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DETERMINATION OF QUERCETIN IN *MICHELIA CHAMPACA* L. (CHAMPA) LEAVES AND STEM BARK BY HPTLC

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ABSTRACT

Background: *Michelia champaca* L. (Magnoliaceae) popularly known as champa is a reservoir of numerous bio-markers, assuming great significance in view of its rich chemistry.

Objective: In the present study a High Performance Thin Layer Chromatography method has been used for detection, and quantification of quercetin in *Michelia champaca* (leaves and stem-bark).

Materials and Methods: Increasing serial dilutions of reference standard quercetin (200 to 1000 µg mL^{-1}) were scanned at 366 nm to detect and quantify the concentrations of quercetin in the test samples.

Results: The estimated values obtained from the same were 682.235, 498.518 µg $mL^{-1}$for leaves and stem bark respectively, amounting to 68.223 and 49.851 mg/g in the drug samples respectively. Leaves were found to be the richest source of quercetin in *Michelia champaca*.

Conclusion: The method provided a rapid and easy approach for detection and the quantitation of the bio-marker quercetin

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KEYWORDS

Michelia champaca, Quercetin, HPTLC, quantitation

INTRODUCTION

Michelia champaca L. (Magnoliaceae), commonly known as champa a native of Southern parts of India is cultivated in various parts of India and planted in gardens and near temples[1-2] . The glorious medicinal plant is a reservoir of numerous active principles and secondary metabolites and is extremely rich in its chemistry and is often widely used traditionally for indolent swellings, fevers and in nervousness[3]. Parthenolide from leaves and root bark, michampanolide, 8-acetoxyparthenolide magnograndiolide, costunolide, dihydroparthenolide and micheliolide from root bark and β-sitosterol, liriodenine, ushinsunine, magnoflorine from stem bark are some of the important chemical moieties reported from this plant[4-10]. High Performance thin layer chromatography is an important tool that can be used qualitatively as well as quantitatively for checking the purity and identity of crude drugs and also for quality control of finished product [11]. HPTLC techniques find applications in a wide range of fields including medicines, pharmaceutical, chemistry, biochemistry and toxicology. An HPTLC detection and quantitation method for quercetin in *Michelia champaca* L. has not been reported in literature; hence an attempt was made to estimate and quantify quercetin in *Michelia champaca* L., (leaves and stem-bark) with the help of HPTLC chromatographic fingerprints in the present investigation.

MATERIALS AND METHODS

Plant Material:
The plant material was collected in and around Lucknow, Uttar Pradesh in the month of August and authenticated by National Botanical Research Institute, Lucknow; also a voucher specimen was submitted for future reference (Ref No. NBRI/CIF/176/2010). The air dried plant material was size communited to a moderately fine powder (#355/180) and stored in an air-tight container for future/further studies.

Solvents: All the solvents used were of AR grade.

***Reference standard*:** The reference standard (Quercetin) was obtained from SD Fine Chemicals, Mumbai, India.

***Chromatographic conditions*:**

Instrument: HPTLC system equipped with a sample applicator device Camag Linomat 5. Camag twin trough chamber, Camag TLC scanner and integration software (Wincats)
***HPTLC Plate*:** Silica gel GF254 (Merck) 15 X 10 cm
***Mobile Phase*:** Toluene: Ethyl acetate: Acetic acid: Methanol (2.5:7:0.25:0.25) [12]
***Wavelength*:** 366 nm

Standard Preparation:
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 [13]. Further dilutions were made with Methanol to obtain working standards 200, 400, 600, 800 and 1000 µg mL^{-1}.

Sample Preparation:
100 mg of size reduced air dried powdered plant material (leaves, stem-bark) was defatted with n-

Hexane and then Soxhlet extracted with Methanol for 16 hours. The methanolic extract was concentrated and 10 mg of the concentrated methanolic extract was redissolved in 10 mL Methanol to obtain a test sample (1000 µg mL^{-1})

Procedure:

The TLC plate was activated by placing in an oven at the temperature of 110 °C for 20 min. the plate was spotted with test and standard preparation maintaining a distance of 15mm from the edge of TLC plate. It was developed upto 75mm in the twin trough chamber using mobile phase, dried in an oven and subjected for TLC scanning at 366 nm [14].

