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Research Article

DEVELOPMENT OF HPTLC DENSITOMETRIC METHOD FOR ESTIMATION OF QUERCETIN IN *BOMBAX CEIBA* L. LEAVES

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ABSTRACT

Bombax ceiba L. is a medicinally valuable herb in the ayurvedic and traditional systems of medicine. Various activities have been reported in almost all parts of *Bombax ceiba*, some of these include hypertensive, antioxidant, hypoglycemic, hepatoprotective, and antipyretic. Quercetin, one of the most important flavonoids is active against various cardio vascular diseases, cancer, tuberculosis, neurological diseases, cataract etc. In the present study High Performance Thin Layer Chromatography method has been developed for detection and quantification of quercetin in *Bombax ceiba* 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 value obtained from the same was 5.38% quercetin in powdered leaf sample. The method provided a rapid and easy approach for detection and the quantization of the bio-marker quercetin.

Keywords: *Bombax ceiba,* Bombacaceae, Quercetin, HPTLC

1. INTRODUCTION

Bombax ceiba L. (syn *Bombax malabaricum, Salmalia malabarica*) belongs to the family Bombacaceae. It is important Ayurvedic medicinal plant distributed in different part of India up to 1500m. It is a large, deciduous tree, commonly known as semal, Shaalmali, Mochaa, Pichhila, Raktapushpa, Tuulini, Purani, Silk cotton [1]. The various parts used include roots, leaves, flowers, seeds etc. Leaves are used as anti-inflammatory property; used as antidysentric, in leucorrhoea, anemia, infertility [2] stem bark and Roots are used as antidysentric, aphrodisiac, astringent, demulcent, emetic & tonic diarrhea, dysentery, boils of burns, diabetes, snake bite, leucorrhoea [3, 4] Flower and fruit have used in anti fertility, uterus protection [5]. Quercetin is a flavonoid that forms the chemical backbone for other flavonoids. Quercetin offers several potential therapeutic uses in cancer, cataract, schizophrenia and prostatitis [6]. The leaves, stem and root bark contain crude protein, crude fiber, calcium, phosphorous and Shamimin, quercetin, beta sterol, gallic acid [7-9]. 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 [10]. The present study aimed at quantitative estimation of concentration of quercetin in *Bombax ceiba*. Leaves extract.

2. MATERIAL AND METHODS

2.1. Plant material

The leaves of *Bombax ceiba* were collected from Allahabad, 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. NRRI/CIF/292/2012).

2.2. Reagents and other materials

Toluene: Ethyl acetate: Formic acid and silica gel F254 precoated TLC aluminum [11] plates.

2.3. Preparation of standard solution

A stock solution of Quercetin (1000 µg mL-1) was prepared by dissolving 10.0 mg of accurately weighed quercetin in Methanol and diluting it to 10.0 mL with methanol [12]. Further dilutions were made with Methanol to obtain working standards 20, 40, 60, 80 and 100µg/ml.

2.4. Preparation of sample solution

Dried powdered leaves were 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)

2.5. 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 [13] and operated by Win cats software.

2.6. Quercetin estimation in *Bombax ceiba*

Stationary Phase- Silica gel F 254 plates
Mobile phase- Toluene: Ethyl acetate: Formic acid (5:4:1)
Standard- Quercetin- 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100 µg/ml (2 µL)
Sample- *Bombax ceiba* extracts 100 µg/ml (2 µL)
Migration distance- 85 cm
Scanning wavelength- 366nm

3. RESULTS AND DISCUSSION

The Rf value of standard quercetin was found to be 0.91 in all tracks. Rf values of all tracks along with their areas are mentioned in table 1. Chromatogram of different concentrations of standard quercetin is given from Fig 1-5. Fig 6 depicts the chromatogram for the test sample.

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

Tracks (Std quercetin conc. in µg/ml)	Rf value	Area Under Curve (AUC)
Track – 1 (20µg/ml)	0.91	1244.9
Track – 2 (40µg/ml)	0.91	1838.6
Track – 3 (60µg/ml)	0.91	2128.9
Track – 4 (80µg/ml)	0.91	3106.0
Track – 5 (100µg/ml)	0.91	4246.8

