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SCREENING OF CLASS OF PHYTOCHEMICAL COMPOUNDS AND ANTIOXIDANT AUTORADIOGRAPHIC EVALUATION IN *PARIS POLYPHYLLA* AND *TRILLIUM GOVANIANUM* USING HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

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ABSTRACT

Paris polyphylla and *Trillium govanianum* are medicinally important Himalayan plants known for their ethnopharmacological applications, particularly in traditional medicine for anti-inflammatory, antimicrobial, and anticancer uses. The present study employs High-Performance Thin-Layer Chromatography (HPTLC) to identify major classes of phytochemicals present in dried powdered samples of these species. Qualitative HPTLC screening was performed using CAMAG HPTLC instrumentation with nine reagent systems targeting major classes of compounds, including flavonoids, glycosides, steroids, sterols, essential oils, saponins, terpenoids, anthraquinones, and phenolic compounds. The results confirmed the presence of essential oils, saponins, triterpenoids, steroids, sterols, glycosides, and flavonoids in both species, whereas anthraquinones and phenolic compounds were absent. Additional DPPH assay indicated significant antioxidant band responses in both plant extracts. The findings demonstrate similarity in phytochemical composition between both species, justifying their reported medicinal equivalence in traditional medicine.

KEYWORDS: *Paris polyphylla*, *Trillium govanianum*, phytochemical profiling, HPTLC, saponins, antioxidant activity.

1. INTRODUCTION

Medicinal plants remain a major source of therapeutic compounds across traditional and modern medicinal systems. Among them, *Paris polyphylla* (family: Melanthiaceae) and *Trillium govanianum* are widely used in Ayurveda and Himalayan folk medicine for their therapeutic activities including antimicrobial, anticancer, anti-inflammatory, and antioxidant properties. Their pharmacological functions have been attributed largely to bioactive secondary metabolites such as steroidal saponins, flavonoids, sterols, glycosides, and other phytochemical groups. Formulations involving *Paris polyphylla* are traditionally employed for the treatment of fevers, wounds, and poisonous or animal bites in Himalayan and Nepalese Ayurvedic systems (Rawat et al., 2023; Thapa et al., 2022).

The rhizomes of *Paris polyphylla* are prescribed in indigenous medical practices for managing chronic and severe conditions such as arthritis, hypertension, cancer, and sepsis, and are incorporated into local Ayurvedic and herbal formulations (Rawat et al., 2023; Thakur et al., 2023). *Trillium govanianum* holds a significant position in Himalayan Ayurveda and folk medicine, with documented applications in inflammation, wound healing, and gastrointestinal disorders (Sharma, 2017; Thakur et al., 2025).

High-performance thin-layer chromatography (HPTLC) is a rapid, reliable, and cost-effective analytical technique widely employed for the phytochemical evaluation and standardization of medicinal plants. The method enables efficient chromatographic fingerprinting of complex herbal matrices and supports the qualitative and semi-quantitative assessment of bioactive compounds. HPTLC has been successfully utilized for the characterization of phenolic constituents and antioxidant potential of medicinal herbs, underscoring its relevance in correlating chemical profiles with biological activity (Saraf & Saraf, 2020; Saraf et al., 2024). Furthermore, the technique has proven effective in generating reproducible alkaloid fingerprint profiles, contributing to authentication and quality control of herbal drugs (Amit, 2015). Its application in pharmacognostic and phytochemical studies facilitates the establishment of quality parameters essential for ensuring consistency, purity, and therapeutic reliability of medicinal plant materials (Aparna & Aruna, 2014). The increasing commercial demand and restricted natural distribution, chemical authentication and comparative phytochemical profiling are essential to validate botanical identity and assess herbal product quality. High-Performance Thin-Layer Chromatography (HPTLC) is a reproducible, rapid, and cost-effective tool widely used in herbal quality control and phytochemical fingerprinting.

