ORIGINAL ARTICLE

Comparative estimation of β-sitosterol in roots, leaves and flowers of *Clerodendrum infortunatum* L.

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Background: *Clerodendrum infortunatum* L. (family Verbenaceae) commonly known as Bhant, plays a significant role in Indian System of Medicine, i.e., Ayurveda, due to its medicinal properties. The plant is used in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy. **Aim:** High performance thin layer chromatography (HPTLC) 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. In the present study, HPTLC has been developed for detection and quantification of β-sitosterol in various parts of *C. infortunatum* L. **Materials and Methods:** Increasing serial dilutions of reference standard β-sitosterol (200-1000 µg/ml) were scanned at 273 nm to detect and quantify the concentrations of β-sitosterol in test samples. **Results and Discussion:** The estimated amount of β-sitosterol on per gram basis of crude powder was found to be 7.96 mg/g, 4.23 mg/g and1.92 mg/g in roots, leaves and flowers, respectively. **Conclusion:** The method provided a rapid and easy approach for detection and the quantitation of the bio-marker β-sitosterol. In the present study, we established the HPTLC profile for the vegetative and reproductive parts of *C.infortunatum* L. to detect and quantify the β-sitosterol.

Key words: Bhant, *Clerodendrum infortunatum* L., high performance thin layer chromatography, β-sitosterol

INTRODUCTION

Clerodendrum infortunatum L. Gaertn. Is known in Ayurveda by the Sanskrit names 'Bhargi', 'Bhrigubhava', 'Padma', 'Fanji' and 'Brahmanyastika', as 'peruvelam' in Kerala, and in Hindi as 'Bhant'or 'Bharangi'.[1] It is well-known as a medicinal plant because of its wide therapeutic uses. The plant is useful as an excellent laxative cholagogue, anthelmintic, ascarides, antiperiodic, febrifuge, in malarial fever, in torpidity of the liver, in dysentery, etc.[2] Various parts of the plant have been used by tribes in colic, scorpion sting, snakebite, tumour and certain skin diseases, also used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy.[3] *C. infortunatum* L. is one of the commonly used plants in ethno medicine for its various medicinal properties. Apart from its application as antipyretic and anthelmintic in ethnic medicine, it is also used for relieving thirst and burning sensation, foul odours and diseases of the blood.[4] The aerial part of the plant contains sterols; the root contains β-sitosterol, lupeol and steroidal glycosides; the leaf contain a diterpene clerodin and the flower contains β-sitosterol, lupeol, cleridine, hentriacontane and Fumaric acid esters of caffeic acid.[5] Plantsterols and stanols have been known for a long time to reduce serum levels of low density lipoprotein-cholesterol by competing with dietary and biliary cholesterol for intestinal absorption. The first therapeutic agent described was β-sitosterol, used to treat hypercholesterolemia about 60 years ago.[6] β-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 gynaecological disorders.[7,8] The present investigation, presents the chromatographic fingerprinting of various parts of *C.infortunatum* L. developed by high performance thin layer chromatography (HPTLC) using β-sitosterol as a marker compound.

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MATERIALS AND METHODS

Collection and Authentication

The plant *C. infortunatum* L. were collected from local areas of Lucknow in the month of August 2011 and authenticated by National Botanical Research Institute (NBRI (CSIR)), Lucknow, also a voucher specimen was submitted for future reference (Ref. No. NBRI/CIF/293/2012).The air dried

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plant material was first washed with running tap water, then again washed twice with double distilled water and the washed specimens (root, leaves and flower) was air-dried and size comminuted to a moderately fine powder and stored in an air-tight container at 25°C for future/ further studies.

Extraction of Plant Material

The powdered drug (root, leaves and flower) was defatted with petroleumether (60-80°C) for 48 h and was successively extracted with methanol and water for 48 h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts Table 1. Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present with in them.

Thin Layer Chromatography Profile

Thin layer chromatography (TLC) fingerprint profiles were carried out by preparing the extract with10 ml methanol and 0.01 g powdered drugs. TLC of this alcoholic extract on Silica Gel G plate using solvent system, Toluene:Ethylacetate: Glacialaceticacid (8:2:0.1)[9,10] spraying with anisaldehyde sulphuric acid and heating the plate for 10 min at 105°C shows the spots [Figures 1a-c and 2].

