

## Research Article

# Detection and Quantitation of $\beta$ -sitosterol in *Clerodendrum infortunatum* and *Alternanthera sessilis* by HPTLC

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**ABSTRACT: Introduction:** High Performance Thin Layer chromatography is an important tool used qualitatively as well as quantitatively for the purity and identity determinations of crude drugs and one of the major recent advances in the area of standardization, and also to keep a check on adulteration. **Methods:** In the present study High Performance Thin Layer Chromatography has been developed for detection, and quantification of  $\beta$ -sitosterol in the leaves of *Clerodendrum infortunatum* and *Alternanthera sessilis*. Increasing serial dilutions of reference standard  $\beta$ -sitosterol (200 to 1000  $\mu\text{g mL}^{-1}$ ) were scanned at 273 nm to detect and quantify the concentrations of  $\beta$ -sitosterol in the test samples. **Results:** The estimated values obtained from the same were 934.18 and 912.80  $\mu\text{g mL}^{-1}$  for *Clerodendrum infortunatum* and *Alternanthera sessilis* respectively. The presence of the biomarker- i.e.  $\beta$ -sitosterol in appreciable amounts shows sufficient scientific promise in both these plants as a good source of the same. **Conclusion:** The method provided a rapid and easy approach for detection and the quantitation of the bio-marker  $\beta$ -sitosterol. The authors also aim to validate the present method in terms of ruggedness and accuracy and undertake the isolation of  $\beta$ -sitosterol from the said plant.

**KEYWORDS:** *Clerodendrum infortunatum*, Bhat, *Alternanthera sessilis*, Matsyakshi, beta sitosterol, HPTLC

## INTRODUCTION

*Clerodendrum infortunatum* (Verbenaceae), commonly known as Bhat, is an important and widely used medicinal plant. It is reported to contain active bitter substances like clerodin and has been widely used as tonic and anthelmintic agent in the countryside's of North India.<sup>[1]</sup> As reported in Ayurveda, the plant has a bitter pungent taste and is a tonic, aphrodisiac, antipyretic and anthelmintic. It also is useful in biliousness, "kapha i.e in Ayurveda, one of the three organizing principles (doshas) that are responsible for maintaining homeostasis. Formed by a combination of water and earth, 'kapha' is responsible for body stability and cohesion.", "tridosha i.e.

in Ayurveda, the collective term for the three fundamental psychosomatic principles that sustain and regulate all psychologic and physiologic functions. Each principle in turn is a combination of two elements (mahabhutas)", leucoderma (a deficiency of skin pigmentation that results in white patches over the surface of the skin), , burning sensations caused by excessive thirst, foul odor and diseases of blood. *Alternanthera sessilis* (Amaranthaceae, commonly known as Matsyaakshi and Gudari saag, is distributed in warmer parts of India ascending to an altitude of 1200m. It is a herbaceous branched weed possessing significant medicinal value. It is used as lactagogue, galactagogue, abortifacient and febrifuge.<sup>[2]</sup> The plant is rich in saturated hydrocarbons, aliphatic ester, stigmasterol and  $\beta$ -sitosterol.<sup>[3]</sup> A petroleum ether extract of this plant was reported to yield nonacosane, 16-hentriacontane,  $\beta$ -sitosterol, stigmasterol and handianol.<sup>[4]</sup>  $\beta$ -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 prostate gland. It is also useful in certain gynecological disorders.<sup>[5,6]</sup> The structure of  $\beta$ -sitosterol is shown in Figure 1. Many methods including UV spectroscopy, HPLC, GC

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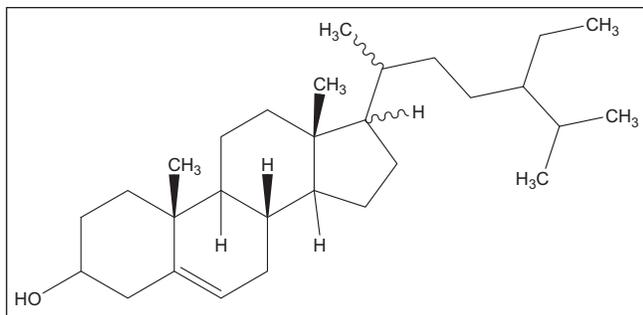


