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Determination of Gallic acid in *Michelia champaca* L. (Champa) Leaves and Stem Bark by HPTLC

Hafsa Ahmad, Anurag Mishra, Rajiv Gupta*, Shubhini A. Saraf

Department of Pharmacognosy, Faculty of Pharmacy, Babu Banarasi Das National Institute of Technology & Management, Sector-I, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow-227105, Uttar Pradesh, India

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ABSTRACT:

Background: Michelia champaca L. (Magnoliaceae) popularly known as Champa is a reservoir of numerous bio-markers, and assumes greater 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 gallic acid in Michelia champaca (leaves and stem-bark). Materials and Methods: Increasing serial dilutions of reference standard gallic acid (200 to 1000 µg mL^{-1}) were scanned at 254 nm to detect and quantify its concentrations in the test samples. Results: The estimated values obtained from the same were 736.963 and 595.287 µg $mL^{-1}$for leaves and stem bark respectively, amounting to 73.696 and 59.287 mg/g in the drug samples respectively. Leaves were found to be the richest source of gallic acid in Michelia champaca. Conclusion: The method provided a rapid and easy approach for detection and the quantitation of the poly-phenol gallic acid. The authors also aim to validate the present method in terms of ruggedness and accuracy and undertake the isolation studies on the said plant.

Keywords: *Michelia champaca*, Gallic acid, HPTLC, quantitation

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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. HPTLC techniques find applications in a wide range of fields including medicines, pharmaceutical, chemistry, biochemistry and toxicology. [11] Gallic acid (chemically 3, 4, 5,-trihydroxybenzoic acid) is a phenylpropanoid (Figure 1). Polyphenolics like gallic acid are known to possess anti-inflammatory, cardio-protective, anti-oxidant and astringent responses and exert beneficial effects on human health. [12] An HPTLC detection and quantitation method for gallic acid in *Michelia champaca* L. has not been reported in literature; hence an attempt was made to estimate and quantify gallic acid 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 (Gallic acid) 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: Formic acid: Methanol (3:3:0.8:0.4) [13]
Wavelength: 254 nm

Standard Preparation:
A stock solution of gallic acid (1000 µg mL^{-1}) was prepared by dissolving 10.0 mg of accurately weighed gallic acid in Methanol and diluting it to 10.0 mL with methanol.[12] 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 $^{\circ}C$ 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 254 nm. [14]

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

S.No.	Start position	Maximum Rf	End position
Track1	0.91	0.97	1.00
Track2	0.91	0.97	1.00
Track3	0.91	0.96	1.00
Track4	0.91	0.96	0.99
Track5	0.91	0.96	0.99
Track6	0.91	0.96	1.00
Track7	0.91	0.96	1.00

Table 2: Area under curve values for different concentrations of working standards of Gallic acid for linear calibration.

S.No.	Concentrations of working standard of gallic acid (µg mL^{-1})	Area under Curve (AU)
Track1	200	2587.50
Track2	400	2746.10
Track3	600	2936.50
Track4	800	3082.50
Track5	1000	3375.00

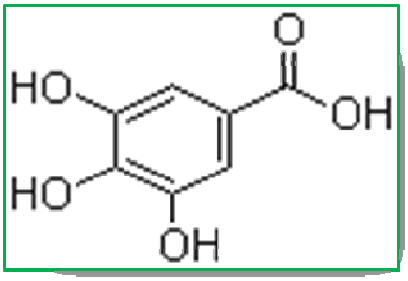
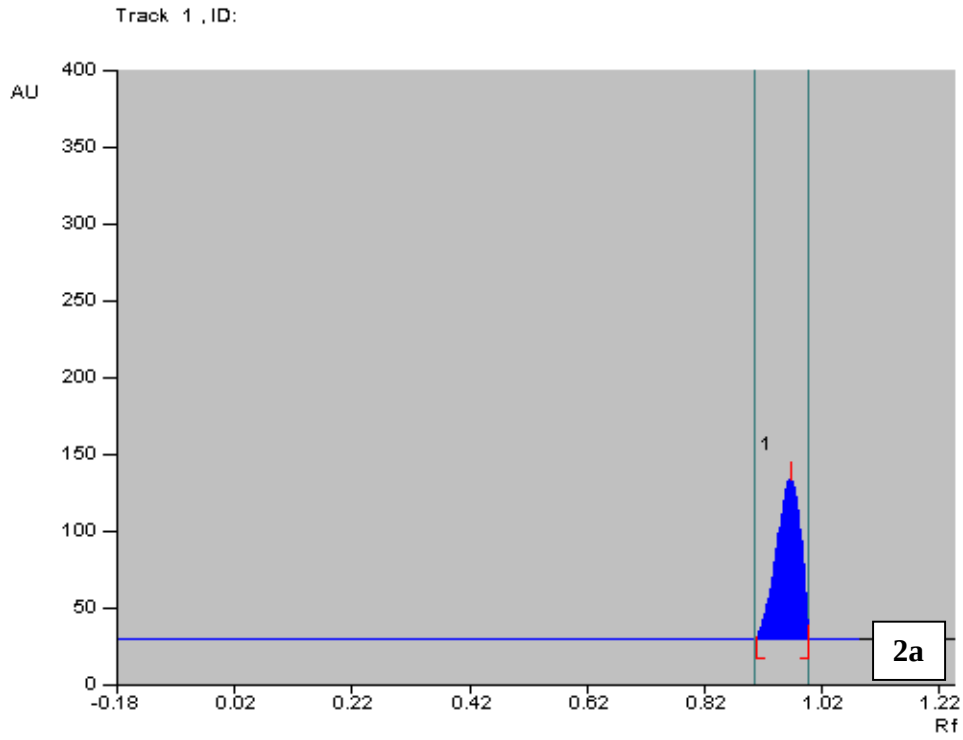