RESULTS AND DISCUSSION

Under the chromatographic conditions described above, the Rf value of quercetin was about 0.90 and 0.91 in leaves and stem-bark of *Michelia champaca* respectively. The respective RF's obtained for each track is shown in Table 1. (Structure of quercetin is shown in Fig 1) The Chromatograms of standard quercetin are shown in Figure 2 (A-E) and that of quercetin in *Michelia champaca* are shown in Figure 3 (A-B). Spectral Comparison of quercetin reference standard with quercetin in samples is shown in Fig 4 (A-D). The 3D spectra of all tracks scanned at 366 nm are shown in Figure 5. The area under the curve (AUC) obtained for various tracks are enumerated in Table 2. The calibration curve was linear in the range of 200 to 1000 µg mL^{-1}, as illustrated in Figure 6. From the regression equation, y = 1.087x – 36.29, the concentrations of the test samples i.e. leaves (Track 6) and stem-bark (Track 7) was estimated to be about 682.235 and 498.518 µg mL^{-1} respectively. The estimated value on per gram basis of drug was about 68.223 and 49.815 mg/g of leaves and stem bark respectively.

Table 1
Rf range and maximum Rf (peak) of tracks 1-7.

S.No.	Start position	Maximum Rf	End position
Track1	0.90	0.91	0.92
Track2	0.88	0.91	0.93
Track3	0.87	0.91	0.92
Track4	0.87	0.90	0.92
Track5	0.87	0.90	0.92
Track6	0.87	0.90	0.92
Track7	0.88	0.91	0.92

Table 2

Area under curve values for different concentrations of working standards of quercetin for linear calibration.

S.No.	Concentrations of working standard of quercetin (µg mL^{-1})	Area under Curve (AU)
Track1	200	198.60
Track2	400	412.50
Track3	600	598.30
Track4	800	794.70
Track5	1000	920.19

Figure 1

Structure of quercetin

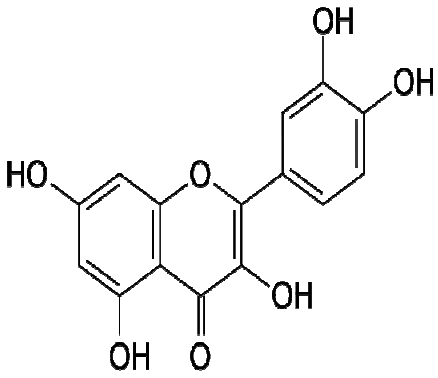
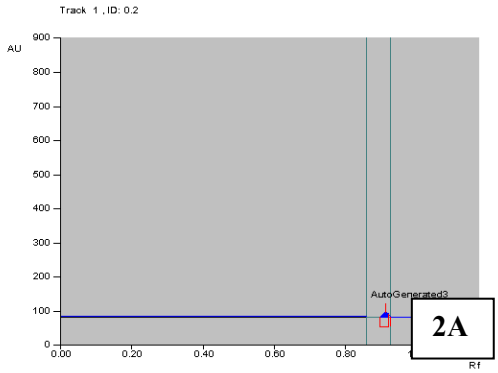
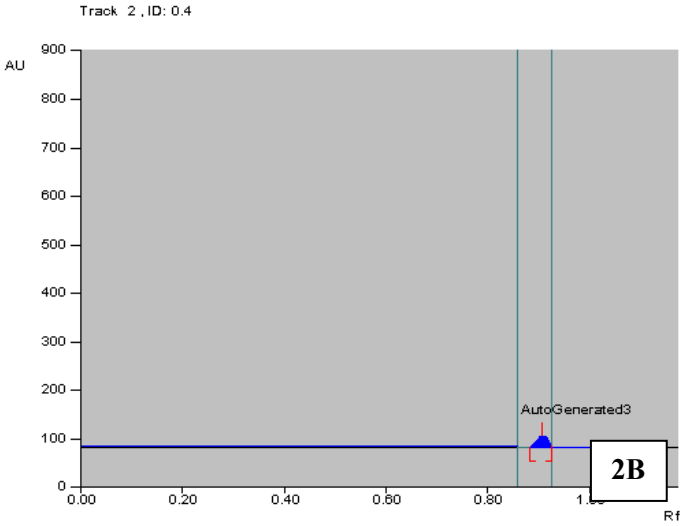
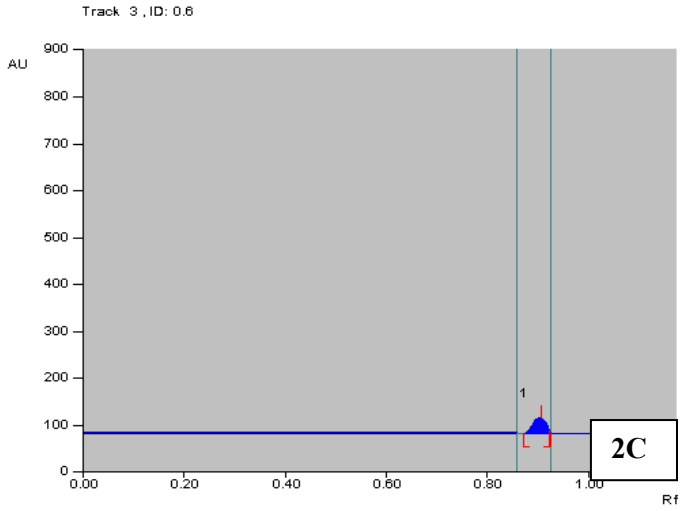


