

HPTLC Fingerprinting, Validated Quantification of β -Sitosterol in *Putranjiva roxburghii* Wall. and Colchicine in *Boswellia serrata* Roxb., and Organ-Wise Marker Compound Distribution

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Highlights

Validated HPTLC methods developed for β -sitosterol in *Putranjiva roxburghii* and colchicine in *Boswellia serrata*.

Both methods show excellent linearity ($r = 0.992$ and 0.995) over 100–1000 ng/band. Method precision (intra-/inter-day RSD < 3.3%) meets ICH validation criteria.

β -Sitosterol accumulates preferentially in flower tissue (59.84 $\mu\text{g/g}$).

Colchicine accumulates preferentially in root tissue (42.34 $\mu\text{g/g}$) of *Boswellia serrata*.

Abstract

High-performance thin-layer chromatography (HPTLC) fingerprinting and validated quantification were carried out for two pharmacologically significant marker compounds — β -sitosterol in *Putranjiva roxburghii* Wall. and colchicine in *Boswellia serrata* Roxb. — across seven and five organs of the respective species. Comparative fingerprinting of steroids in *P. roxburghii* at 200, 366 and 540 nm resolved bands with R_f values ranging from 0.03 to 0.78, with several bands (R_f 0.06, 0.13, 0.17 at 200 nm; R_f 0.25, 0.77 at 366 nm; R_f 0.33, 0.78 at 540 nm) common to all sampled organs, indicating a conserved core steroidal profile across the plant body. Alkaloid fingerprinting of *B. serrata* at 254, 366 and 540 nm resolved bands with R_f values from 0.05 to 0.74, with R_f 0.38 common across all organs at both 254 and 366 nm. The HPTLC method for β -sitosterol ($R_f = 0.51$; toluene:ethyl acetate:methanol, 17:2:1) was linear over 100–1000 ng/band ($r = 0.99219$; $Y = 1638.387 + 0.404X$), with a limit of detection (LOD) of 185.1 ng/band and limit of quantification (LOQ) of 447.3 ng/band, intra-/inter-day precision of 2.68% and 2.87% RSD, and mean recovery of 68.70%. The method for colchicine ($R_f = 0.22$; toluene:methanol:acetone, 16:4:1) was linear over the same range ($r = 0.99491$; $Y = 8519.241 + 0.810X$), with LOD 147.6 ng/band, LOQ 447.3 ng/band, intra-/inter-day precision of 2.74% and 3.27% RSD, and mean recovery of 93.12%. Applying these validated methods, β -sitosterol content in *P. roxburghii* ranged from 10.17 $\mu\text{g/g}$ (leaf) to 59.84 $\mu\text{g/g}$ (flower, the highest-yielding organ), while colchicine content in *B. serrata* ranged from 24.27 $\mu\text{g/g}$ (fruit) to 42.34 $\mu\text{g/g}$ (root, the highest-yielding organ). These validated, organ-resolved HPTLC methods provide simple, precise and accurate quality-control tools for standardising raw material and extracts of both species, and identify flower and root tissue, respectively, as the preferred organs for marker-compound-targeted collection.

Keywords

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Introduction

High-performance thin-layer chromatography (HPTLC) is a widely adopted analytical technique for the fingerprinting, standardisation and quantitative quality control of herbal raw materials, owing to its simplicity, cost-effectiveness, capacity for simultaneous multi-sample analysis, and suitability for the generation of chemical fingerprints that capture the overall phytochemical identity of a plant sample rather than a single isolated marker. For medicinal plants marketed on the basis of specific bioactive constituents, validated HPTLC quantification of a representative marker compound provides a practical and regulatorily recognised basis for batch-to-batch quality assurance.

Putranjiva roxburghii Wall. is traditionally valued for its steroidal and phenolic constituents, among which β -sitosterol — a phytosterol with documented anti-inflammatory, hypocholesterolaemic and immunomodulatory activity — is an important quality marker.

Boswellia serrata Roxb., in addition to its characteristic boswellic acids, has also been examined for alkaloidal constituents including colchicine-type compounds, whose quantification provides a complementary chemical marker for this species. Despite the pharmacological relevance of both compounds, validated, organ-resolved HPTLC quantification data for β -sitosterol in *P. roxburghii* and colchicine in *B. serrata* remain limited.