Figure 1: Structure of gallic acid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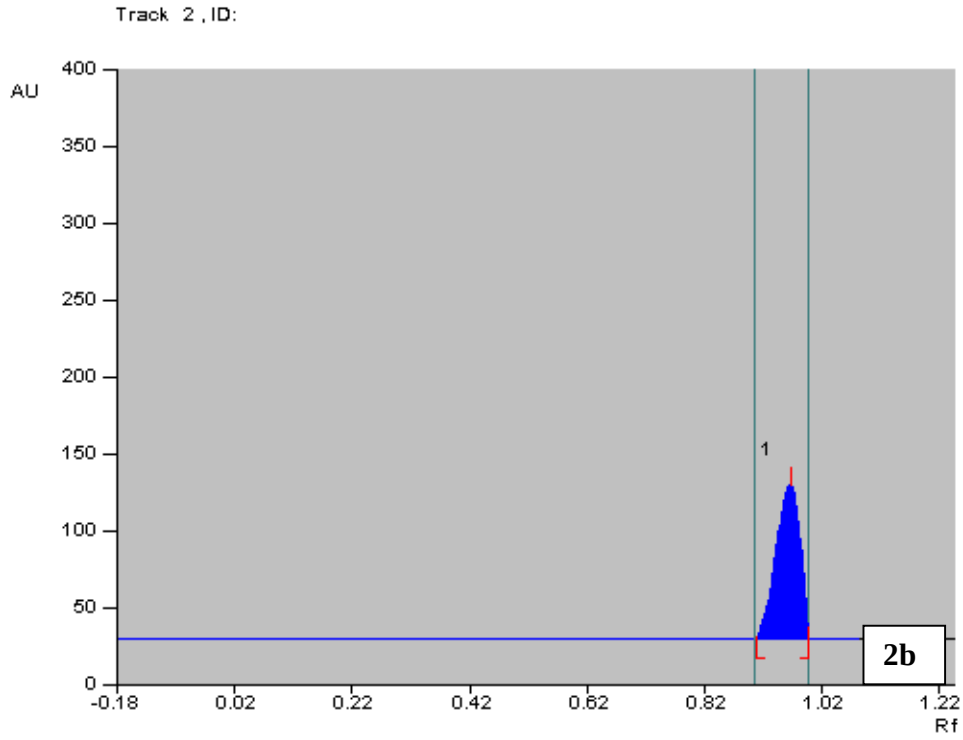
AU
400
350
300
250
200
150
100
50
0
1
2a
-0.18
0.02
0.22
0.42
0.62
0.82
1.02
1.22
Rf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AU
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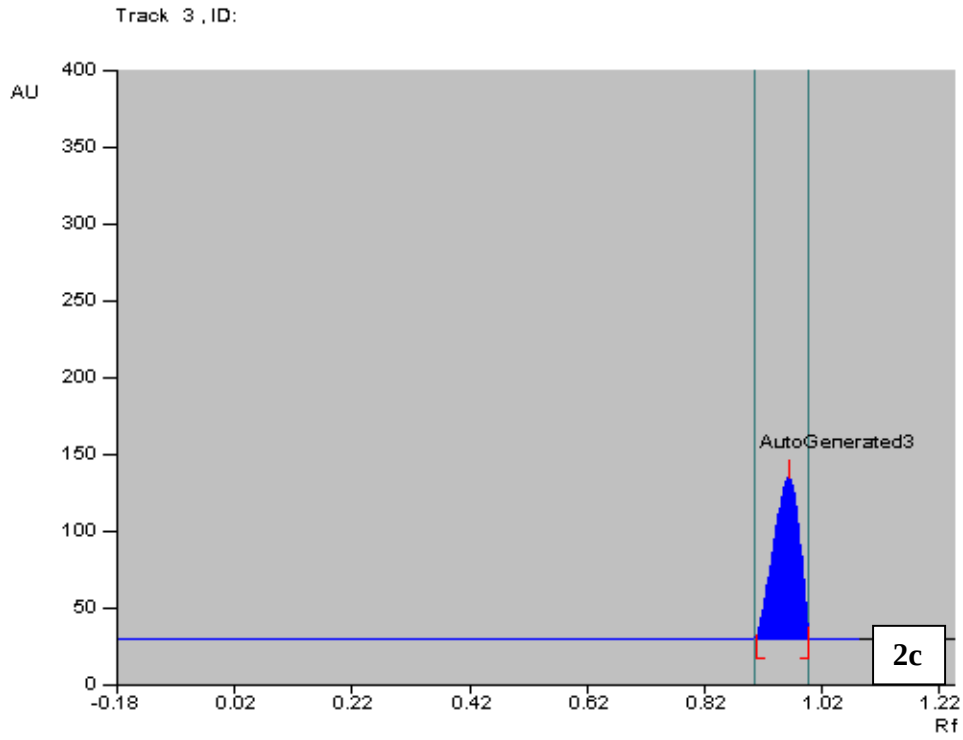
350
300
250
200
150
100
50
0
1
2b
-0.18
0.02
0.22
0.42
0.62
0.82
1.02
