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RESEARCH ARTICLE

PHARMACOGNOSY

DETECTION AND QUANTITATION OF ß-SITOSTEROL IN *DIOSPYROS MONTANA* ROXB. BY HPTLC

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ABSTRACT

Diospyros montana Roxb. is a medicinally valued herb in the Ayurvedic and traditional systems of medicine. ß-sitosterol is an important plant sterol present in Diospyros which is reported to posses anti-cancer and adaptogenic properties. In the present study High Performance Thin Layer Chromatography has been developed for detection and quantification of ß-sitosterol in *Diospyros montana* (leaves, stem bark, roots and seeds).Increasing serial dilutions of reference standard ß-sitosterol (200 to 1000 µg mL^{-1}) were scanned at 273 nm to detect and quantify the concentrations of ß-sitosterol in the test samples. The estimated values obtained from the same were 651.99,467.06,447.14 and 323.87µg mL^{-1} for leaves, stem bark, roots and seeds respectively. Leaves were found to be the richest source of ß-sitosterol in *Diospyros montana* Roxb. The method provided a rapid and easy approach for detection and the quantitation of the bio-marker ß-sitosterol. The authors also aim to validate the present method in terms of ruggedness and accuracy and undertake the isolation of ß-sitosterol from the said plant.

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KEYWORDS

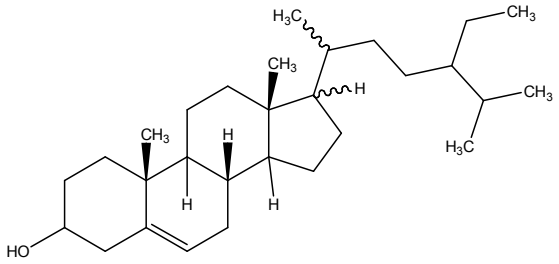
Diospyros montana, ß-sitosterol, HPTLC, quantitation

INTRODUCTION

Diospyros montana Roxb. (Ebenaceae) also known as Bistendu (Hindi), Mottled ebony (Eng.), Tumala (Sans.). It is distributed throughout India, Malaya, Australia [1]. Ethnomedicinally, it is used in boils, fever, dysuria, neuralgia, pleurisy, menorrhagia, diarrhea, spider bite, jaundice, deep wound etc [2]. Chemically, ethanolic extract of fruit pulp yields viscous, yellow liquid, containing fatty acid esters of alpha-amyrin, ursolic acid, oleanolic acid and betulinic acid [3]. The seeds showed the presence of crude proteins, pentosan and water soluble mucilage [4]. The benzene extract of the leaves afforded diospyrin, lupeol and betulinic acid [5]. Presence of saponins, tannins, alkaloids, flavonoids and terpenoids has also been reported [6].

ß-sitosterol is reported to help in the management of ageing, hyperlipidaemia, cholesterol absorption, and as an immunomodulator. It is beneficial in the treatment of breast cancer and cancer of the prostrate gland. It is also useful in certain gynecological disorders [7,8]. The structure of β-sitosterol is shown in Figure I. Many methods like UV spectroscopy; HPLC, GC and HPTLC are available for determination of ß-sitosterol in plants and plant products. In the present investigation, chromatographic fingerprint of the *Diospyros Montana* Roxb, on the different parts of plant (leaves, bark, root and seeds) has been developed by HPTLC method using ß-sitosterol as a marker compound. This method is found to be rapid, sensitive, precise and accurate.

Figure I
Structure of ß-sitosterol



MATERIALS AND METHODS

Plant Material:
Whole plant of *Diospyros montana* Roxb. was collected from local areas of District Sultanpur, Uttar Pradesh and identified and authenticated by National Botanical Research Institute, Lucknow; also a voucher specimen was submitted for future reference (Ref No. NBRI/CIF/180/2010).

Solvents:
All the solvents used were of AR grade.

Reference standard:
The reference standard (ß-sitosterol) was obtained from Sigma Aldrich, USA.

