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HPTLC-Based Qualitative Identification of Gallic Acid in Rauvolfia serpentina Roots Collected from Diverse Agro-Climatic Zones of India

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ABSTRACT

Gallic acid is a bioactive phenolic compound widely recognized for its antioxidant and pharmacological properties. The present study qualitatively investigates the presence of gallic acid in root samples of Rauvolfia serpentina (Sarpagandha) collected from four distinct agro-climatic zones of India using High Performance Thin Layer Chromatography (HPTLC). Methanolic extracts of root samples were analyzed alongside a gallic acid standard employing a standardized solvent system. The chromatographic profiles of all samples exhibited peaks corresponding to the retention factor (Rf) range of the reference standard, confirming the presence of gallic acid across all evaluated samples. This study supports the phytochemical consistency of Rauvolfia serpentina roots and contributes to quality control and standardization efforts of this important medicinal plant.

Keywords: *Rauvolfia serpentina, Gallic acid, HPTLC, Qualitative Analysis, Medicinal Plant Standardization.*

INTRODUCTION

Rauvolfia serpentina (L.) Benth. ex Kurz is an important medicinal plant extensively used in traditional systems of medicine, particularly Ayurveda, for the management of hypertension, neurological disorders, and insomnia (Shah and Gilani, 2010). In addition to its well-documented alkaloidal constituents, the plant is known to contain phenolic compounds that contribute significantly to its antioxidant and therapeutic potential (Patel *et al.,* 2012). Gallic acid, a naturally occurring phenolic acid, has been reported to possess antioxidant, anti-inflammatory, antimicrobial, and cardioprotective activities, thereby enhancing the pharmacological relevance of medicinal plants containing this compound (Kumar and Pandey, 2013).

Phytochemical standardization using chromatographic techniques such as HPTLC is a reliable and reproducible approach for the qualitative and quantitative assessment of marker compounds in herbal drugs (Harborne, 1998). The present investigation aims to qualitatively confirm the presence of gallic acid in *R. serpentina* root samples collected from different agro-climatic regions of India using HPTLC as a marker-based quality assessment tool.

MATERIALS AND METHODS

Qualitative studies of Gallic acid in root specimens were analyzed by following the method of Wagner and Bladt, 2009; Reich and Schibli, 2007 with modification.

Plant Material

Root samples of *Rauvolfia serpentina* were collected from four different agro-climatic zones of India and authenticated using standard pharmacognostical parameters. The samples were shade-dried, powdered, and stored in airtight containers until further analysis.

Chemicals and Reagents

Analytical grade methanol, toluene, ethyl acetate, formic acid, and ninhydrin reagent were used. Gallic acid standard was procured from a certified reference supplier.

Sample Preparation

Ten milliliters of each root extract sample were transferred into sterilized tubes and diluted with 20 ml of methanol. The mixture was refluxed at 40°C for 2 hours, followed by filtration. The clear filtrate was used for HPTLC analysis.

HPTLC Conditions

Chromatographic analysis was carried out on Merck silica gel 60 F254 HPTLC plates. Samples and standards were applied as bands using a calibrated applicator. Plate development was performed using a solvent system consisting of toluene : ethyl acetate : formic acid (5:4:1 v/v/v) until the solvent front reached approximately 80% of the plate height. After development, the plates were air-dried and derivatized by spraying with ninhydrin reagent.

Application Details

The application positions, volumes, and sample identifiers used during HPTLC analysis are summarized in Table 1.