1.22
Rf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350
300
250
200
150
100
50
0
AutoGenerated3
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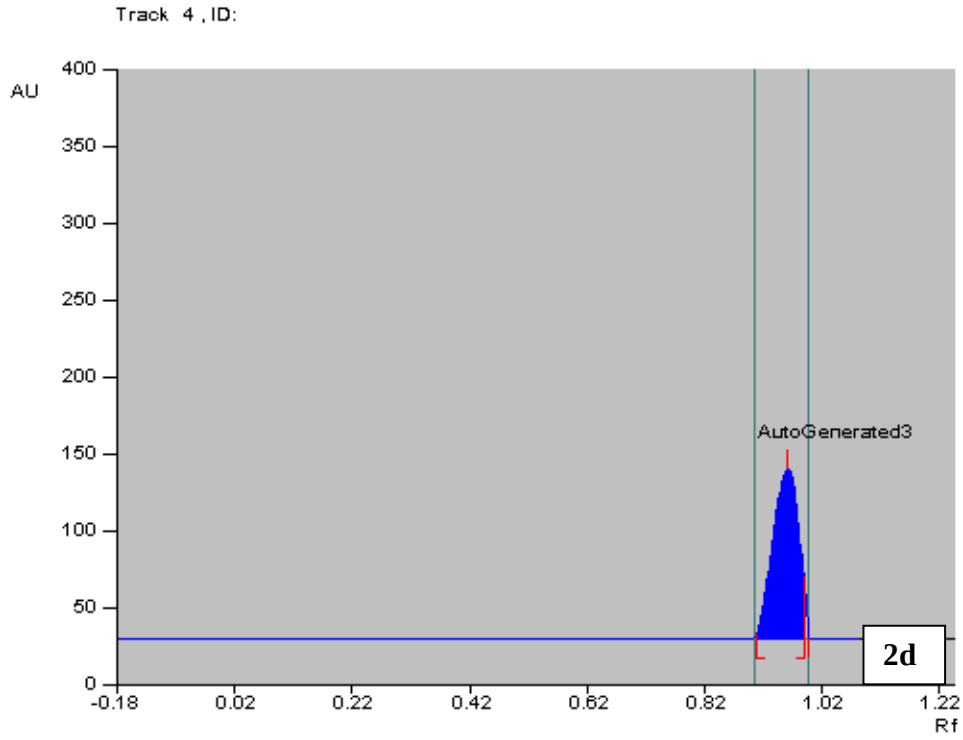
0.02
0.22
0.42
0.62
0.82
1.02
1.22
Rf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AU
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350
300
250
200
150
100
50
0
AutoGenerated3
2d
-0.18
0.02
0.22
0.42
0.62
0.82
1.02
1.22
Rf