The objective of the present study was to qualitatively profile major classes of compounds in *Paris polyphylla* and *Trillium govanianum* using specific HPTLC derivatization reagents and to compare their phytochemical composition.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Powdered raw samples of *Paris polyphylla* and *Trillium govanianum* were processed as follows:

Step	Procedure
Sample weight	500 mg plant powder
Solvent used	1 mL methanol
Sonication	20 minutes
Centrifugation	10 minutes at 3000 rpm
Extraction condition	Overnight at room temperature
Final preparation	Supernatant transferred to vials for spotting

2.2 HPTLC Instrumentation and Conditions

Instrument:	CAMAG HPTLC System
Stationary Phase:	Silica gel 60 F254 aluminum-backed plates (Merck, #1.05554.0007)
Development Distance:	70 mm
Plate Dimensions:	
Application position:	8 mm
First Track position:	15 mm
Track distance:	11.4 mm
Application length:	8 mm
Application width:	5 mm
Track 1,2 &3	*Paris polyphylla*
Track 4,5 & 6	*Trillium govanianum*
Saturation Time:	20 minutes
Application Volume:	1–2 µL depending on assay

2.3 Mobile Phase Systems and Reagents

Nine mobile phases and derivatization reagents were used to detect respective phytochemical classes (Table 1).

Table 1: HPTLC Systems Used for Qualitative Class Analysis.

Class of Compounds	Mobile Phase	Derivatizing Reagent
Essential Oils	Toluene: Ethyl acetate (93:7)	ASR
Steroids	n-Butanol: Methanol: Water (3:1:1)	ASR
Triterpenoids	n-Hexane: Ethyl acetate (1:1)	ASR
Saponins	Chloroform: Acetic acid: Methanol: Water (6.4:3.2:1.2:0.8)	ASR
Sterols	Toluene: Ethyl acetate (8:2)	ASR
Glycosides	Ethyl acetate: Methanol: Water (81:11:8)	ASR
Anthraquinones	Ethyl acetate: Methanol: Water (16:4:0.2)	Alcoholic KOH
Flavonoids	Ethyl acetate: Methanol: Water (16:4:0.2)	10% Methanolic sulfuric acid
Phenolics	Ethyl acetate: MEK: Formic acid: Water (5:3:1:1)	Alcoholic $FeCl_3$

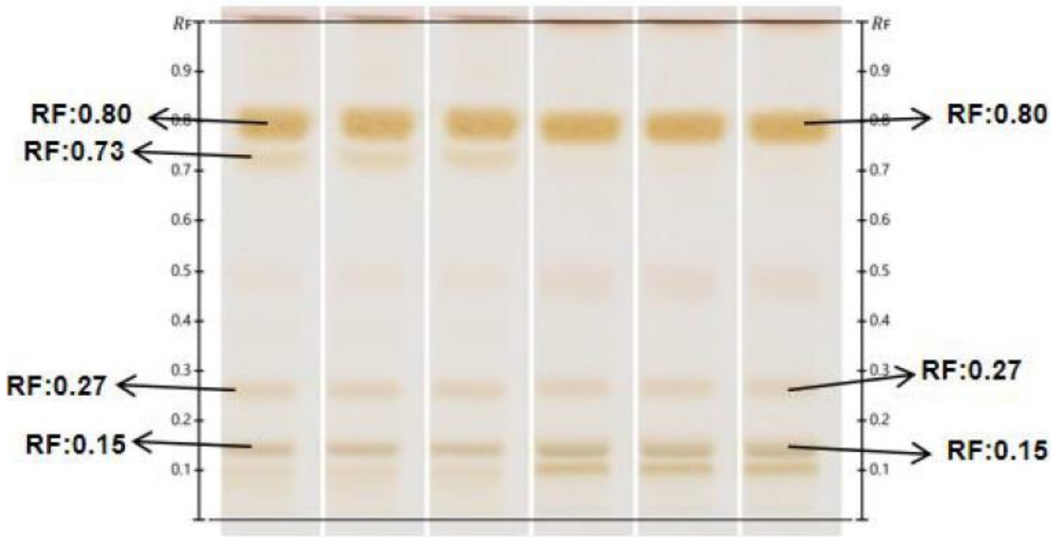
2.4 Antioxidant Activity (DPPH Assay)

The antioxidant activity was assessed by derivatization using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), prepared at 200 mg in 200 mL ethanol. Plates were dipped under dark conditions and evaluated after 4–5 minutes. Yellow bands indicated antioxidant presence.