Table 1. Application position and volume of samples and standard on HPTLC plate

No.	Application Position (mm)	Volume (µl)	Vial	Sample ID
1	15.0	8.0	1	SZ1
2	25.0	8.0	2	SZ4
3	35.0	8.0	3	SZ9
4	45.0	8.0	4	SZ10
5	55.0	4.0	5	Standard
6	65.0	6.0	6	Standard
7	75.0	8.0	7	Standard

RESULTS

HPTLC fingerprinting of methanolic root extracts of *Rauvolfia serpentina* revealed distinct and reproducible chromatographic bands. All test samples (SZ1, SZ4, SZ9, and SZ10) exhibited spots with Rf values corresponding closely to those of the gallic acid reference standard, indicating the presence of gallic acid in all samples irrespective of agro-climatic origin. Comparable band intensity and alignment with the standard track further support the qualitative consistency of gallic acid distribution among samples. The developed HPTLC plate (Figure 1) demonstrated well-resolved spots under post-derivatization with ninhydrin reagent, a method previously reported for enhancing visualization of phenolic compounds in herbal matrices. The densitometric scan (Figure 2) showed characteristic peaks of gallic acid in both standard and test tracks, with sample peaks falling within the standard Rf start and end range, confirming identity and chromatographic specificity.

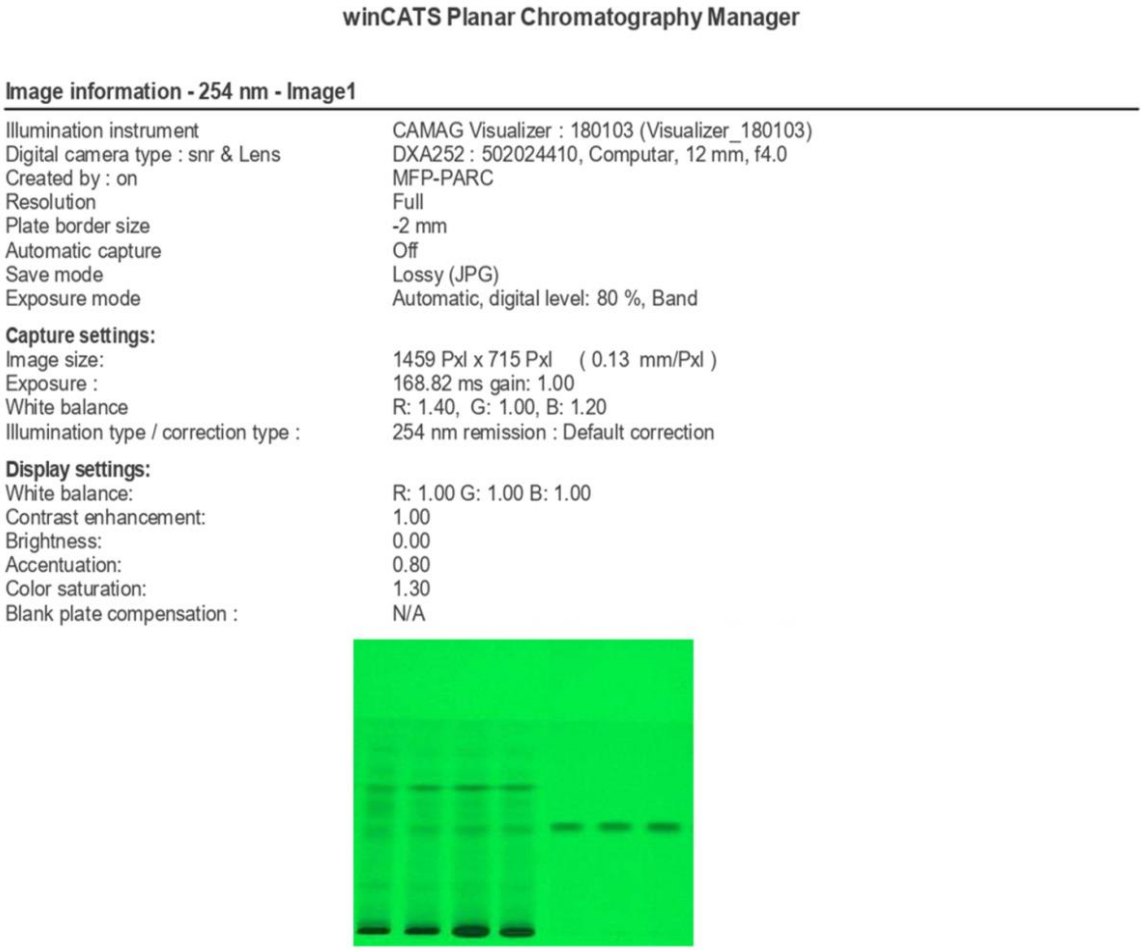
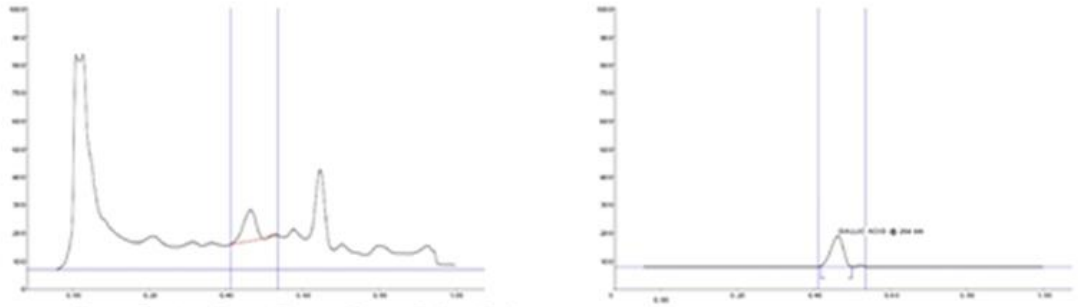