HPTLC Fingerprinting

The preliminary phytochemical investigation of the petroleumether extract of roots, leaves and flower of *C. infortunatum* L. showed the presence of Sterols. Hence, the petroleumether fraction was used for HPTLC studies to detect and quantify the β-sitosterol in the above mentioned extracts.[10-12]

- Solvents: All the solvents used were of AR grade from Sigma Aldrich and SD Fine Chem. Mumbai, Maharashtra, India
- Reference standard: The reference standard (β-sitosterol) was obtained from Sigma Aldrich, USA, through an authorized institutional supplier M/S SohanLal and Sons, Lucknow, Uttar Pradesh, India
- HPTLC plate:Silicagel GF_{254} (Merck) 10 cm × 10 cm
- Mobile phase:Toluene:Ethylacetate:Glacialacetic acid (8:2:0.1)
- Wavelength: 273 nm.

Table 1: Extraction of *Clerodendrum infortunatum* L.

Plant part	Extract	%Yield
Root	Methanol	0.356
Leaves	Methanol	2.22
Flower	Methanol	2.82

Standard Preparation

A stock solution of β-sitosterol (1000 µg/ml) was prepared by dissolving 10.0 mg of accurately weighed β-sitosterol in methanol and diluting it to 10.0 ml with methanol. Further dilutions were made with methanol to obtain working standards 200, 400, 600, 800 and 1000 µg/ml.

Sample Preparation

A 100 mg quantity of size reduced air dried powdered plant material (root, leaves and flower) was separately defatted with petroleumether and the petroleumether extract was dried and10mg of the dried extract was re-dissolved to a final volume of 10 ml with methanol to obtain a test samples (1000 µg/ml).

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 15 mm from the edge of TLC plate. It was developed upto 75 mm in the twin trough chamber using mobile phase, dried in an oven and subjected for TLC scanning at 273 nm.

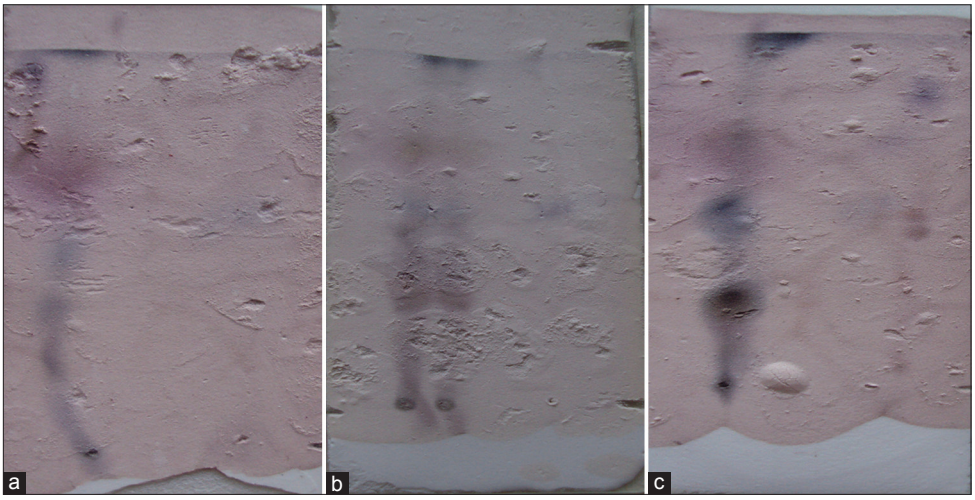

Figure 1: Thin layer chromatography profile of standard β-sitosterol in (a) root; (b) leaves and (c) flower