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 (9:1) [9]

Wavelength: 273 nm

Standard Preparation:
A stock solution of β-sitosterol (1000 µg mL^{-1}) was prepared by dissolving 10.0 mg of accurately weighed β-sitosterol in Methanol and diluting it to 10.0 mL with methanol. [10] 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, bark, root and seed) 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 273nm [11].

RESULTS

Under the chromatographic conditions described above, the Rf value of ß-sitosterol was about 0.76. The Chromatograms of standard ß-sitosterol are shown in Figure II (a-e) and that of ß-sitosterol in *Diospyros montana* are shown in Figure III (a-d). The respective Rf's obtained for each track is shown in Table I. Spectral Comparison of ß-sitosterol reference standard with ß-sitosterol in samples is shown in Fig IV (a-h). The 3D spectra of all tracks scanned at 273 nm are shown in Figure V (a-d). The area under the curve (AUC) obtained for various tracks are enumerated in Table II. The calibration curve was linear in the range of 200 to 1000 µg mL^{-1}, as illustrated in Figure VI. From the regression equation, y = 5.574x + 1436, the concentrations of the test samples i.e. leaves (Track 6), stem-bark (Track 7), roots (Track 8) and seeds (Track 9) was estimated to be about 651.99, 467.06,447.14 and 323.87 µg mL^{-1} respectively

.

Figure II:

A Typical HPTLC chromatogram of ß-sitosterol 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})