3. RESULTS

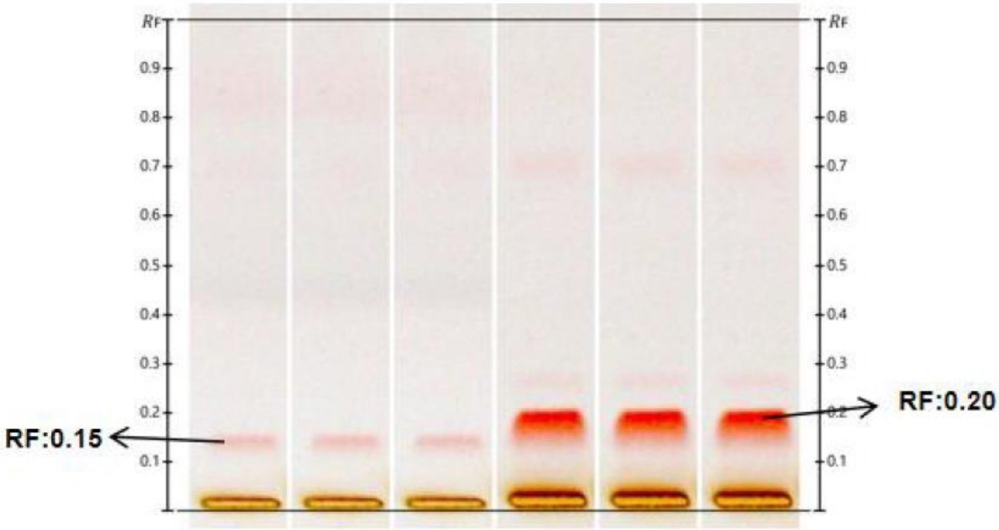
3.1 Detection of various class of compounds by HPTLC

3.1.1: Saponins



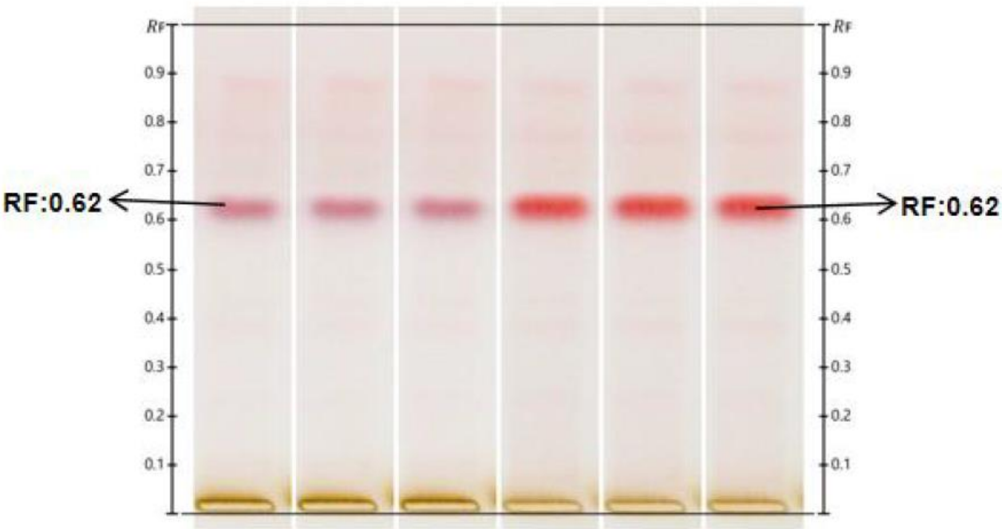
Saponins were detected in both *Paris polyphylla* and *Trillium goranianum*. The derivatization of the developed plate with anisaldehyde–sulphuric acid reagent (ASR) shows distinct yellow-colored bands in the sample tracks, confirming the presence of saponins.