RESULTS AND DISCUSSION

Under the chromatographic conditions described above, the R_f value of β-sitosterol was determined to be approximately 0.62 for *C. infortunatum* L. Thechromatograms of standard β-sitosterol, i.e., track peaks are shown in Figure 3a-e and that of β-sitosterol in different part of *C. infortunatum* L. petroleumether extract are shown in Figure 4a-c. The respective R_f's obtained for each track is shown in Table 2. Spectral comparison of the β-sitosterol reference standard with β-sitosterol in plant extracts samples is shown in Figure 5. Spectral comparison brings out the overlaid spectra between the selected tracks at a selected wavelength, which in the present case was 278 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 273 nm are shown in Figure 6. 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 curveobtained for various tracks are enumerated in Table 2. The calibration curve was linear in the range of 200-1000 µg/ml, as illustrated in Figure 7. From the regression equation, $y = 101.53x + 1050.5$, the amount of β-sitosterol on per gram basis of crude powder was found to be maximum in petroleumether extract of root followed by the petroleumether extract of leaves and flower, as shown in Table 3.

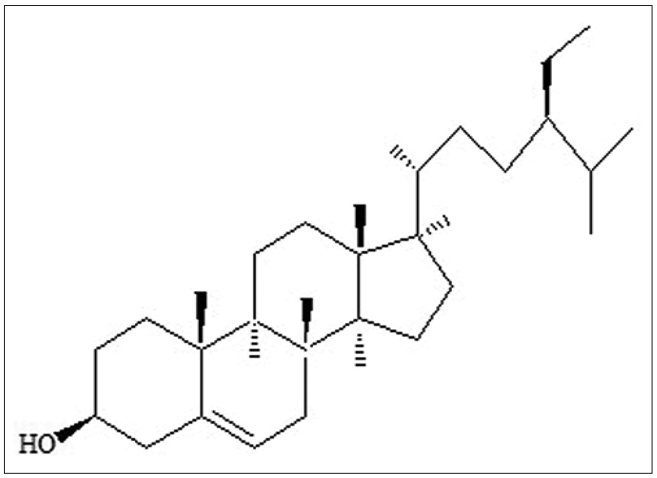

Figure 2: Chemical structure of β-sitosterol

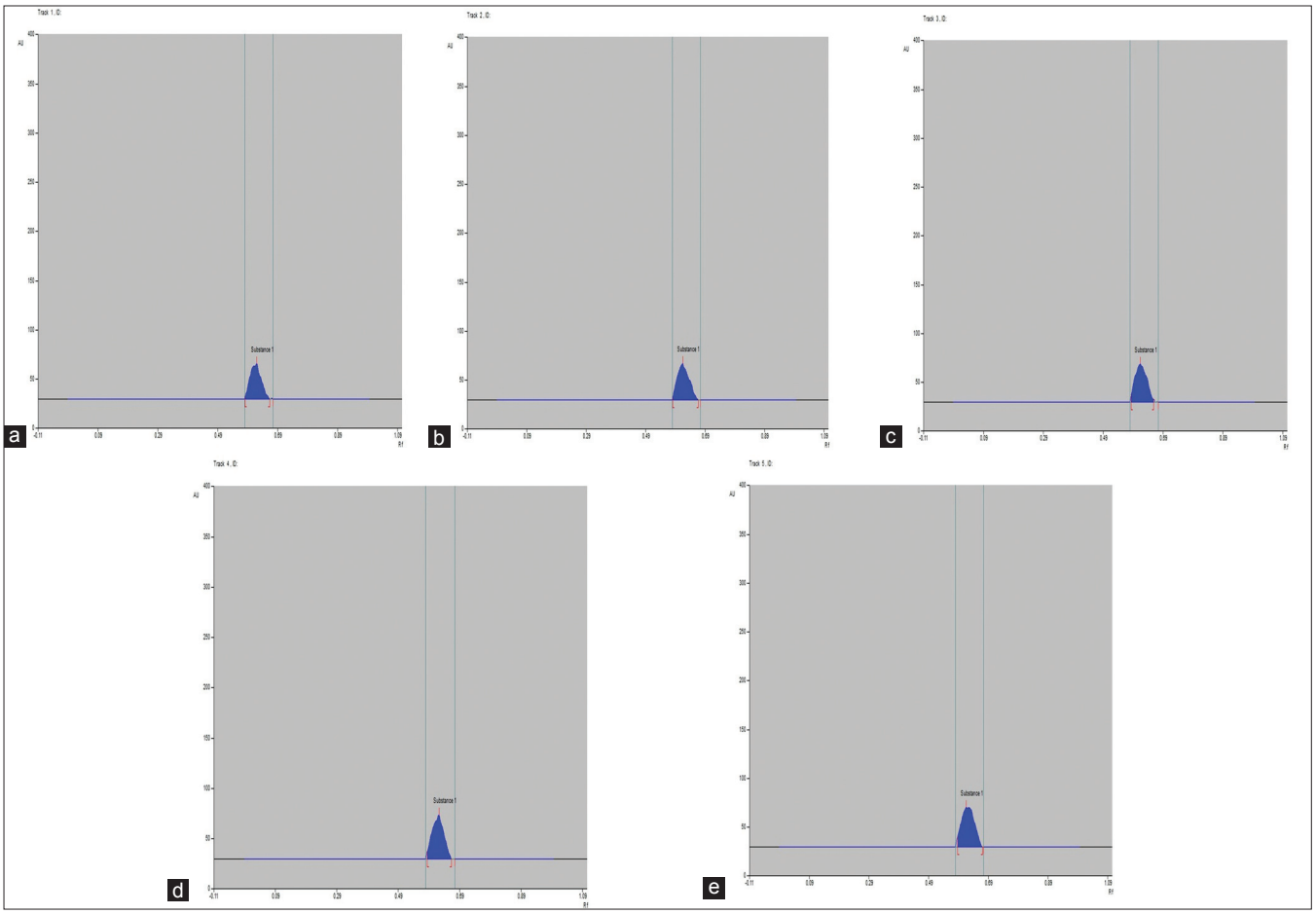

Figure 3: (a) HPTLC chromatogram of β-sitosterol working standard track 1 (200 µg/ml). (b) HPTLC chromatogram of β-sitosterol working standard track 2 (400 µg/ml); (c) HPTLC chromatogram of β-sitosterol working standard track 3 (600 µg/ml); (d) HPTLC chromatogram of β-sitosterol working standard track 4 (800 µg/ml) and (e) HPTLC chromatogram of β-sitosterol working standard track 5 (1000 µg/ml)