The present study was therefore designed to (i) generate comparative HPTLC fingerprints of steroids across seven organs of *P. roxburghii* and of alkaloids across five organs of *B. serrata* at multiple wavelengths; (ii) develop and validate, as per International Council for Harmonisation (ICH, 2005) guidelines, HPTLC methods for the quantification of β -sitosterol and colchicine respectively; and (iii) apply these validated methods to determine the organ-wise distribution of each marker compound, thereby identifying the

most chemically enriched plant part for standardisation and quality-control purposes.

1. Materials and Methods

1.1 Plant material and extraction

Air-dried, powdered samples (1 g) of leaf, stem bark, stem, flower, root, root bark and fruit of *P. roxburghii*, and of leaf, bark, stem, fruit and root of *B. serrata*, collected from plants growing around Gokul Global University, Sidhpur, Patan district, Gujarat, India, were extracted with 10 mL methanol for 24 h in a shaker. Extracts were filtered and concentrated under reduced pressure prior to HPTLC analysis.

1.2 Stationary and mobile phase

Pre-coated silica gel 60 F₂₅₄ HPTLC plates (Merck, 20 × 20 cm, cut to 20 × 10 cm) served as the stationary phase. For steroid (β -sitosterol) fingerprinting and quantification in *P. roxburghii*, the mobile phase was toluene:ethyl acetate:methanol (17:2:1), with anisaldehyde–sulphuric acid reagent used for derivatisation. For alkaloid (colchicine) fingerprinting and quantification in *B. serrata*, the mobile phase was toluene:methanol:acetone (16:4:1), also with anisaldehyde–sulphuric acid derivatisation.

1.3 Sample application, development and scanning

Samples were applied to pre-activated plates using a Camag Linomat-5 sample applicator under WinCATS Planar Chromatography Manager control for precise, track-wise loading. Plates were developed in a solvent-saturated glass twin-trough chamber (20 × 10 cm) to 80% of the plate length, dried, and scanned using a Camag TLC Scanner 3 at 200/254 nm (deuterium lamp), 366 nm (mercury lamp) and 520/540 nm (white light, post-derivatisation), with data processed in WinCATS.

1.4 Method validation

Both methods were validated for linearity, specificity, precision (repeatability) and accuracy (recovery) as per ICH (2005) guidelines. For linearity, 1, 2, 4, 6, 8 and 10 μ L of standard solution were applied to achieve 100, 200, 400, 600, 800 and 1000 ng/band, and calibration curves were constructed by plotting peak area against applied concentration. LOD and LOQ were derived from the calibration data. Specificity was assessed by comparing R_f values and spectra (peak start, apex and end) of standard and sample spots. Precision was evaluated as intra-day and inter-day %RSD at 600 ng/spot (n = 8), and recovery was assessed by the standard-addition method at 80%, 100% and 120% spiking levels.

1.5 Quantification of marker compounds

Following validation, methanolic extracts of each organ were applied alongside the respective standard (β -sitosterol or colchicine), developed and scanned under the validated conditions, and marker compound content was calculated from the calibration curve and expressed as μ g/g dry weight.

2. Results

2.1 HPTLC fingerprinting of steroids in *Putranjiva roxburghii*

Comparative HPTLC fingerprinting of steroidal constituents across the seven organs of *P. roxburghii* was performed at 200 nm, 366 nm (before and after derivatisation) and 540 nm (after derivatisation). At 200 nm, the maximum number of bands was recorded, with overall R_f values ranging from 0.03 to 0.75 across all samples; bands at R_f 0.06, 0.13 and 0.17 were common to all seven organs, indicating a conserved core set of steroidal/co-migrating constituents. At 366 nm (post-derivatisation), R_f values ranged from 0.06 to 0.77, with bands at R_f 0.25 and 0.77 common across all organs, while at 540 nm (post-derivatisation) the highest number of distinctly steroidal bands was resolved, with R_f 0.33 and 0.78 common to all samples.

Figure 1. HPTLC chromatogram of steroid fingerprinting in *Putranjiva roxburghii* at 254 nm (tracks 1–7: Leaf, Stem bark, Stem, Flower, Root bark, Root, Fruit).