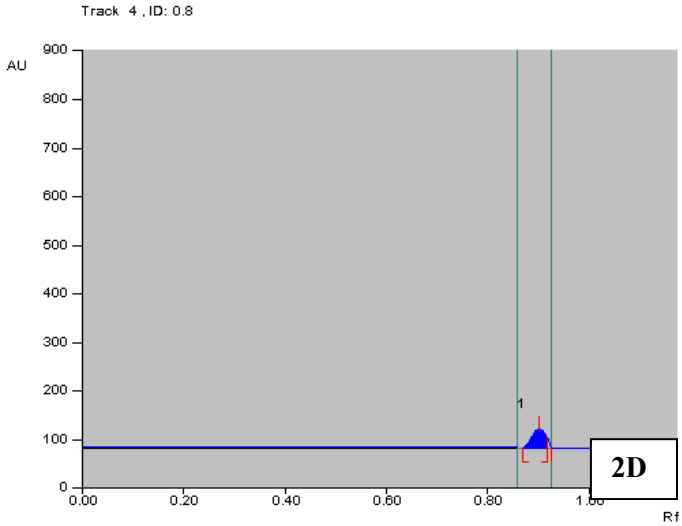
Figure 2

A Typical HPTLC chromatogram of quercetin working standard (A) Track 1 (200µg mL^{-1}) (B) Track 2 (400 µg mL^{-1}) (C) Track 3 (600 µg mL^{-1}) (D) Track 4 (800 µg mL^{-1}) (E) Track 5 (1000 µg mL^{-1})




Track 2 , ID: 0.4
AU
900
800
700
600
500
400
300
200
100
0
0.00
0.20
0.40
0.60
0.80
Rf
AutoGenerated3
2B


Track 3 , ID: 0.6
AU
900
800
700
600
500
400
300
200
100
0
0.00
0.20
0.40
0.60
0.80
1.00
Rf
1
2C


Track 4 , ID: 0.8
AU
900
800
700
600
500
400
300
200
100
0
0.00
0.20
0.40
0.60
0.80
Rf
1
2D

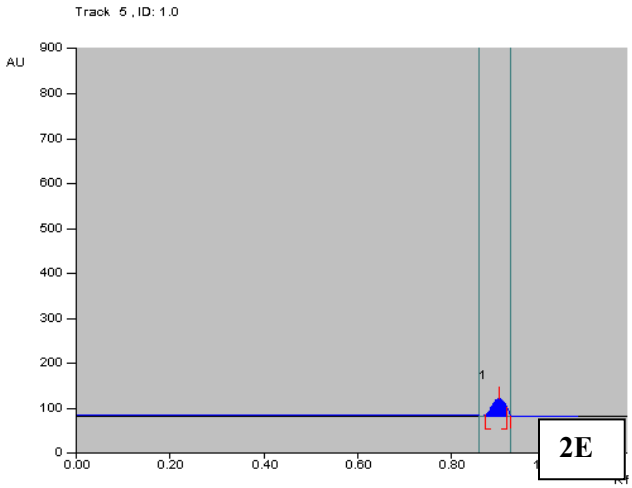


Figure 3
A Typical HPTLC chromatogram of quercetin in Michelia champaca L. (A) Track 6 (leaves) (B) Track 7 (stem bark)