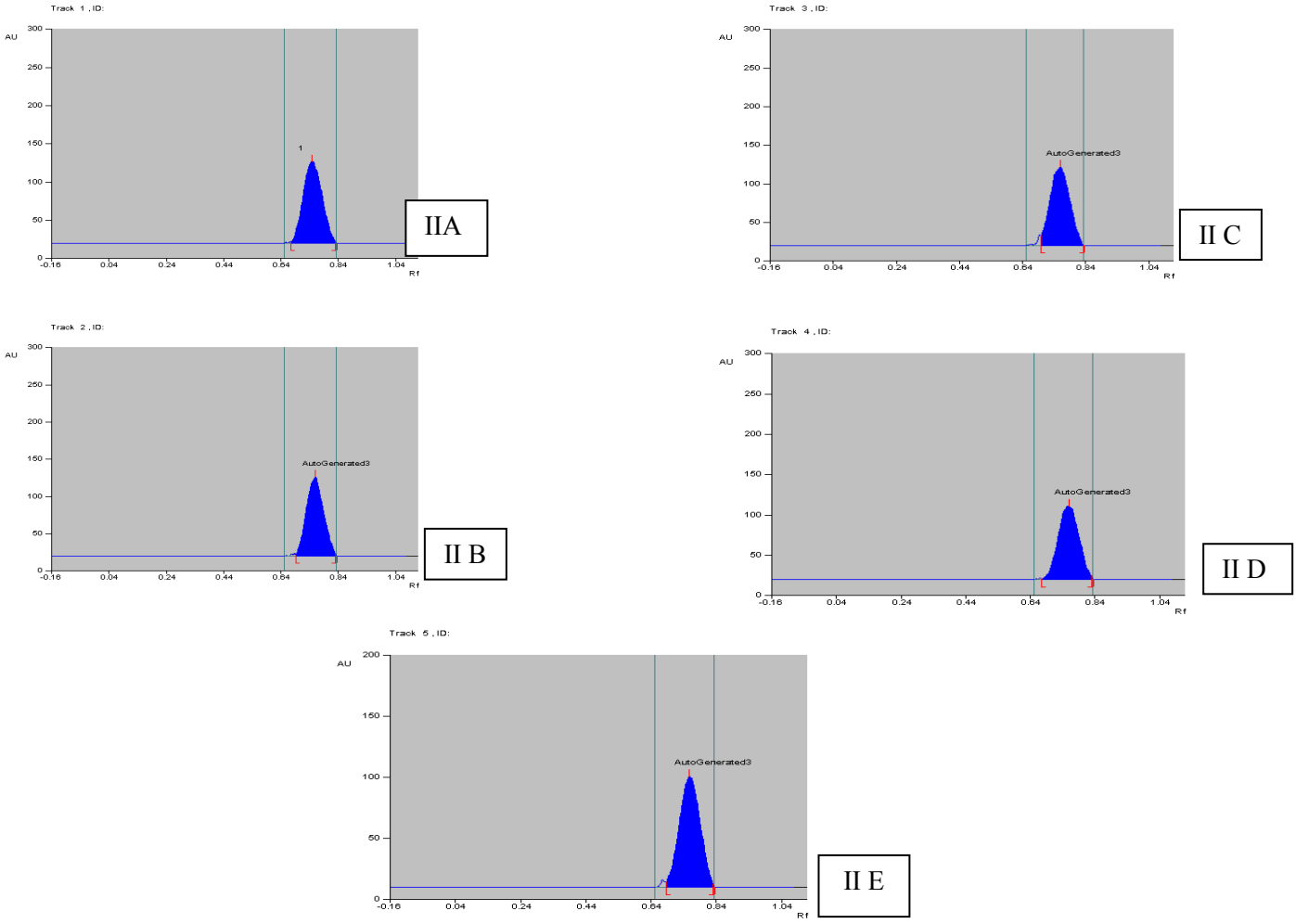


Figure III

A Typical HPTLC chromatogram of ß-sitosterol in Diospyros montana (A) Track 6 (leaves) (B) Track 7 (stem