Figure 1: Structure of  $\beta$  sitosterol.

and HPTLC are available for determination of  $\beta$ -sitosterol in plants and plant products. The present investigations presents chromatographic fingerprints of *Clerodendrum infortunatum* and *Alternanthera sessilis* leaves developed by HPTLC using  $\beta$ -sitosterol as a marker compound. In Ayurveda, traditional practitioners like Charak and Shushruta have used various plants, or their combinations to study their effect on C.N.S. or on memory, also modern writers like Sukhdev and Khare have also discussed such plants in their books, further there are reports of role of  $\beta$  sitosterol in overall health like improving cardiovascular efficiency and memory. An attempt has been made to quantify the amount of  $\beta$ -sitosterol present in the said plants, by using HPTLC as it provides a rapid quick and precise method of detection in comparison to conventional methods like UV and GC. Furthermore the plants selected as the subjects of the study finds mention in several ancient Indian writings but lack real-time and modern scientific approaches being applied to them for better utilization of their full potential as sources of useful pharmaceuticals. Since  $\beta$ -sitosterol is a pharmaceutically significant moiety, its levels were estimated from the said plants.

## MATERIALS AND METHODS

### Plant Material

Leaves of *Clerodendrum infortunatum* and *Alternanthera sessilis* were collected from local areas of Lucknow, Uttar Pradesh and identified and authenticated by Dr. A. K. S. Rawat, National Botanical Research Institute (N.B.R.I., CSIR), Lucknow; also a voucher specimen was submitted for future reference (Ref No. NBRI/PH/4-5-1/51).

**Solvents:** All the solvents used were of AR grade from Sigma Aldrich and SD Fine Chem.

**Reference standard:** The reference standard ( $\beta$ -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: acetone (9:1) [7]

**Wavelength:** 273 nm

### Standard Preparation:

A stock solution of  $\beta$ -sitosterol ( $1000 \mu\text{g mL}^{-1}$ ) was prepared by dissolving 10.0 mg of accurately weighed  $\beta$ -sitosterol in methanol and diluting it to 10.0 mL with methanol.<sup>[8]</sup> Further dilutions were made with methanol to obtain working standards 200, 400, 600, 800 and  $1000 \mu\text{g mL}^{-1}$ .