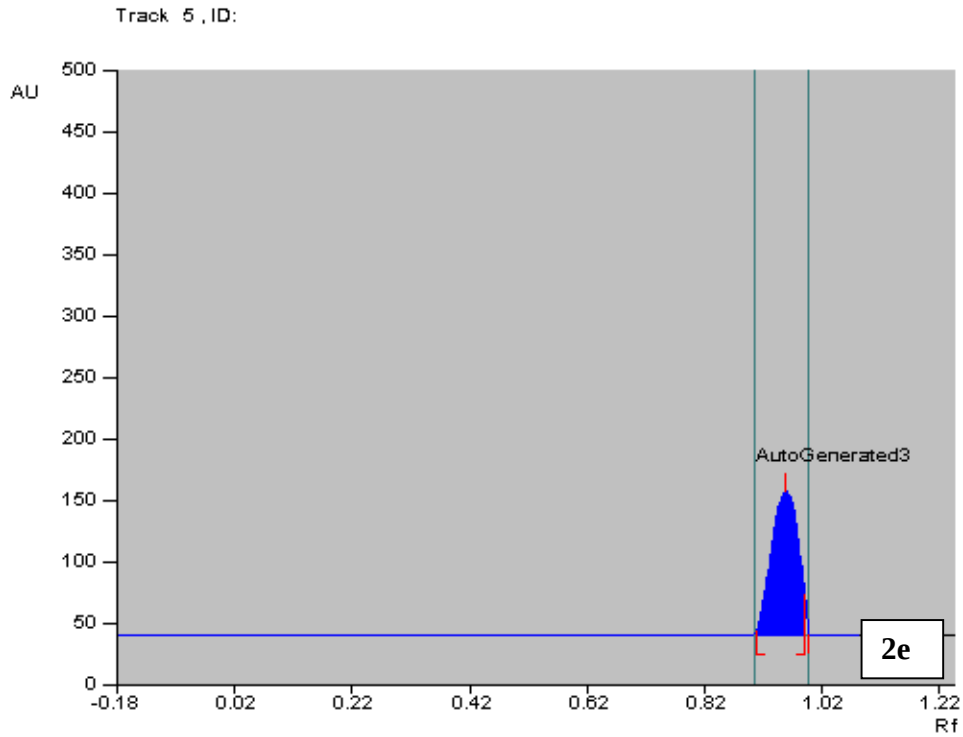
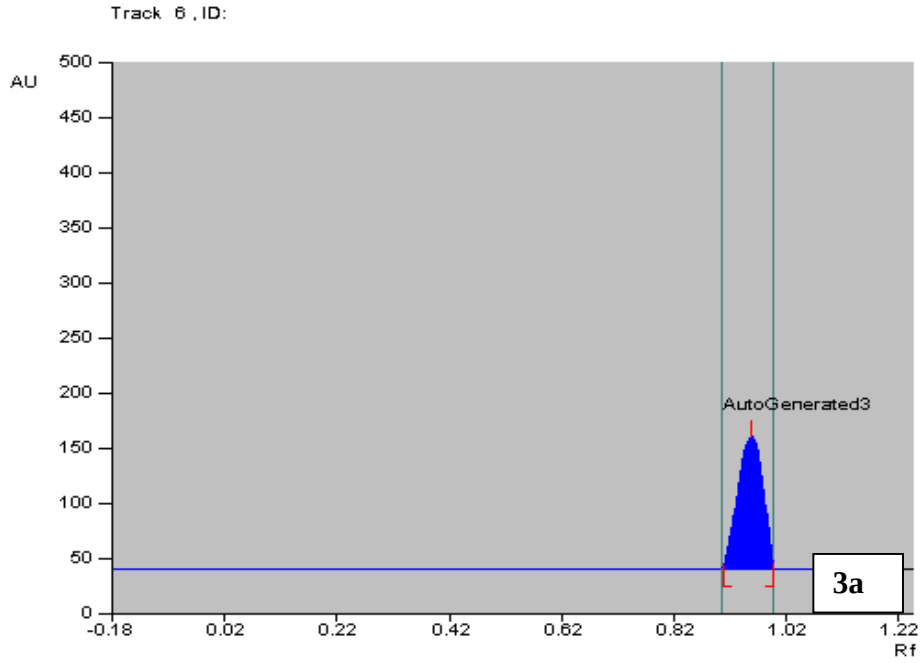