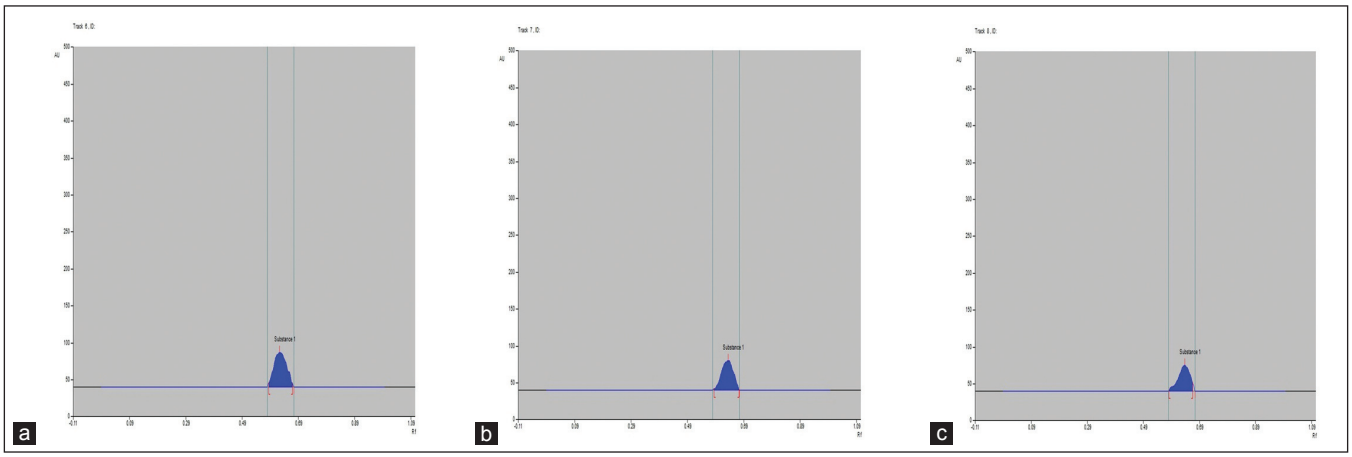

Figure 4: (a) HPTLC chromatogram of β-sitosterol in root of *C. infortunatum* L. track 6 (1000 µg/ml). (b) HPTLC chromatogram of β-sitosterol in leaves of *C. infortunatum* L. track 7 (1000 µg/ml) and (c) HPTLC chromatogram of β-sitosterol in flower of *C. infortunatum* L. track 8 (1000 µg/ml)

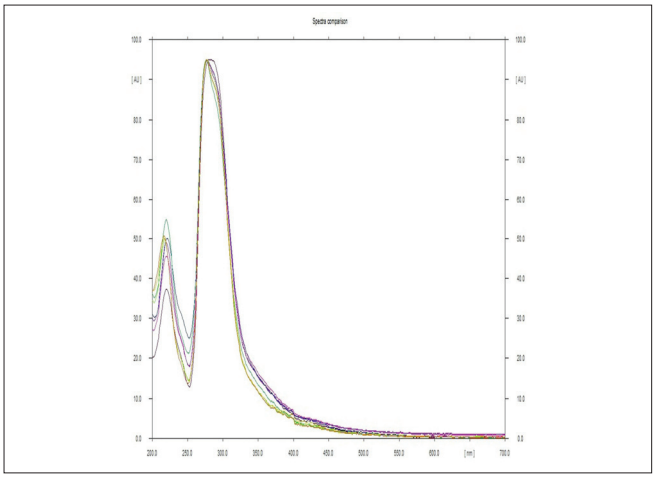

Figure 5: Spectral comparison of track 1-8 at 278 nm

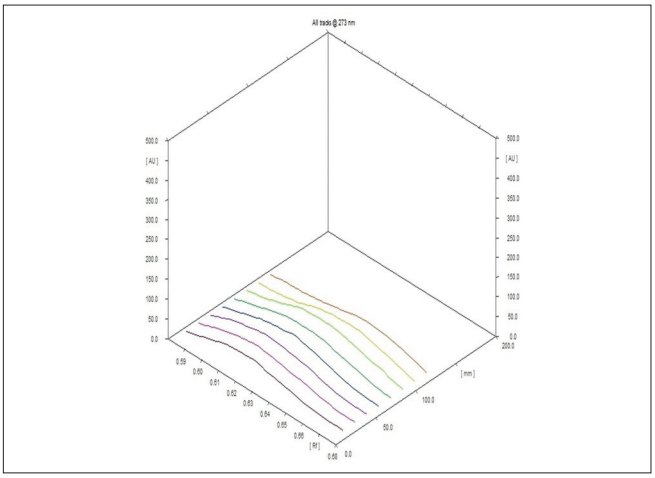

Figure 6: 3D Spectra of tracks 1-8 scanned at 273 nm

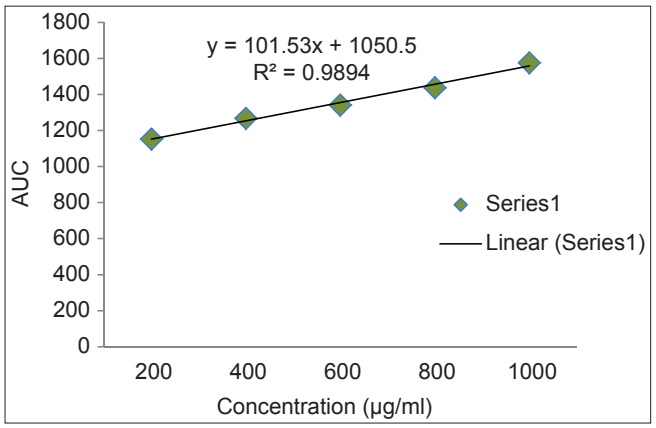

Figure 7: Calibration curve for β-sitosterol standard

Table 2: R_f range and area under the curve of standard β-sitosterol (tracks 1-5), track 6 (roots), track 7 (leaves) and track 8 (flowers)