### Sample Preparation:

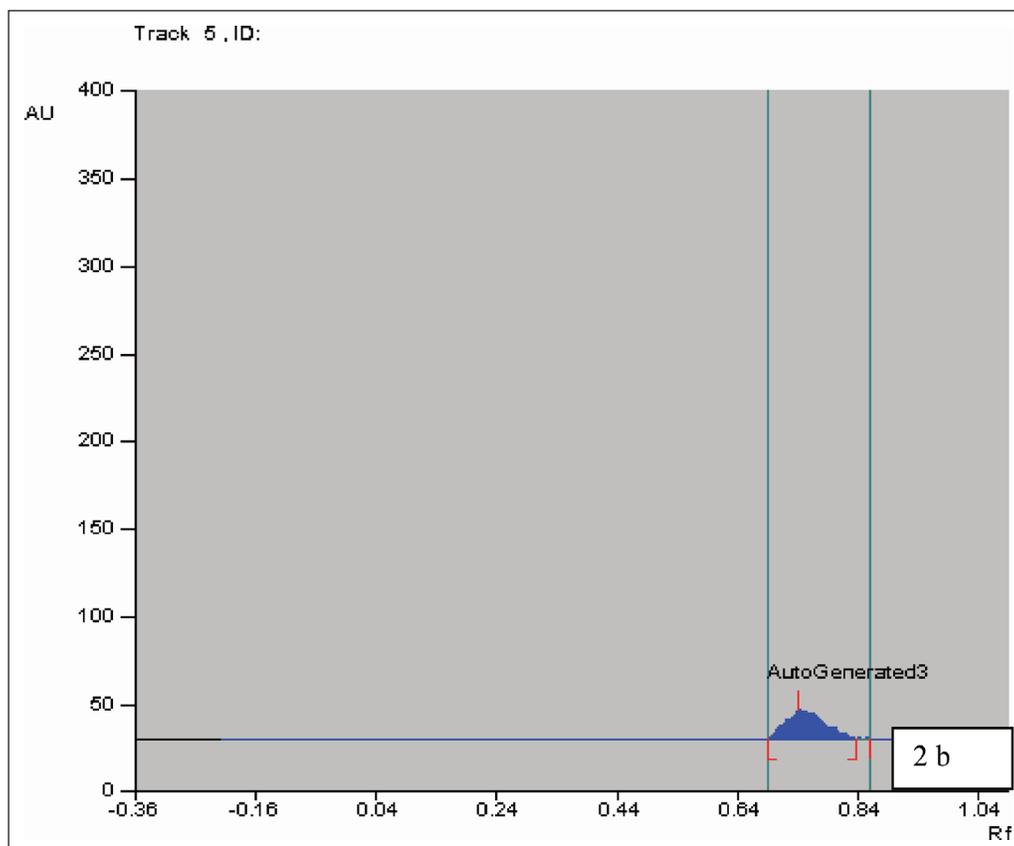
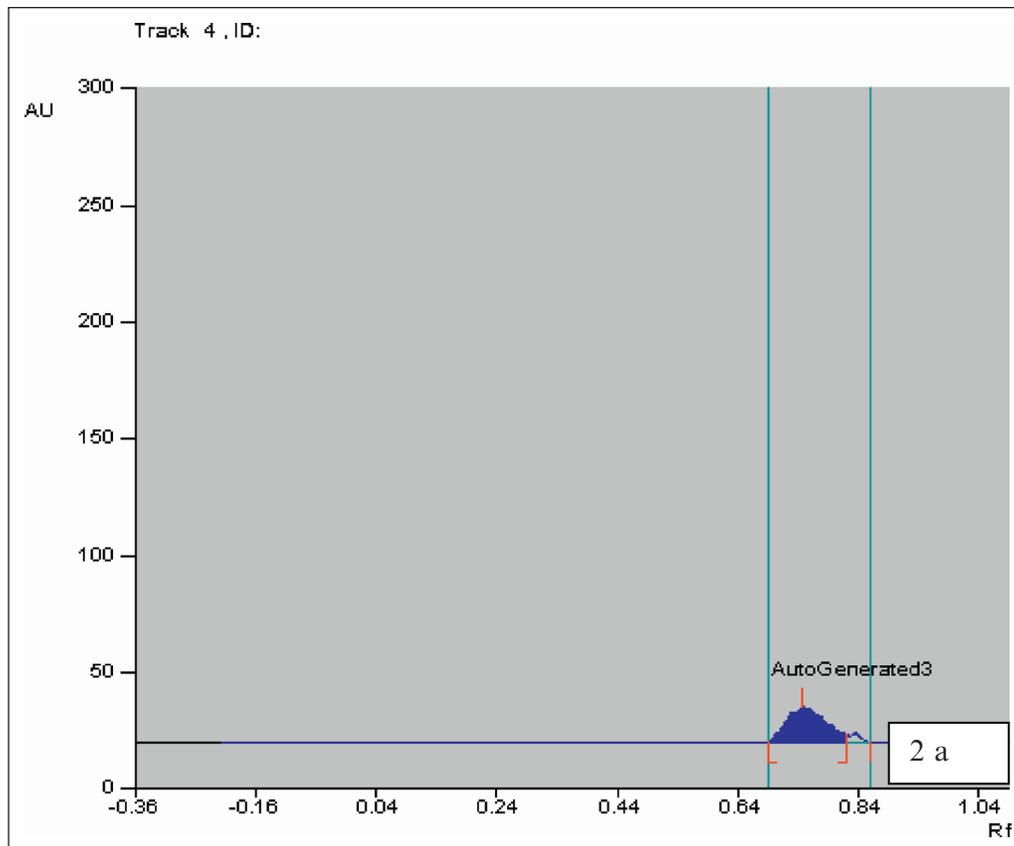
A 100 mg quantity of size reduced air dried powdered plant material (leaves of both the plants) was separately defatted with n-hexane and then Soxhlet extracted with methanol for 16 hours. The methanolic extract was dried and 10 mg of the dried methanolic extract was redissolved to a final volume of 10 mL in methanol to obtain a test sample ( $1000 \mu\text{g mL}^{-1}$ )