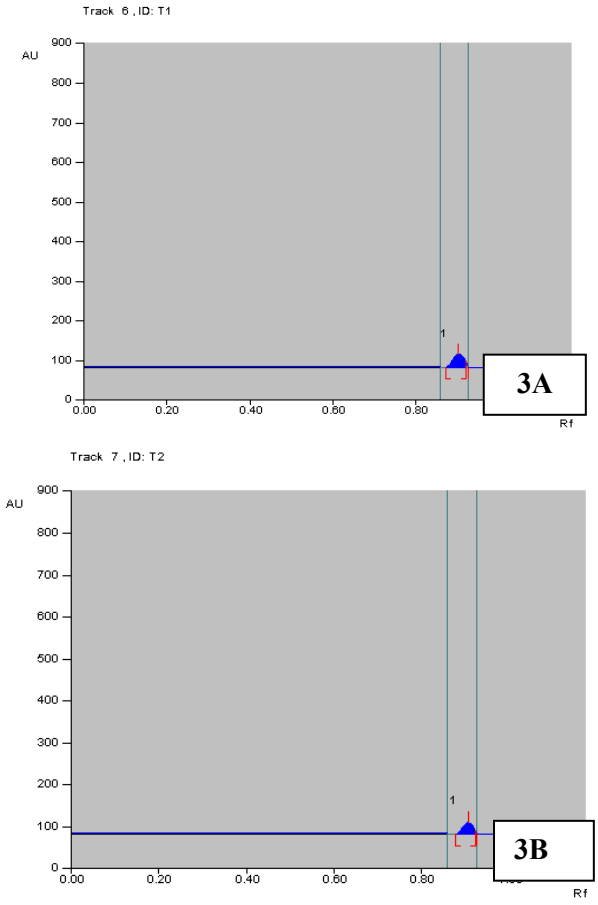
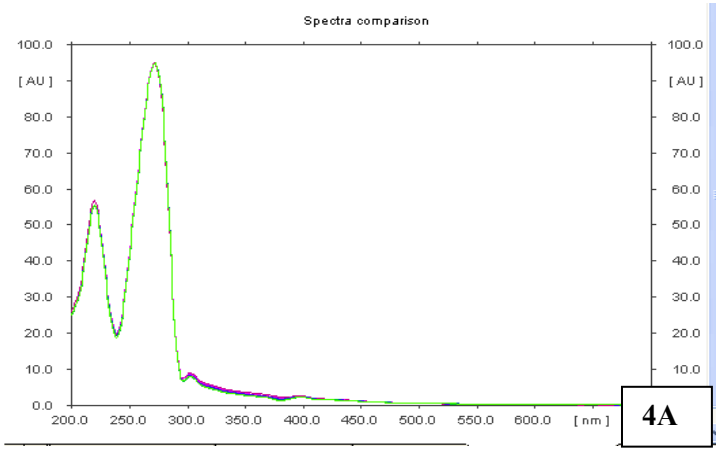
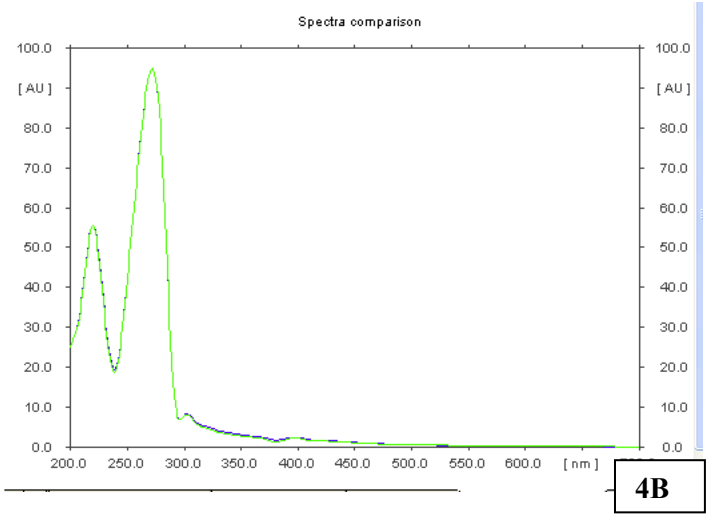


Figure 4

Spectral comparison of sample tracks with standards at selected wavelength. (A) Track 6 with Tracks (1-5) at 272 nm (B) Track 6 with Track 3 at 272 nm (C) Track 7 with Tracks (1-5) at 272 nm (D) Track 7 with Track 3 at 272 nm



