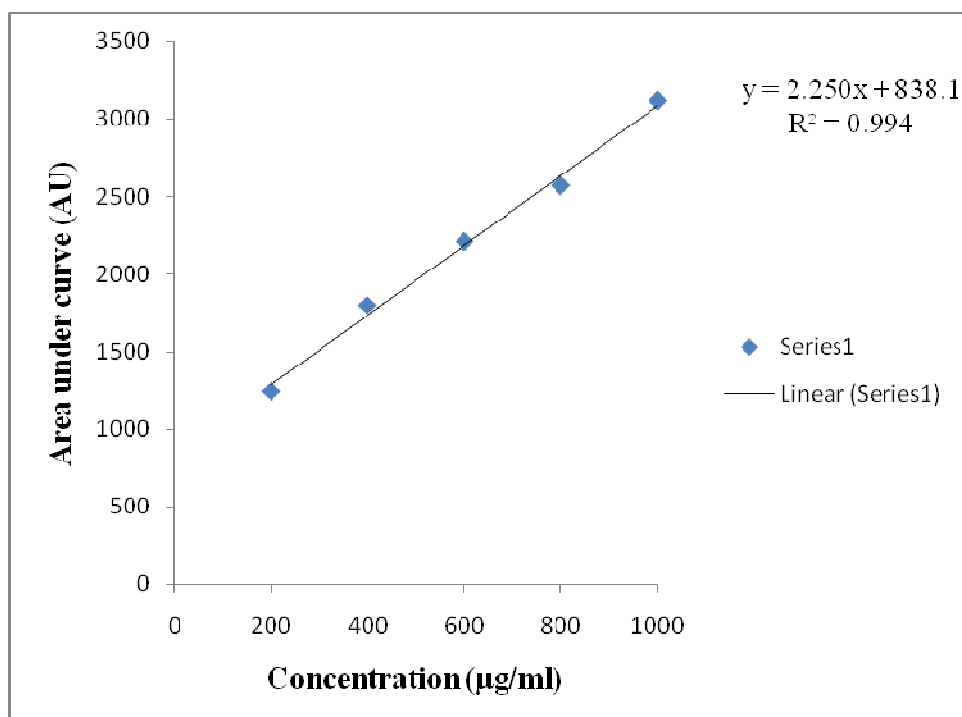
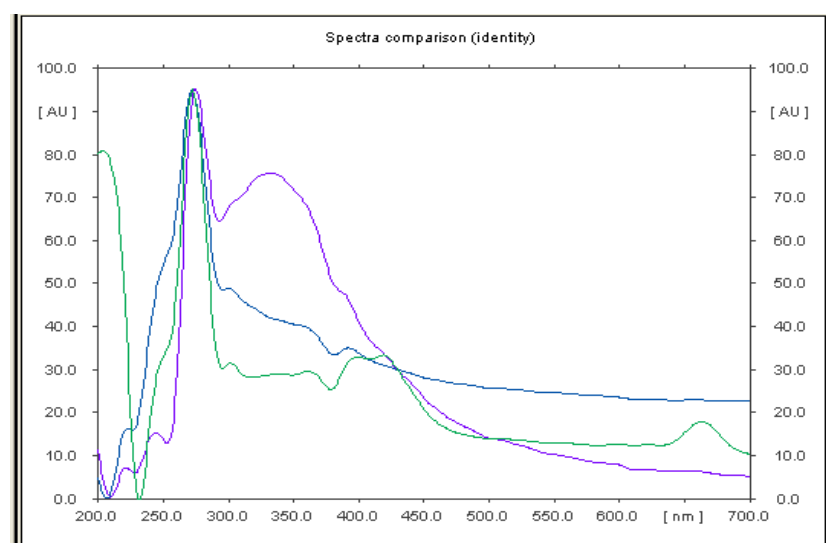


Fig 8. Spectral comparison of Track 6(test sample) with track 4 and track 5 (standard quercetin)



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