Figure 2. HPTLC chromatogram of steroid fingerprinting in *Putranjiva roxburghii* at 366 nm after derivatisation with anisaldehyde–sulphuric acid reagent.

Table 1. Summary of steroid HPTLC fingerprint bands in *Putranjiva roxburghii* across seven plant organs (full band-by-organ matrix available on request).

Wavelength	R _f range observed	R _f values common to all seven organs
200 nm	0.03–0.75	0.06, 0.13, 0.17
366 nm (derivatised)	0.06–0.77	0.25, 0.77
540 nm (derivatised)	0.03–0.78	0.33, 0.78

2.2 Method validation for β -sitosterol

The developed HPTLC method for β -sitosterol (R_f = 0.51) showed maximum spectral absorbance at 519 nm and a linear response over the range 100–1000 ng/band (r = 0.99219; regression equation $Y = 1638.387 + 0.404X$; %SD = 6.87). The limit of detection was 185.1 ng/band and the limit of quantification was 447.3 ng/band. Specificity testing showed complete superimposition of standard and sample spectra at peak start, apex and end positions, confirming the absence of co-eluting interferents. Intra-day and inter-day precision at 600 ng/spot (n = 8) were 2.68% and 2.87% RSD respectively, meeting ICH acceptance criteria for repeatability. Mean recovery across the 80–120% spiking range was 68.70%, with an individual recovery of 60.72% recorded at the 80% spiking level.

Table 2. Validation parameters for the HPTLC method for β -sitosterol.

Parameter	β -Sitosterol
R _f	0.51

Linearity range (ng/spot)	100–1000
Correlation coefficient (r)	0.99219
Regression equation	$Y = 1638.387 + 0.404X$
SD (%)	6.87
LOD (ng/spot)	185.1
LOQ (ng/spot)	447.3
Intra-day precision (RSD%, n = 8)	2.68
Inter-day precision (RSD%, n = 8)	2.87
Mean recovery (%)	68.70

2.3 Organ-wise quantification of β -sitosterol in *Putranjiva roxburghii*

Applying the validated method, β -sitosterol content was quantified across all seven organs of *P. roxburghii*. The highest content was recorded in flower tissue (59.84 $\mu\text{g/g}$), followed by stem (30.09 $\mu\text{g/g}$), root bark (22.76 $\mu\text{g/g}$), fruit (14.75 $\mu\text{g/g}$), stem bark (13.79 $\mu\text{g/g}$) and root (12.05 $\mu\text{g/g}$), with the lowest content recorded in leaf tissue (10.17 $\mu\text{g/g}$). This nearly six-fold difference between the highest- and lowest-yielding organs confirms marked organ-specific accumulation of this steroidal marker.

Table 3. Organ-wise β -sitosterol content in *Putranjiva roxburghii*.

Organ	β -Sitosterol content ($\mu\text{g/g}$)
Leaf	10.17
Stem bark	13.79
Stem	30.09
Flower	59.84
Root bark	22.76
Root	12.05
Fruit	14.75

Figure 3. Organ-wise β -sitosterol content ($\mu\text{g/g}$) in *Putranjiva roxburghii*.

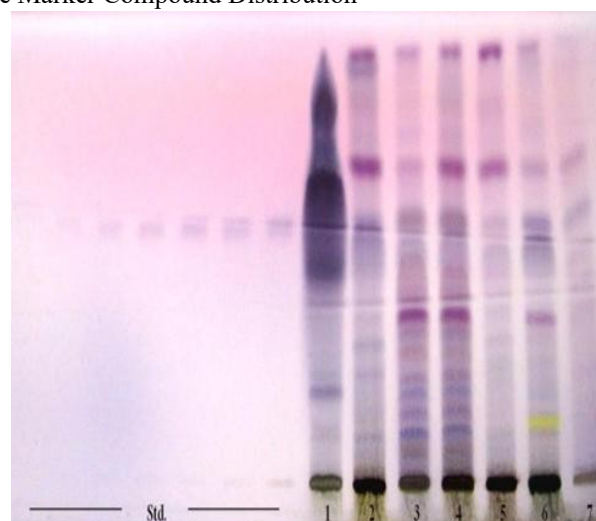


Figure 4. HPTLC quantification plate for β -sitosterol in *Putranjiva roxburghii* at 540 nm after derivatisation (Std. = standard β -sitosterol; tracks 1–7 correspond to the seven organs analysed).