bark) (C) Track 8 (roots) (D) Track 9 (seeds).

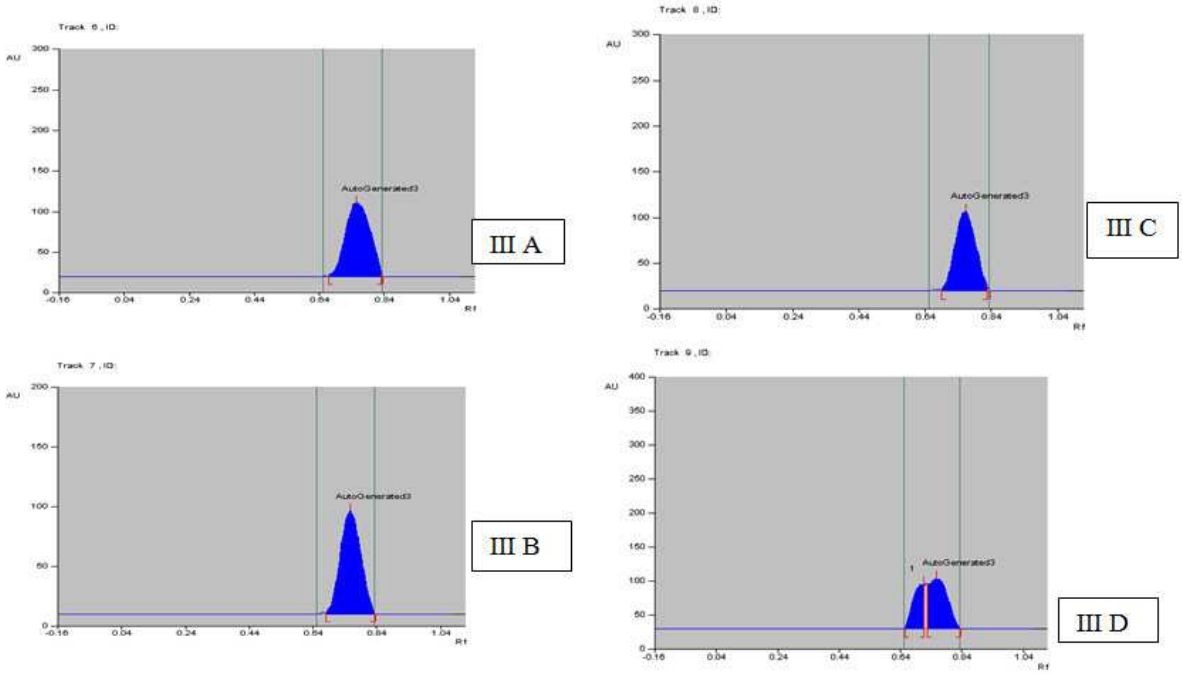
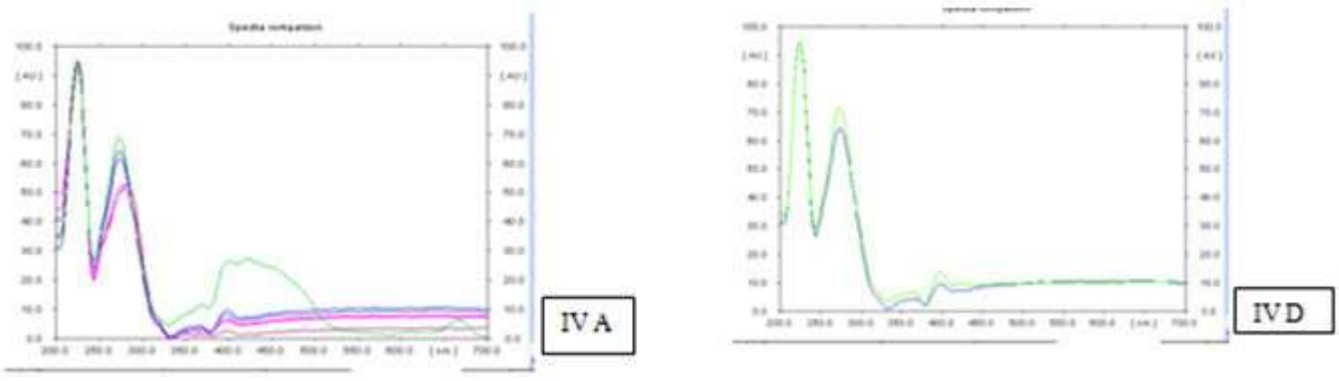
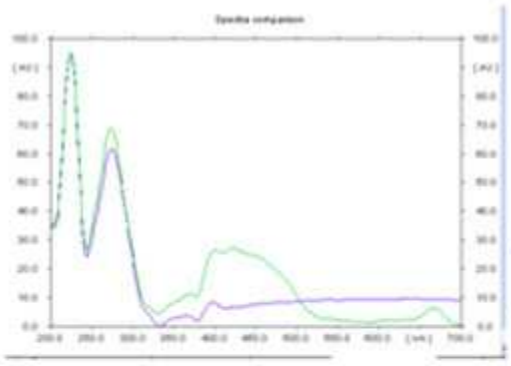