### Procedure:

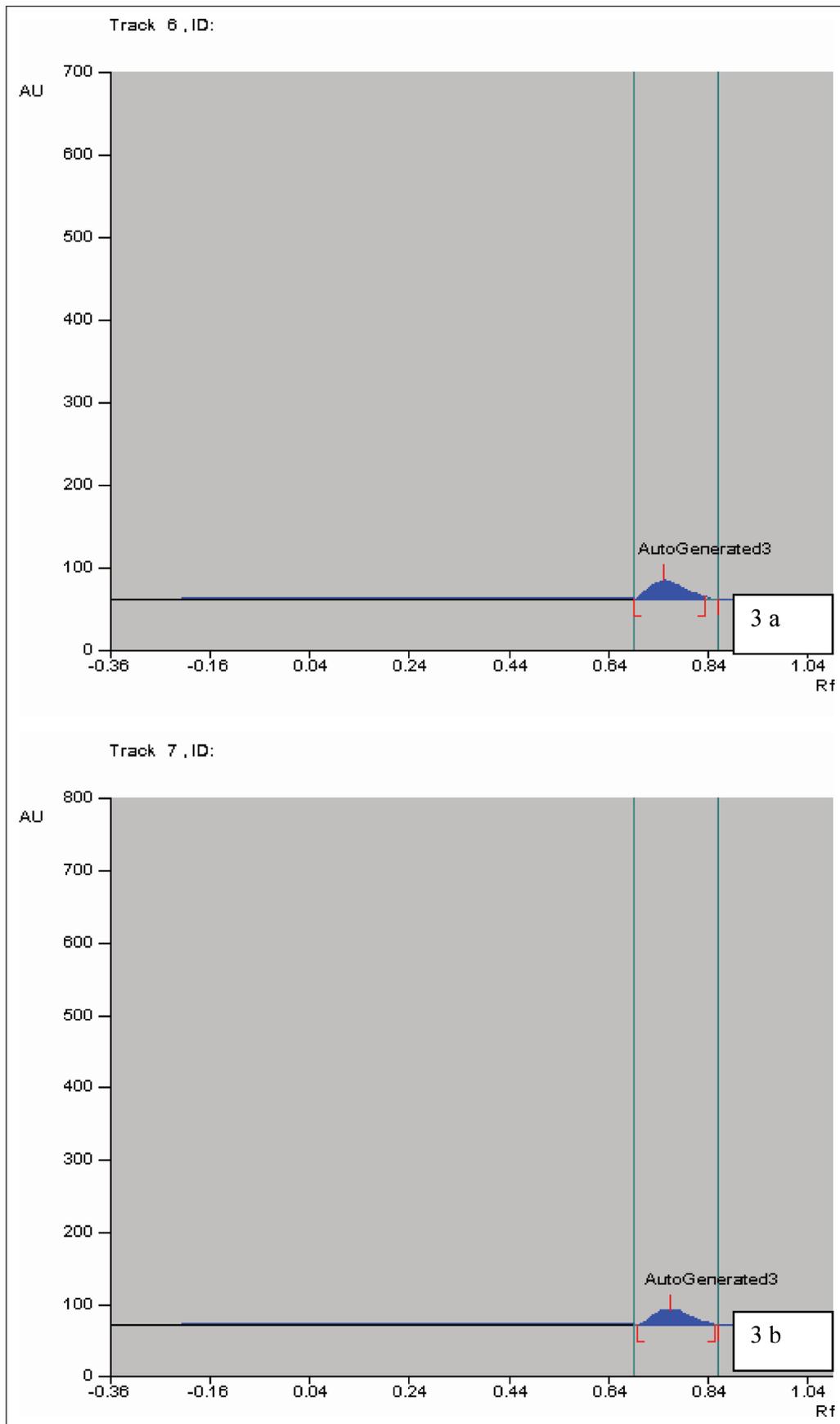
The TLC plate was activated by placing in an oven at the temperature of  $110^\circ\text{C}$  for 20 min. the plate was spotted with test and standard preparation maintaining a distance of 15mm from the edge of TLC plate. It was developed up to 75mm in the twin trough chamber using mobile phase, dried in an oven and subjected for TLC scanning at 273nm.<sup>[9]</sup>