Figure 2: A Typical HPTLC chromatogram of gallic acid working standard (a) Track 1 (200µg mL^{-1}) (b) Track 2 (400 µg mL^{-1}) (c) Track 3 (600 µg mL^{-1}) (d) Track 4 (800 µg mL^{-1}) (e) Track 5 (1000 µg mL^{-1})



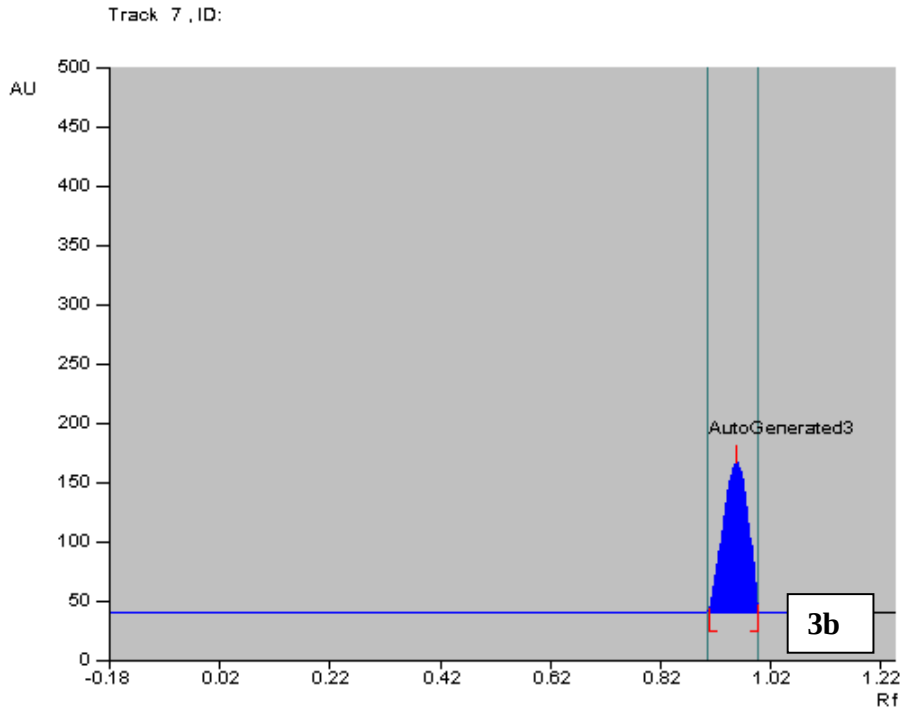
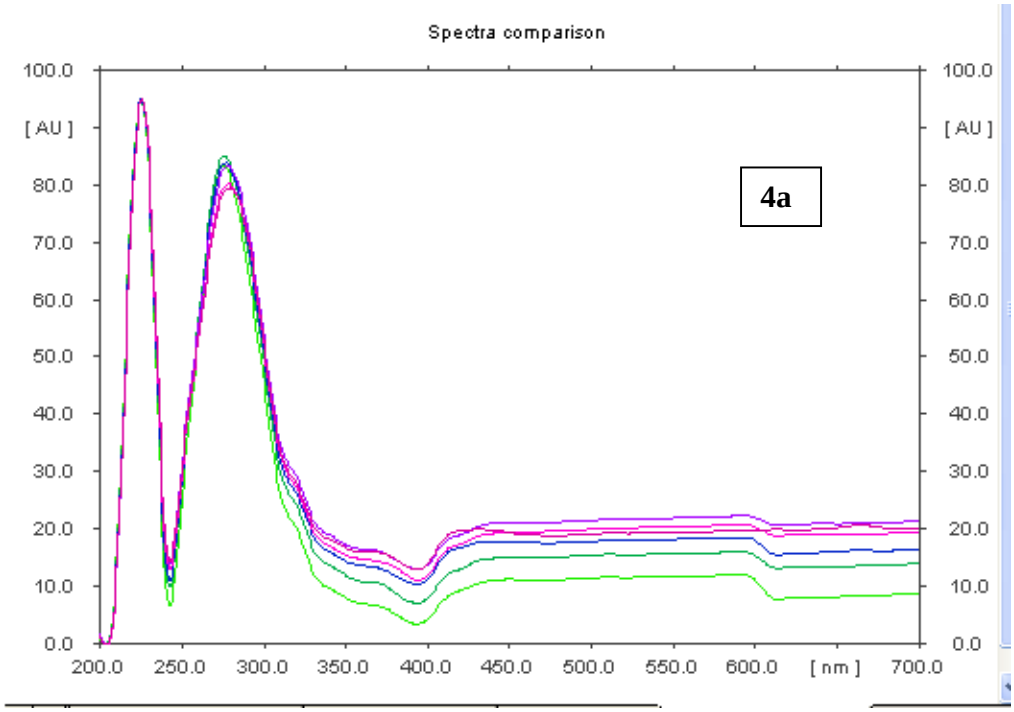
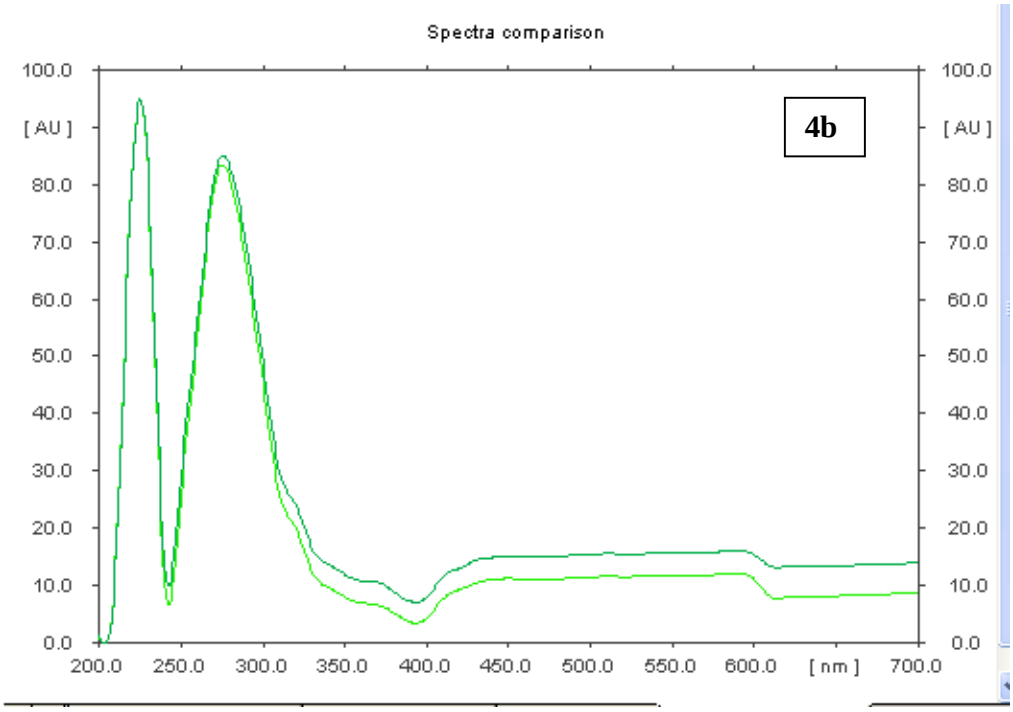
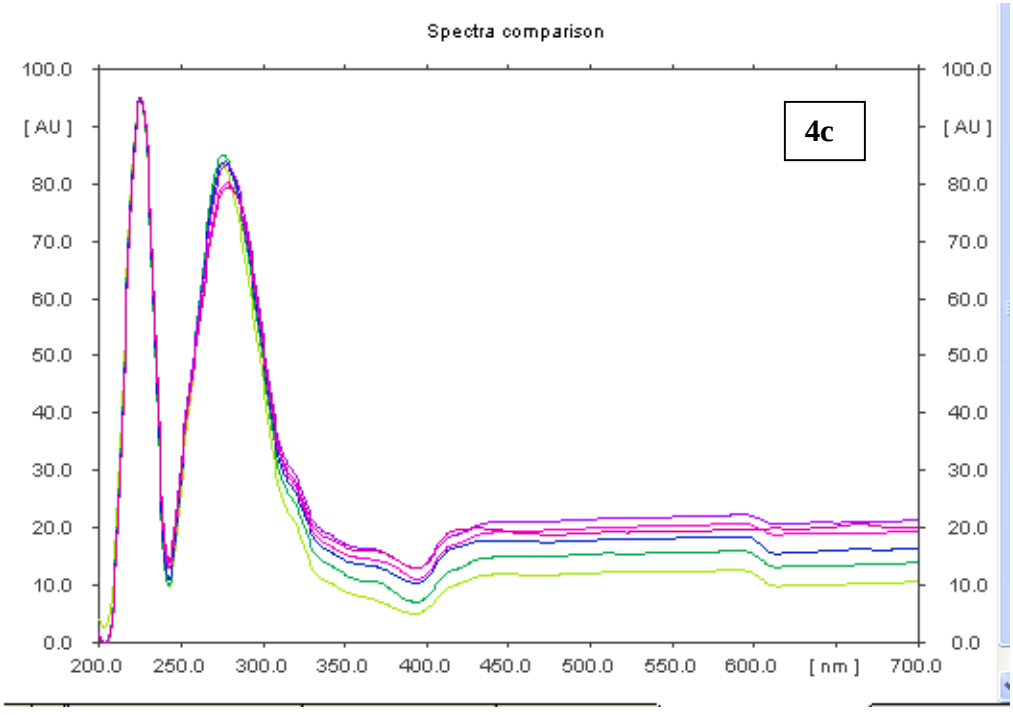