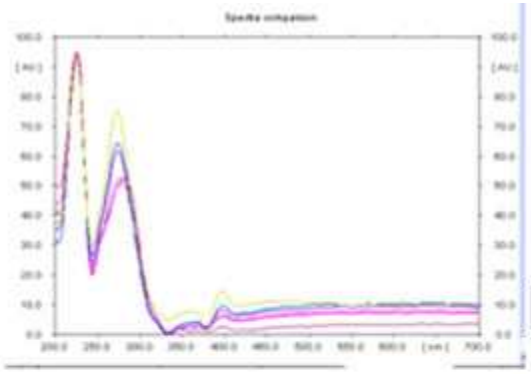
Figure IV

Spectral comparison of sample tracks with standards at selected wavelength. (A) Track 6 with Tracks (1-5) at 225 nm (B) Track 6 with Track 4 at 225 nm (C) Track 7 with Tracks (1-5) at 225 nm (D) Track 7 with Track 5 at 224 nm (E) Track 8 with Tracks (1-5) at 225 nm (F) Track 8 with Track 5 at 224 nm (G) Track 9 with Tracks (1-5) at 225 nm (H) Track 9 with Track 3 at 225 nm.





IVB



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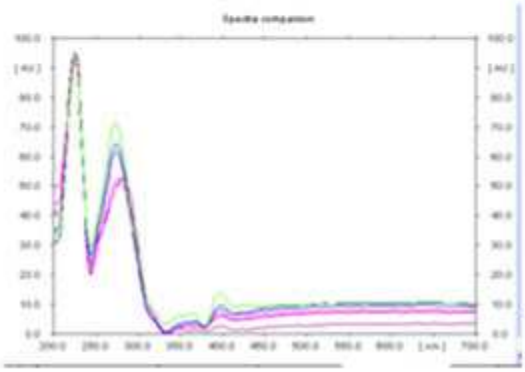
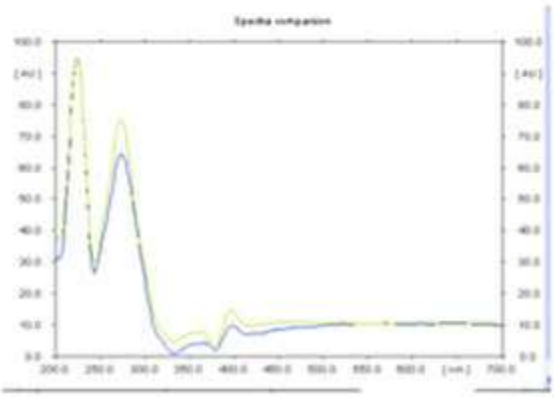
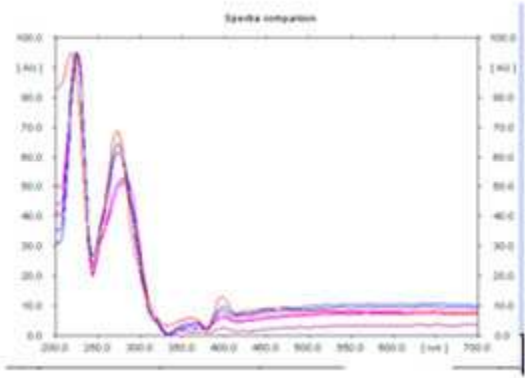


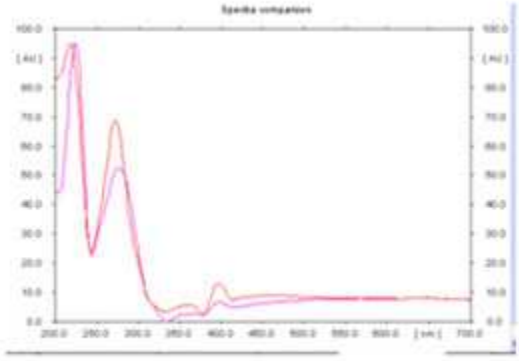
IVC



IVF



IVG



IVH

Figure V

3D spectra of Tracks 1-9 scanned at 273 nm at different vertices (a) 40° (b) 75° (c) 65° (d) 90°