4A



4B

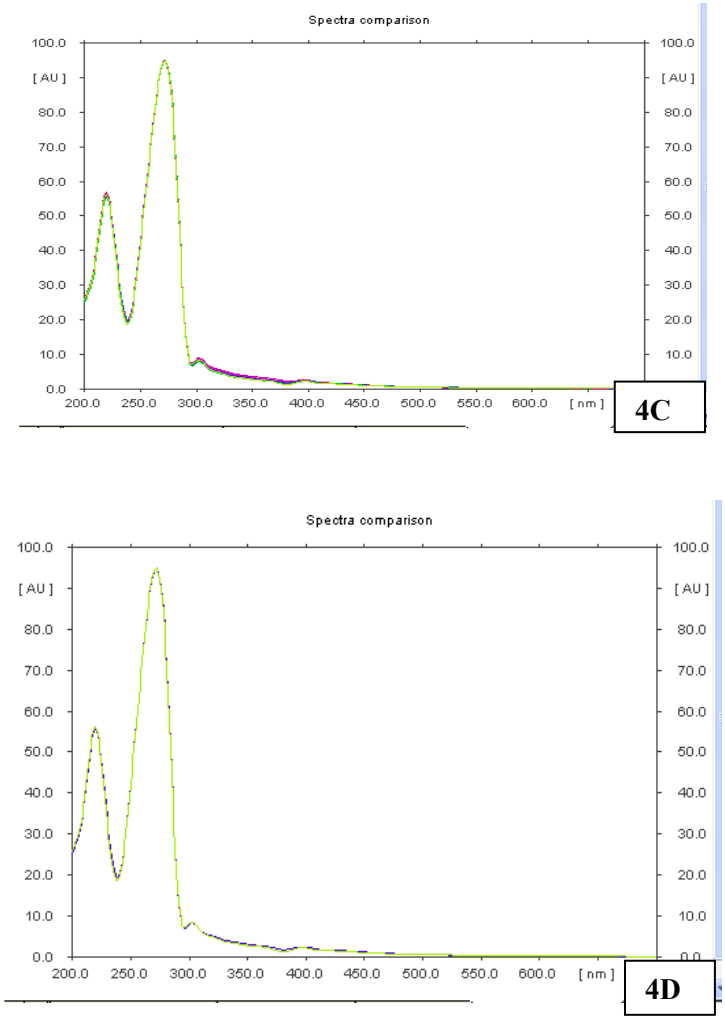


Figure 5
3D spectra of Tracks 1-7 scanned at 366 nm

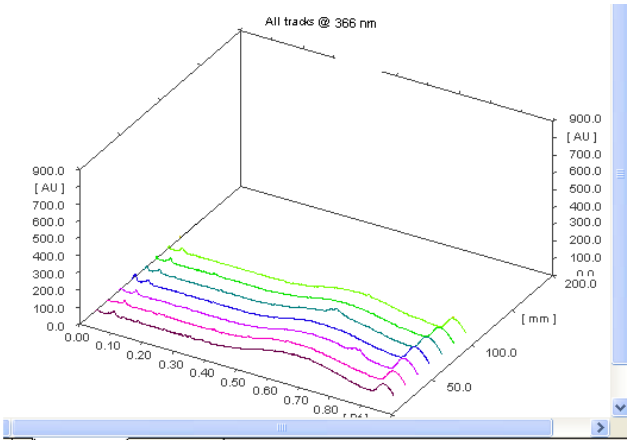
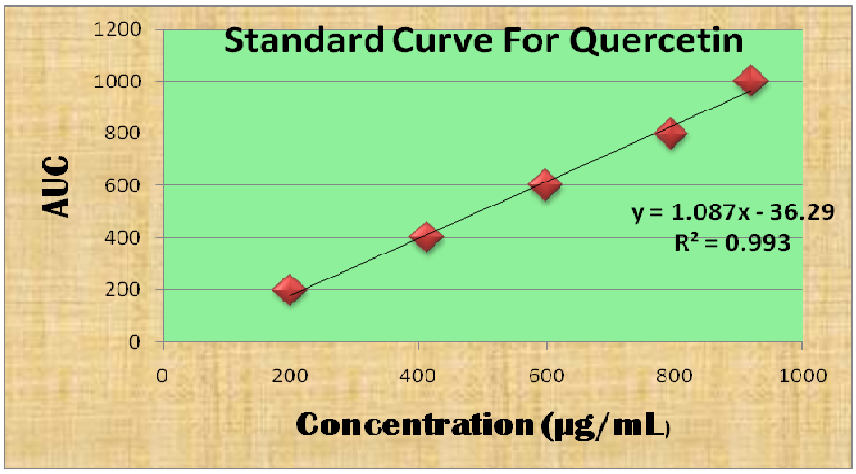


Figure 6
Standard curve (line of best fit) for quercetin



CONCLUSION

The present method provided a quick and easy approach for detection and quantitation of biomarker quercetin in *Michelia champaca* and the estimated values indicate that the leaves are the richest source of the said marker in the plant. The authors further aim to validate the method in terms of robustness, accuracy and percentage recovery.

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