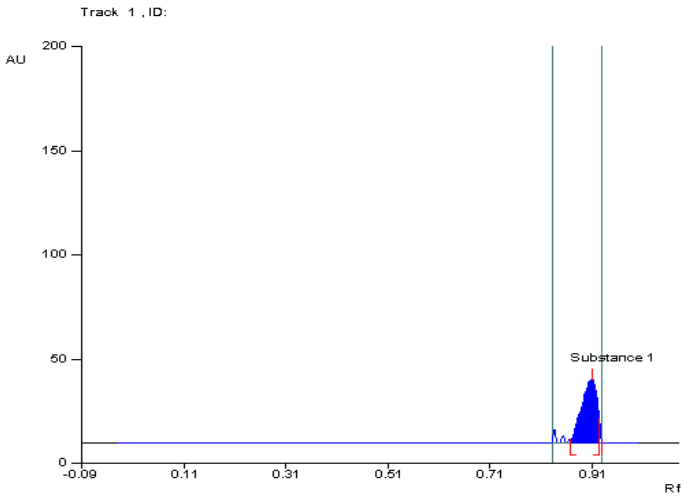

Fig. 1 - HPTLC Chromatogram of Track 1 (Standard) (200µg/ml)

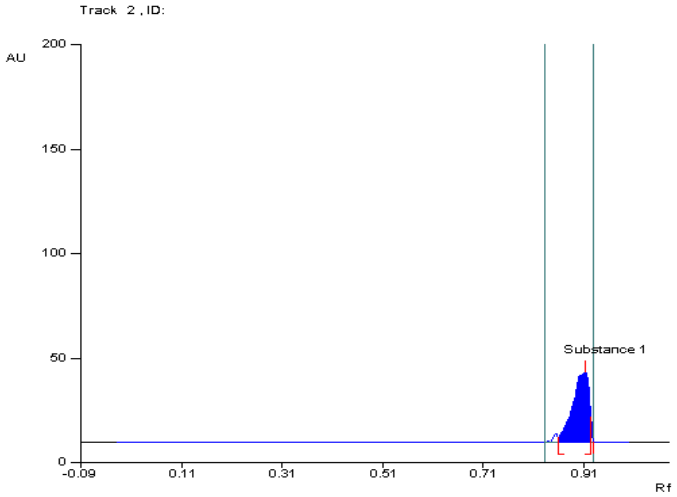

Fig. 2 - HPTLC Chromatogram of Track 2 (Standard) (400µg/ml)

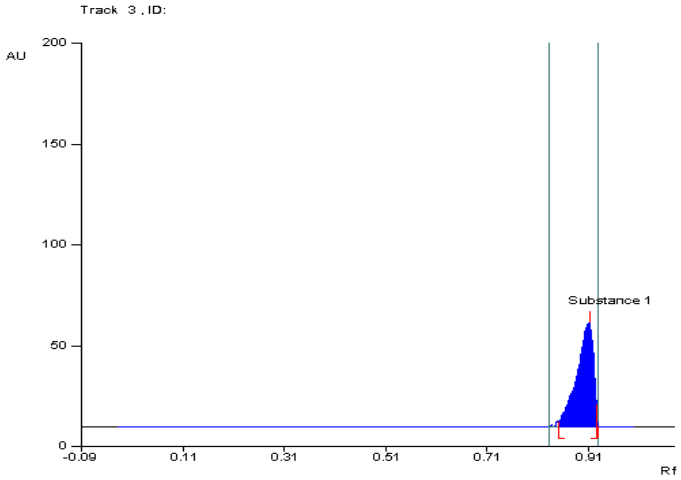

Fig. 3 - HPTLC Chromatogram of Track 3 (Standard) (600µg/ml)

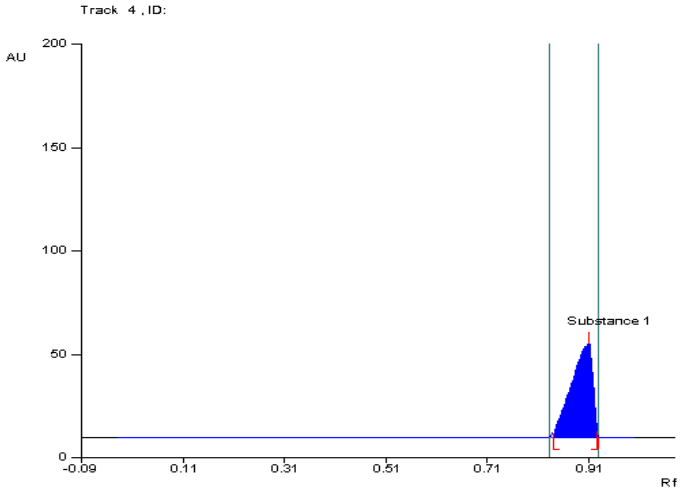

Fig. 4 - HPTLC Chromatogram of Track 4 (Standard) (800µg/ml)