Figure 3: A Typical HPTLC chromatogram of gallic acid in *Michelia champaca* L. (a) Track 6 (leaves) (b) Track 7 (stem bark)




Spectra comparison
4b
100.0
[AU]
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
200.0
250.0
300.0
350.0
400.0
450.0
500.0
550.0
600.0
[nm]
700.0


Spectra comparison
4c
100.0
[AU]
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
200.0
250.0
300.0
350.0
400.0
450.0
500.0
550.0
600.0
[nm]
700.0

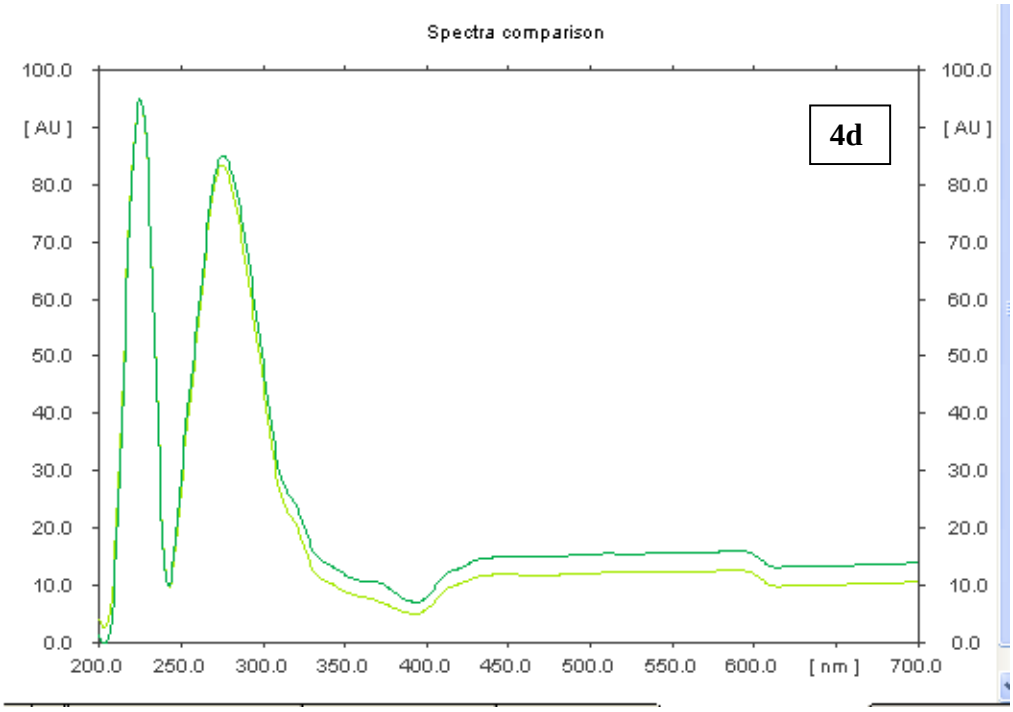


Figure 4: Spectral comparison of sample tracks with standards at selected wavelength. (a) Track 6 with Tracks (1-5) at 226 nm (b) Track 6 with Track 5 at 226 nm (c) Track 7 with Tracks (1-5) at 226 nm (d) Track 7 with Track 5 at 226 nm