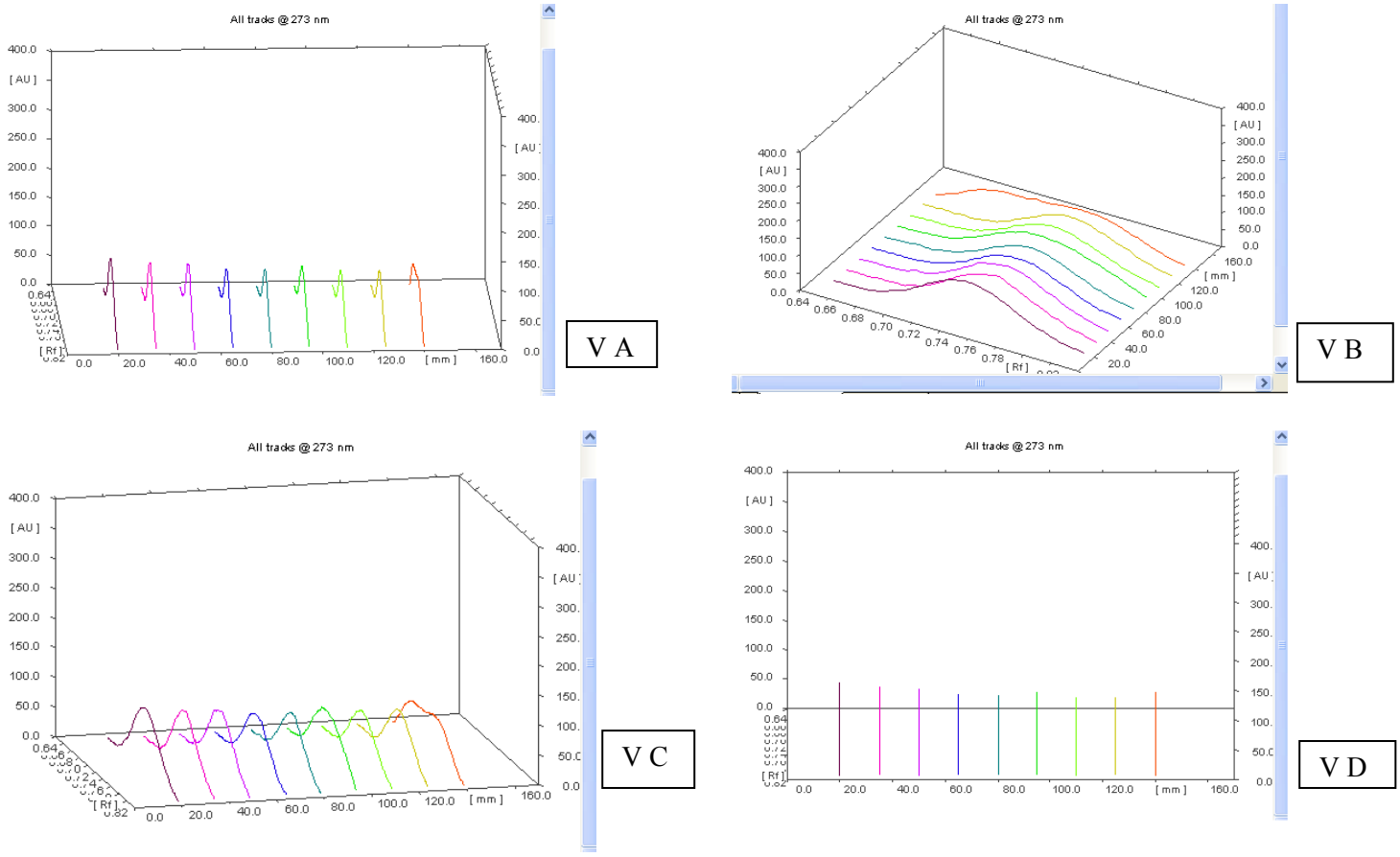


Figure VI

Standard curve (line of best fit) for ß-sitosterol

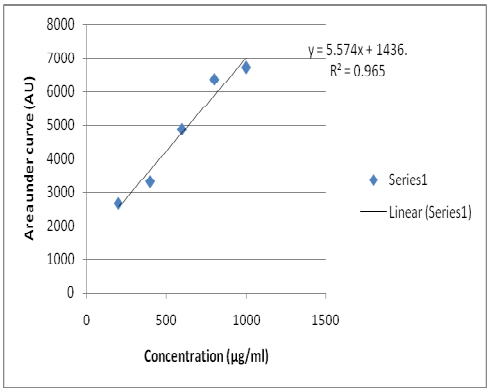


Table 1
Rf range and maximum Rf (peak) of tracks 1-9.

S.No.	Start position	Maximum Rf	End position
Track1	0.68	0.76	0.83
Track2	0.69	0.76	0.83
Track3	0.70	0.76	0.83
Track4	0.68	0.76	0.83
Track5	0.69	0.76	0.83
Track6	0.67	0.75	0.83
Track7	0.68	0.76	0.83
Track8	0.65	0.71	0.83
Track9	0.73	0.76	0.83

Table 2
Area under curve values for different concentrations of working standards of ß-sitosterol for linear calibration.

S.No.	Concentrations of working standard of ß-sitosterol (µg mL^{-1})	Area under Curve (AU)
Track1	200	2660.3
Track2	400	3307.3
Track3	600	4870.0
Track4	800	6356.4
Track5	1000	6710.0

CONCLUSION

The present method provided a quick and easy approach for detection and quantitation of biomarker ß-sitosterol in *Diospyros montana* Roxb. and the estimated values indicate that the leaves are the richest source of the said marker in *D. montana*, the order being leaves > stem bark > roots > seeds. The authors further aim to validate the method in terms of robustness, accuracy and percentage recovery.

ACKNOWLEDGEMENT

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