3.1.2: Essential Oils



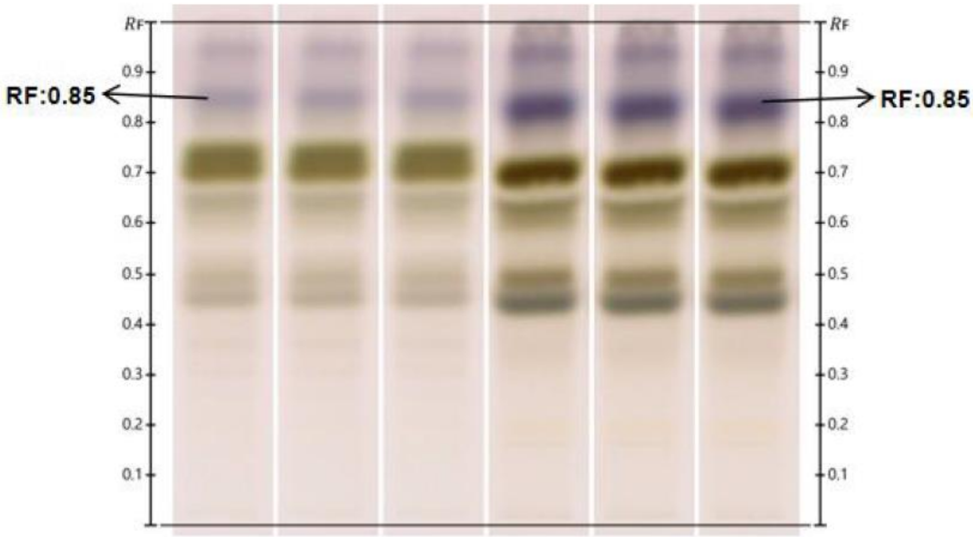
Essential oils were also identified in both plant samples. Derivatization with ASR resulted in the appearance of red-colored bands in the chromatographic tracks of *Paris polyphylla* and *Trillium goranianum*, indicating the presence of essential oils.

3.1.3: Triterpenoids



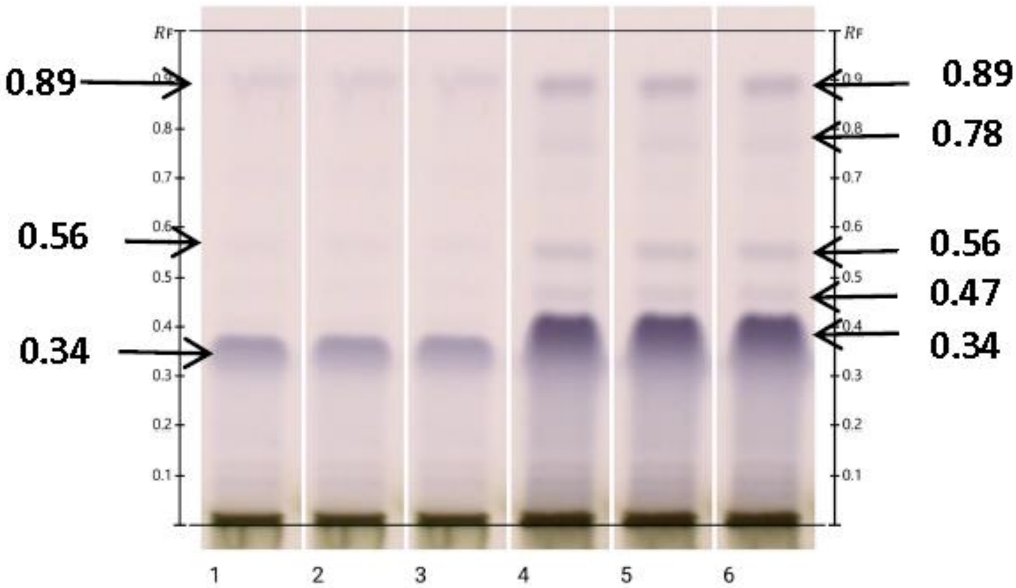
The developed plate, when treated with ASR, exhibited red-colored bands in the corresponding tracks, confirming the presence of triterpenoids in *Paris polyphylla* and *Trillium goranianum*.