2.4 HPTLC fingerprinting of alkaloids in *Boswellia serrata*

Comparative HPTLC fingerprinting of alkaloidal constituents across the five organs of *B. serrata* was performed at 254, 366 and 540 nm. At 254 nm, R_f values ranged from 0.11 to 0.73. At 366 nm, the largest number of unknown alkaloid bands was resolved, indicating the greatest number of detectable phytoconstituents at this wavelength, with the band at R_f 0.38 common to all five organs. At 540 nm, the maximum number of bands overall was present, with R_f values spanning 0.09 to 0.74.

Figure 5. HPTLC chromatogram of alkaloid fingerprinting in *Boswellia serrata* under UV 366 nm.

Table 4. Summary of alkaloid HPTLC fingerprint bands in *Boswellia serrata* across five plant organs (full band-by-organ matrix available on request).

Wavelength	R_f range observed	R_f value common to all five organs
254 nm	0.11–0.73	0.38 (band present in leaf, bark, stem, fruit, root)
366 nm	0.05–0.71	0.38
540 nm	0.09–0.74	0.38

2.5 Method validation for colchicine

The developed HPTLC method for colchicine ($R_f = 0.22$) showed maximum spectral absorbance at 355 nm and a linear response over 100–1000 ng/band ($r = 0.99491$; regression equation $Y = 8519.241 + 0.810X$; %SD = 3.64). The limit of detection was 147.6 ng/band and the limit of quantification was 447.3 ng/band. Specificity testing confirmed complete spectral superimposition of standard and sample spots with no interference from co-extracted plant constituents. Intra-day and inter-day precision at 600 ng/spot ($n = 8$) were 2.74% and 3.27% RSD

respectively. Mean recovery across the 80–120% spiking range was 93.12%, with individual recoveries of 83.04%, 77.52% and 111.8% at the 80%, 100% and 120% spiking levels respectively.

Table 5. Validation parameters for the HPTLC method for colchicine.

Parameter	Colchicine
R ^f	0.22
Linearity range (ng/spot)	100–1000
Correlation coefficient (r)	0.99491
Regression equation	Y = 8519.241 + 0.810X
SD (%)	3.64
LOD (ng/spot)	147.6
LOQ (ng/spot)	447.3
Intra-day precision (RSD%, n = 8)	2.74
Inter-day precision (RSD%, n = 8)	3.27
Mean recovery (%)	93.12

Table 6. Recovery study of the colchicine HPTLC method (mean recovery = 93.12%).

Spiking level (%)	Amount added (ng)	Recovery (%)
80	240	83.04
100	300	77.52
120	360	111.8

2.6 Organ-wise quantification of colchicine in *Boswellia serrata*

Applying the validated method, colchicine content was highest in root tissue (42.34 $\mu\text{g/g}$), followed by stem (34.30 $\mu\text{g/g}$), bark (32.40 $\mu\text{g/g}$) and leaf (28.65 $\mu\text{g/g}$), with the lowest content recorded in fruit tissue (24.27 $\mu\text{g/g}$). Root tissue thus accumulated approximately 1.7-fold more colchicine than fruit tissue, indicating that below-ground tissue is the preferred source for standardised colchicine-content raw material from this species.

Table 7. Organ-wise colchicine content in *Boswellia serrata*.

Organ	Colchicine content ($\mu\text{g/g}$)
Leaf	28.65
Bark	32.40
Stem	34.30
Fruit	24.27
Root	42.34

Figure 6. Organ-wise colchicine content ($\mu\text{g/g}$) in

Boswellia serrata.

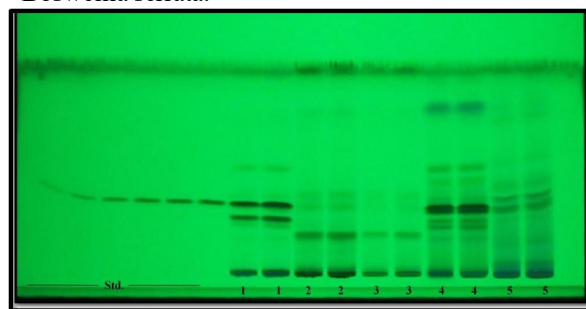


Figure 7. HPTLC quantification plate for colchicine in *Boswellia serrata* at 254 nm (Std. = standard colchicine track; tracks 1–5 correspond to Leaf, Bark, Stem, Fruit and Root, each applied in duplicate).