## RESULTS AND DISCUSSION:

Under the chromatographic conditions described above, the Rf value of  $\beta$ -sitosterol was determined to be approximately 0.75 and 0.76 for *Clerodendrum infortunatum* and *Alternanthera sessilis* respectively. The Rf's obtained for the said plant extracts closely replicate the Rf's found for Tracks 4 and 3 (working standards of  $\beta$ -sitosterol), thus making it a significant fingerprint parameter. The Chromatograms of standard  $\beta$ -sitosterol i.e. track peaks are shown in Figure 2 (a-b) and that of  $\beta$ -sitosterol in *Clerodendrum infortunatum* and *Alternanthera sessilis* are shown in Figure 3 (a-b). The respective Rf's obtained for each track is shown in Table 1. Spectral Comparison of the  $\beta$ -sitosterol reference standard with  $\beta$ -sitosterol in plant extract samples is shown in Fig 4 (a-d). Spectral comparison brings out the overlaid spectra between the selected tracks at a selected wavelength which in the present case was 272 nm thus facilitating a match between the spectra of the plant extract and that of the working standard. The 3D spectra of all tracks scanned at 272 nm are shown in



**Figure 2:** A Typical HPTLC chromatogram of  $\beta$ -sitosterol working standard (a) Track 4 ( $800\mu\text{g mL}^{-1}$ ) (b) Track 5 ( $1000\mu\text{g mL}^{-1}$ )



**Figure 3:** A Typical HPTLC chromatogram of  $\beta$ -sitosterol in (a) *Clerodendrum infortunatum* (Track 6) (b) *Alternanthera sessilis* (Track 7)

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

Tracks	Start position	Maximum Rf	End position
Track1	0.68	0.72	0.76
Track2	0.70	0.74	0.76
Track3	0.70	0.76	0.83
Track4	0.69	0.75	0.82
Track5	0.69	0.74	0.84
Track6	0.69	0.75	0.83
Track7	0.70	0.76	0.85