3.1.4: Steroids



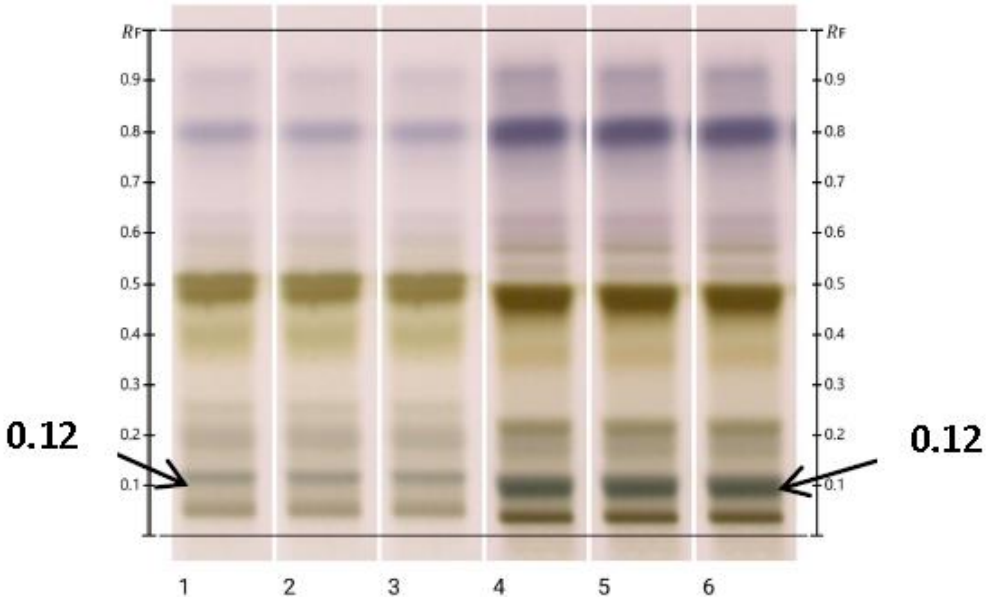
Purple-colored bands observed after derivatization with ASR on the developed plate confirmed the presence of steroids in *Paris polyphylla* and *Trillium goranianum*.

3.1.5: Sterols



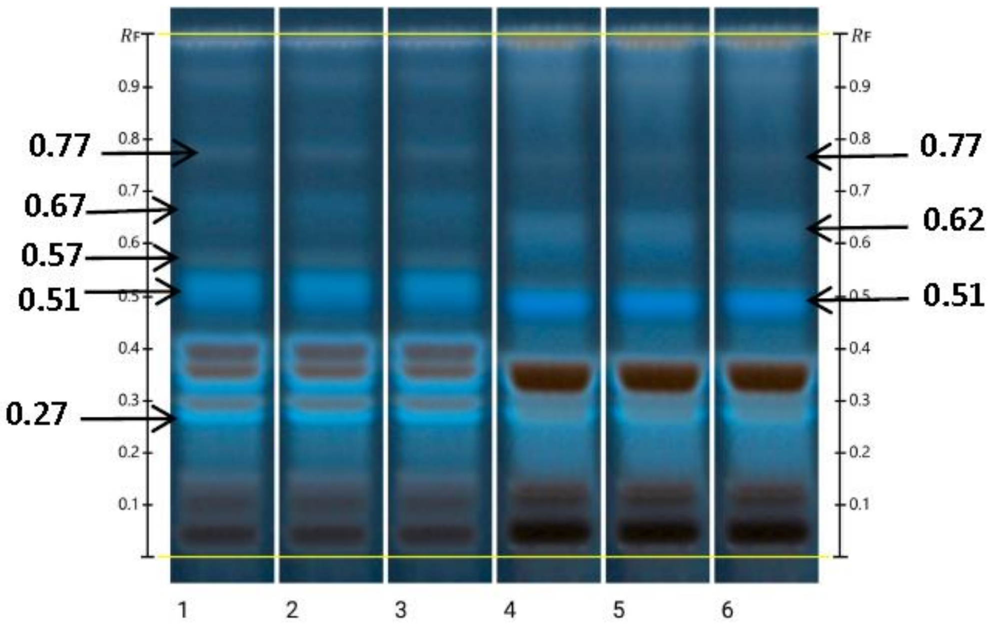
Sterols were also detected in both samples. Upon derivatization with ASR, purple-colored bands appeared in the chromatographic tracks of both *Paris polyphylla* and *Trillium goranianum*, indicating the presence of sterols.