3. Discussion

Both HPTLC methods developed in this study satisfied ICH (2005) validation criteria for linearity, specificity, precision and accuracy, confirming their suitability as quality-control tools. The β -sitosterol method achieved a correlation coefficient of 0.99219 with RSD values below 3% for both intra- and inter-day precision, comparable to previously reported HPTLC methods for phytosterol quantification in other medicinal plant matrices, such as the HPTLC quantification of schisandrol A and schisandrol B reported by Ekiert *et al.* (2013) across fruit, leaf and root preparations. The comparatively modest mean recovery of 68.70% for β -sitosterol reflects the challenges commonly encountered in the standard-addition recovery of moderately lipophilic phytosterols from complex plant matrices, and indicates that while the method is precise and suitable for comparative organ-wise profiling, absolute quantification may be conservatively biased; this should be borne in mind when the method is used for regulatory quality-control applications.

The colchicine method showed superior recovery (93.12%) alongside excellent linearity ($r = 0.99491$) and precision, indicating a robust and accurate quantification protocol for this alkaloid marker in *B. serrata*. The preferential accumulation of colchicine in root tissue (42.34 $\mu\text{g/g}$) is consistent with reports of alkaloid accumulation patterns in other colchicine-producing taxa; for example, Kolar and Ghouse Basha (2014) similarly reported maximum colchicine accumulation in the tuber (an underground storage organ) of *Gloriosa* species, suggesting a broader physiological tendency for below-ground storage tissues to serve as sinks for this class of alkaloid. As noted by Baskaran *et al.* (2014), the biosynthesis and accumulation of such secondary metabolites can be strongly influenced by tissue-specific nutrient status and metabolic sink strength, which may explain the comparatively higher colchicine content of root and stem tissue relative to fruit in the present study.

The identification of flower tissue as the principal site of β -sitosterol accumulation in *P. roxburghii*, in contrast to the comparatively lower content in leaf tissue, and the corresponding identification of root tissue as the principal site of colchicine accumulation in *B. serrata*, provide directly actionable guidance for the sustainable and chemically optimised collection of raw material from each species. Where flower or root harvesting is impractical for conservation or renewability reasons, the fingerprint data presented here — which identify conserved marker bands (e.g., R_f 0.33 and 0.78 for steroids; R_f 0.38 for alkaloids) common to all organs — additionally support the use of more renewable tissues such as leaf or stem for qualitative identity confirmation, even where quantitative marker yield is lower.

4. Conclusion

This study presents validated, ICH-compliant HPTLC methods for the quantification of β -sitosterol in *Putranjiva roxburghii* and colchicine in *Boswellia serrata*, alongside comparative multi-wavelength fingerprinting of the steroidal and alkaloidal profiles of both species across their principal organs. Both methods demonstrated good linearity, specificity, precision and (for colchicine, particularly high) accuracy, and their application revealed pronounced organ-specific accumulation of each marker compound, with flower tissue identified as the optimal source of β -sitosterol in *P. roxburghii* and root tissue identified as the optimal source of colchicine in *B. serrata*. These validated methods and organ-wise reference data provide a practical foundation for the chemical standardisation and quality control of raw material and extracts of both medicinally important species.

References

- Baskaran, P., Moyo, M., & Van Staden, J. (2014). In vitro plant regeneration, phenolic compound production and pharmacological activities of *Coleonema pulchellum*. *South African Journal of Botany*, 90, 74–79.
- Ekiert, H., Klimek-Szczykutowicz, M., & Szopa, A. (2013). HPTLC quantification of schisandrol A and schisandrol B in fruit, leaf and root preparations of *Schisandra* species. *Journal of Planar Chromatography*, 26(6), 494–499.
- International Council for Harmonisation (ICH). (2005). *Validation of Analytical Procedures: Text and Methodology Q2(R1)*. ICH Harmonised Tripartite Guideline, Geneva.
- Kolar, F.R., & Ghouse Basha, M. (2014). Comparative HPTLC studies on colchicine content in different plant

parts of *Gloriosa superba* L. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(9), 486–488.