S. No.	Concentration (µg/ml)	Start position	Maximum R_f	End position	AUC
Track 1	200	0.58	0.62	0.67	1153
Track 2	400	0.58	0.62	0.67	1268
Track 3	600	0.59	0.62	0.66	1341.4
Track 4	800	0.59	0.63	0.67	1437.1
Track 5	1000	0.59	0.62	0.67	1576.1
Track 6	1000	0.59	0.63	0.67	1858.7
Track 7	1000	0.59	0.64	0.68	1479.6
Track 8	1000	0.58	0.64	0.67	1245.8

AUC – Area under the curve

CONCLUSION

The method was found to be simple, precise, accurate, specific and sensitive for the simultaneous quantitation of β-sitosterol in roots, leaves and flowers powder. It can also be used for routine quality control of herbal raw materials. The estimated quantity of β-sitosterol in various parts of the plant indicates that *C. infortunatum* L. is the richer source of β-sitosterol particularly, the root part are promising model for the extraction of β-sitosterol. The authors further aim to validate the method in terms of robustness, accuracy and percentage recovery.

Table 3: Amount of β-sitosterol in various parts of *Clerodendrum infortunatum* L.

Plant part	Extract	Amount of β-sitosterol (mg/g)
Root	Petroleumether extract	7.96
Leaves	Petroleumether extract	4.23
Flower	Petroleumether extract	1.92

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REFERENCES

1. Rajurkar BM. Morphological study and medicinal importance of *Clerodendrum infortunatum* Gaertn. (Verbenaceae), found in Tadoba National Park, India. JPRHC 2010;2:216-20.
2. Haque MZ, Rouf MA, Jalil MA, Islam MB, Islam MR. Screening of phytochemical and biological potential of *Clerodendronviscosum* leaves extracts. Bangladesh J Sci Ind Res 2010;45:381-6.
3. Modi AJ, Khadabadi SS, Deore SL, Kubde MS. Antioxidant effects of leaves of *Clerodendrum infortunatum* (Linn.). Int J Pharm Sci Res 2010;1:67-72.
4. Gouthamchandra K, Mahmood R, Manjunatha H. Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from *Clerodendrum infortunatum* L. Environ Toxicol Pharmacol 2010;30:11-8.
5. Lobo R, Punitha IS, Rajendran K, Shirwaikar A, Shirwaikar A. Preliminary study on antisnake venom activity of alcoholic root extract of *Clerodendrumviscosum* (Vent.) in *Najanaja* Venom. Nat Prod Sci 2006;12:153-6.
6. Izar MC, Tegani DM, Kasmas SH, Fonseca FA. Phytosterols and phytosterolemia: Gene-diet interactions. Genes Nutr 2011;6:17-26.
7. Becker M, Staab D, Von Bergman K. Long-term treatment of severe familial hypercholesterolemia in children: Effect of sitosterol and bezafibrate. Pediatrics 1992;89:138-42.
8. Best MM, Duncan CH. Modification of abnormal serum lipid patterns in atherosclerosis by administration of sitosterol. Ann Intern Med 1956;45:614-22.
9. Gupta AK, Tandon N, Sharma M. *Leucas cephalotes* (Roth) spreng. in Quality Standards of Indian Medicinal Plants. Vol. II. New Delhi, India: Indian Council of Medical Research; 2005. p. 151-3.
10. Rohini RM, Das AK. Determination of lupeol, β-sitosterol and quercetin from ethyl acetate extract of *Rhizophoramucronata* bark by HPTLC technique. Asian J Pharm Clin Res 2011;4:103-5.
11. Vaidya V, Raote A, Jangid A, Tale R. Estimation of two bioactive compounds from *Artocarpuslakoocha* Roxb. leaves using HPTLC. Int J Pharma Bio Sci 2011;2:860-6.
12. Gupta R, Singh HK. Detection and quantitation of ß-sitosterol in *Clerodendrum infortunatum* and *Alternantherasessilis* by HPTLC. Pharmacogn Commun 2012;2:31-6.

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