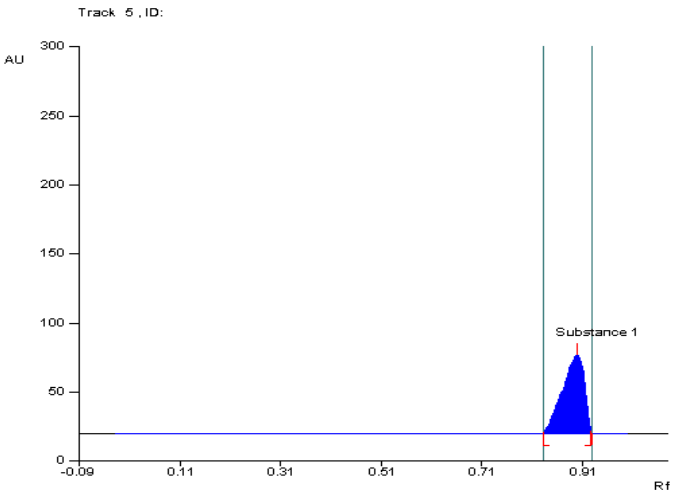

Fig. 5 - HPTLC Chromatogram of Track 5 (Standard) (1000µg/ml)

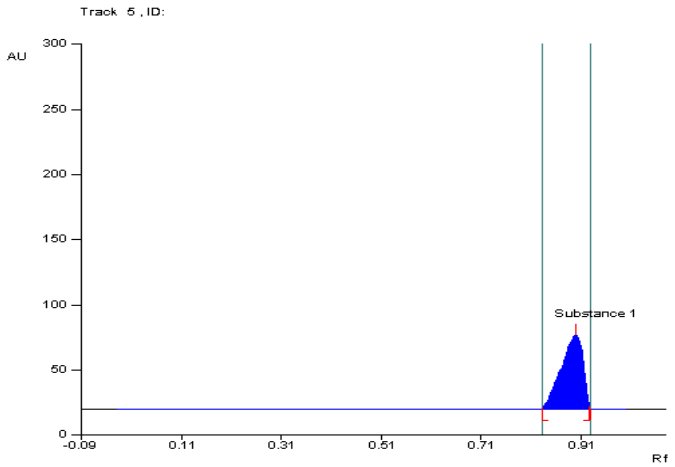

Fig. 6 - HPTLC Chromatogram of Track 6 (Test sample)

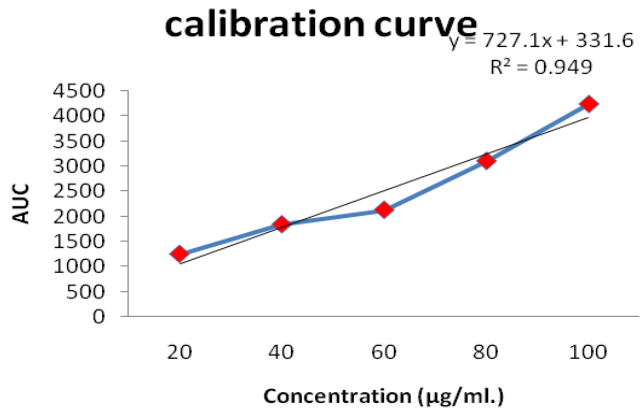

Fig. 7 - Calibration curve for Quercetin

Calibration curve for the standard quercetin is given in Fig 7. On putting the values in the regression equation, y= 727.1x+331.6, the concentration of quercetin in test sample was calculated to be 5.38%. Fig 8 depicts 3D-Display spectral comparison of test sample track along with standard tracks.

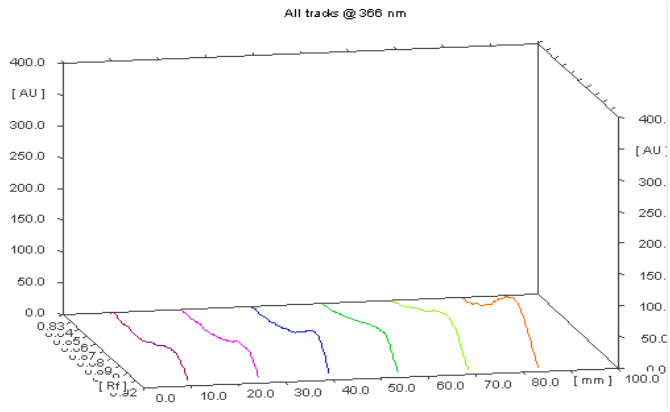

Fig. 8 - 3D-Display of all Tracks (1-6) at 366nm

4. CONCLUSION

The present method provided a quick and easy approach for detection and quantitation of biomarker quercetin in *Bombax ceiba L.* and the estimated values indicates that the leaves are the good source of the said marker in the plant.

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