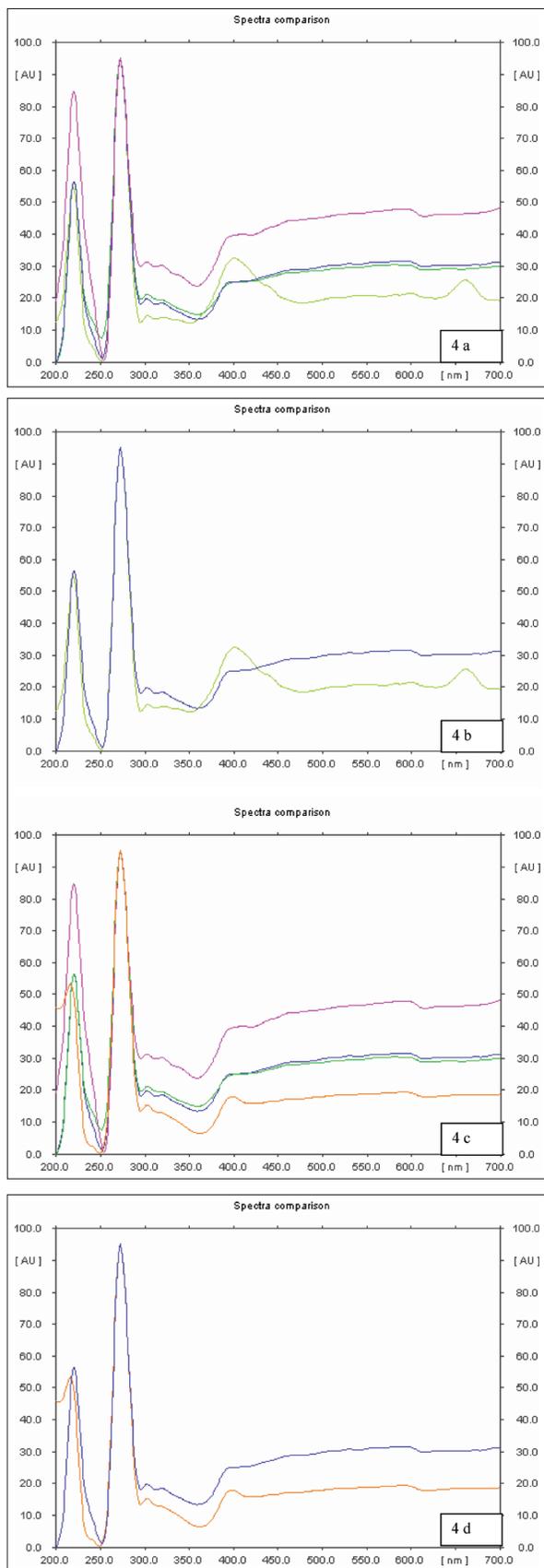
Figure 5 (a-c). A three dimensional view enhances the visible similarities amongst all spectral tracks at a selected wavelength which in the present study was 273 nm at different vertices thus bringing out the desired fingerprints. The 3D spectra obtained from the present study bring out the spectra's for all tracks viewed together and are suggestive of similarities between the test tracks and the standard tracks also elucidating strong presence of the biomarker in the plant extracts. 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  $\mu\text{g mL}^{-1}$ , as illustrated in Figure 6. From the regression equation,  $y = 0.664x + 44.5$ , the concentrations of the test samples i.e. *Clerodendrum infortunatum* (Track 6) and *Alternanthera sessilis* (Track 7) was estimated to be about 934.18 and 912.80  $\mu\text{g mL}^{-1}$  respectively.