3.1.6: Glycosides



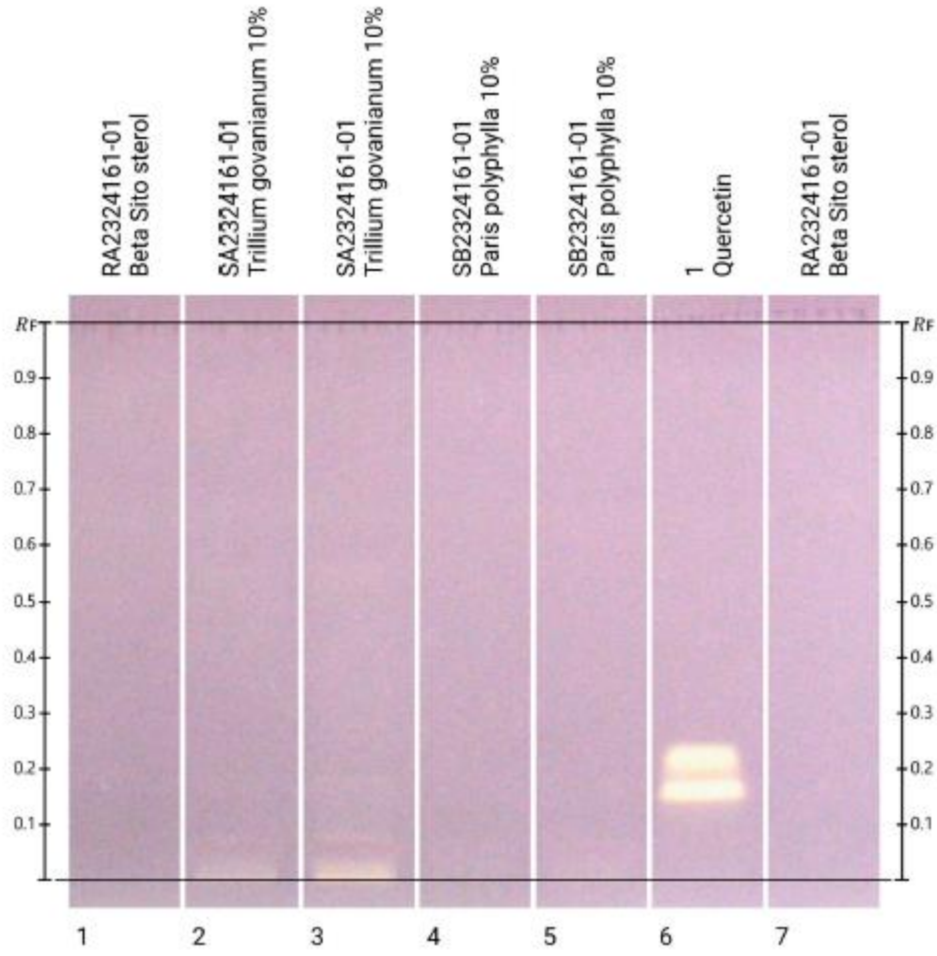
The developed plate derivatized with ASR showed blue-green colored bands in both sample tracks, signifying the presence of Glycosides in *Paris polyphylla* and *Trillium goranianum*.

3.1.7: Flavanoids



Flavonoids were detected in both plant samples. Derivatization of the developed plate with 10% methanolic sulphuric acid reagent resulted in the appearance of blue fluorescent bands in the sample tracks, confirming the presence of flavonoids in *Paris polyphylla* and *Trillium goranianum*.

3.2 Antioxidant Activity



Both plant samples showed multiple yellow scavenging zones after DPPH derivatization, confirming the presence of antioxidant constituents.

4. DISCUSSION

HPTLC analysis of *Paris polyphylla* and *Trillium goranianum* revealed the presence of multiple classes of phytoconstituents. Derivatization with anisaldehyde–sulphuric acid reagent confirmed the presence of saponins (yellow bands), essential oils and triterpenoids (red bands), steroids and sterols (purple bands), and glycosides (blue-green bands) in both samples. Flavonoids were detected in both samples based on the appearance of blue fluorescent bands following derivatization with 10% methanolic sulphuric acid. Yellow zones on the plate dipped in DPPH reagent confirms the presence of antioxidant compounds.