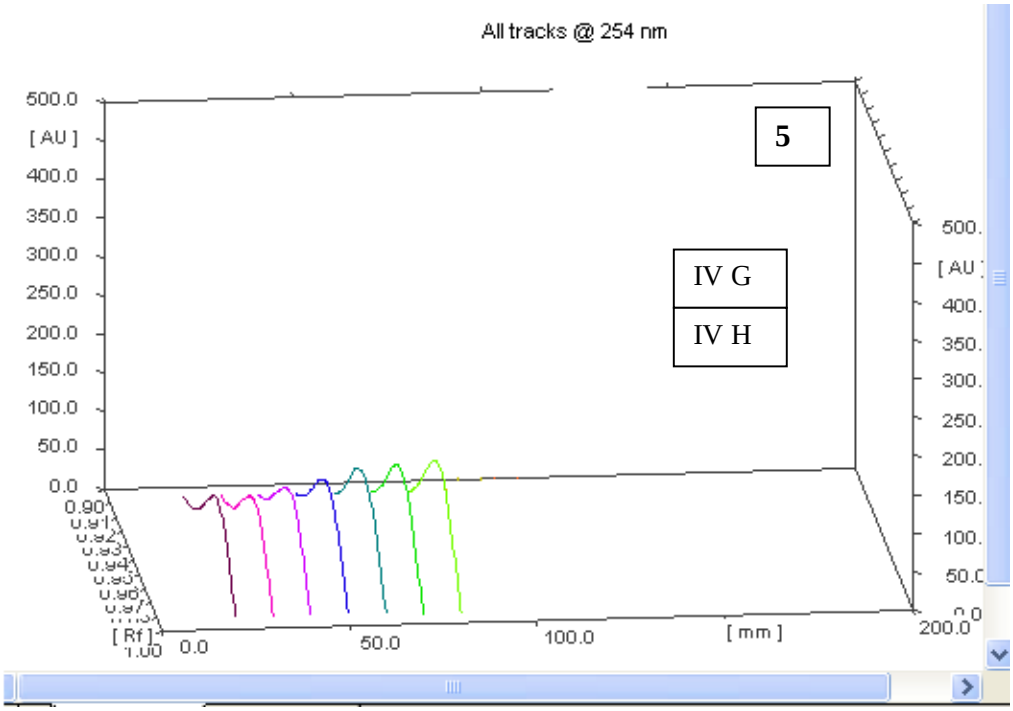


Figure 5: 3D spectra of Tracks 1-7 scanned at 254 nm

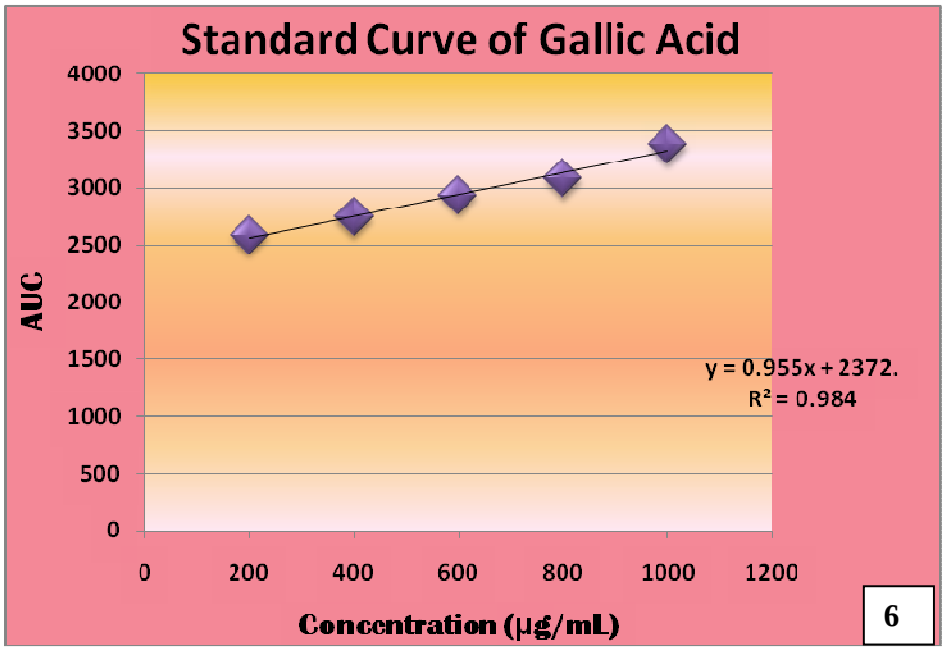


Figure 6: Standard curve (line of best fit) for gallic acid.

RESULTS AND DISCUSSION

Under the chromatographic conditions described above, the Rf value of gallic acid was about 0.96 in leaves and stem-bark of *Michelia champaca* respectively. The respective RF's obtained for each track is shown in Table 1. The Chromatograms of standard gallic acid are shown in Figure 2 (a-e) and that of gallic acid in *Michelia champaca* are shown in Figure 3 (a-b). Spectral Comparison of gallic acid reference standard with gallic acid in samples is shown in Fig 4 (a-d). The 3D spectra of all tracks scanned at 254 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 = 0.955x + 2372, the concentrations of the test samples i.e. leaves (Track 6) and stem-bark (Track 7) was estimated to be about 736.963 and 595.287 µg mL^{-1} respectively. The estimated value on per gram basis of drug was about 73.696 and 59.287 mg/g of leaves and stem bark respectively.

CONCLUSION

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

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