## CONCLUSION

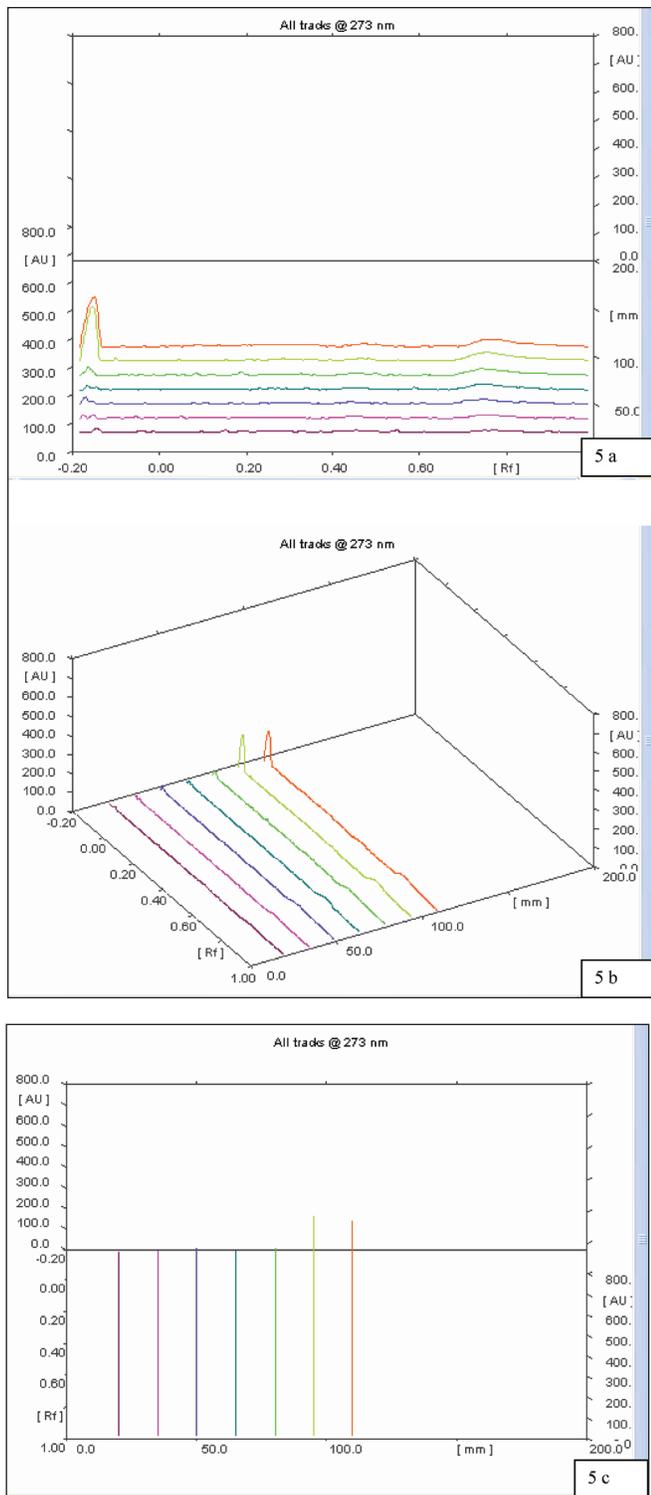
The present method provided a quick and easy approach for detection and quantitation of biomarker  $\beta$ -sitosterol in *Clerodendrum infortunatum* and *Alternanthera sessilis*. The estimated  $\beta$ -sitosterol values for the two extracts indicate that *Clerodendrum infortunatum* is the richer source of  $\beta$ -sitosterol and that both the plants are promising models for extraction of  $\beta$ -sitosterol as it is present in appreciable quantities in both plant extracts. The present study supports the usage of plants in era of Charak and Shushruta in intellect improving ayurvedic preparations as these are rich source of responsible moieties. The authors further aim to validate the method in terms of robustness, accuracy and percentage recovery.

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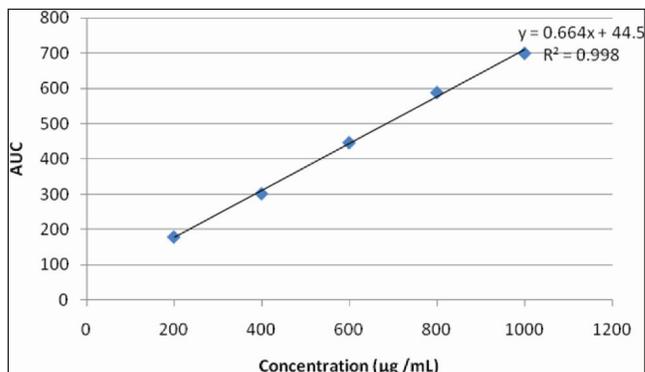
**Figure 4:** Spectral comparison of sample tracks with standards at selected wavelength. (A) Track 6 with Tracks (3-5) at 272 nm (b) Track 6 with Track 4 at 272 nm (c) Track 7 with Tracks (3-5) at 272 nm (d) Track 7 with Track 4 at 272 nm



**Figure 5:** 3D spectra of Tracks 1-7 scanned at 273 nm at different vertices (a) 0° (b) 60° (c) 90°

**Table 2: Area under curve values for different concentrations of working standards of  $\beta$ -sitosterol for linear calibration.**

Tracks	Concentrations of working standard of $\beta$ -sitosterol ( $\mu\text{g mL}^{-1}$ )	Area under Curve (AU)
Track1	200	179.0
Track2	400	302.0
Track3	600	446.8
Track4	800	589.0
Track5	1000	700.4



**Figure 6:** Standard curve (line of best fit) for  $\beta$ -sitosterol.

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