The study demonstrates a clear similarity in phytochemical composition between *Paris polyphylla* and *Trillium govanianum*. Both species contained key metabolites such as saponins, flavonoids, steroids, terpenoids, and glycosides. These compounds are associated with reported antioxidant, anticancer, antimicrobial, and anti-inflammatory properties.

HPTLC proved effective for rapid and reproducible qualitative phytochemical profiling and may serve as an authentication tool for herbal quality assessment.

5. CONCLUSION

HPTLC-based phytochemical screening successfully identified major bioactive compound classes in both *Paris polyphylla* and *Trillium govanianum*. The comparative results suggest strong phytochemical overlap, supporting their interchangeable use in traditional medicine. Further quantitative and compound-specific analytical studies (LC-MS, NMR, and HPTLC densitometry) are recommended for authentication and standardization.

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REFERENCES

1. Amit, S. Chromatographic fingerprint profile of alkaloids of *Abrus precatorius* Linn. by HPTLC. *European Journal of Academic Essays*, 2015; *2*(6): 45–50.
2. Aparna, S., & Aruna, S. Pharmacognostic and phytochemical studies for the establishment of quality parameters of leaf of *Achyranthes aspera* Linn. *International Journal of Pharmacognosy and Phytochemical Research*, 2014; *6*(1): 122–127.
3. Kumar, P., Singh, K., Lone, J. F., Bhushan, A., Gupta, P., & Gairola, S. Morpho-anatomical, molecular, and chemical standardization of *Trillium govanianum* Wall. ex D. Don: An endangered medicinal herb native to the Himalayas. *Pharmacognosy Magazine*, 2023; *19*(1): 128–143. https://doi.org/10.1177/09731296221145070
4. Rawat, J. M., Pandey, S., Rawat, B., Rai, N., Preeti, P., Thakur, A., & Bachheti, R. K. Traditional uses, active ingredients, and biological activities of *Paris polyphylla* Smith: A comprehensive review of an important Himalayan medicinal plant. *Journal of Chemistry*, 2023; *2023:* 7947224. https://doi.org/10.1155/2023/7947224
5. Saraf, A. Y., & Saraf, A. A. HPTLC fingerprinting: A tool for simplified analysis of phenolics in medicinal plants. *Indian Journal of Pharmaceutical Education and Research*, 2020; *54*(3): 567–574.

6. Saraf, A. Y., Saraf, A. A., Apurva, P., & Shinde, P. S. Fast and simple HPTLC chemical fingerprinting and antioxidant analysis of medicinal herbs. *Journal of Pharmacognosy and Phytochemistry*, 2024; *13*(2): 806–809. https://doi.org/10.22271/phyto.2024.v13.i2f.14927
7. Sharma, D. K. Review on traditional medicinal plant *Trillium govanianum* (Nagchatri). *Journal of Medicinal Plants Studies*, 2017; *5*(2): 120–122.
8. Thakur, A., et al. Conservation and medicinal applications of *Trillium govanianum*: A review. *International Journal of Science & Engineering Development*, 2025; *10*(2): a173–a186. https://doi.org/10.6084/m9.doione.IJRTI2502023
9. Thakur, U., Shashni, S., Thakur, N., Rana, S. K., & Singh, A. A review on *Paris polyphylla* Smith: A vulnerable medicinal plant species of global significance. *Journal of Applied Research on Medicinal and Aromatic Plants*, 2023; *33:* 100447. https://doi.org/10.1016/j.jarmap.2022.100447
10. Thapa, C. B., Paudel, M. R., Bhattarai, H. D., Pant, K. K., Devkota, H. P., Adhikari, Y. P., & Pant, B. Bioactive secondary metabolites in *Paris polyphylla* Sm. and their biological activities: A review. *Heliyon*, 2022; *8*(2): e08982. https://doi.org/10.1016/j.heliyon.2022.e08982
11. Wagner H., Bladt S. (1996). *Plant Drug Analysis: A Thin Layer Chromatography Atlas.*
12. WHO. (2011). *Quality control methods for herbal materials.*