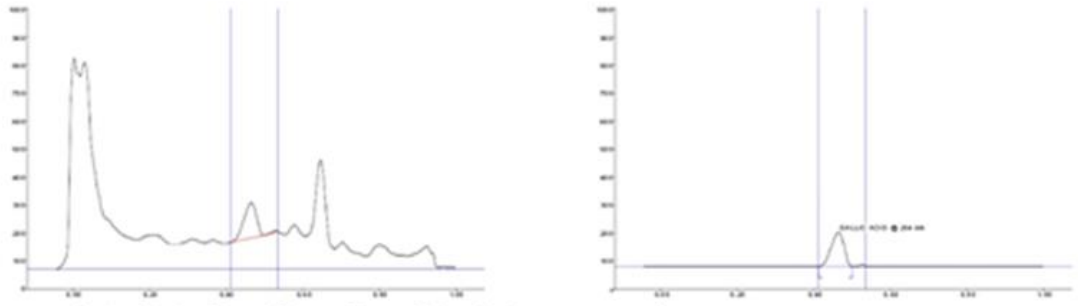
Figure 1. HPTLC plate showing separation of gallic acid in standard and *R. serpentina* root samples (visualized after derivatization).

Track 1, ID: SZ1



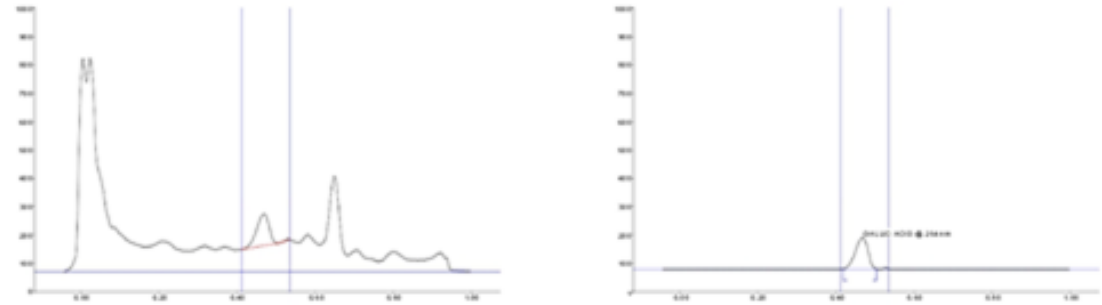
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	4.4	0.46	110.1	100.00	0.50	0.1	2793.4	100.00	GALLIC ACID

Track 2, ID: SZ4



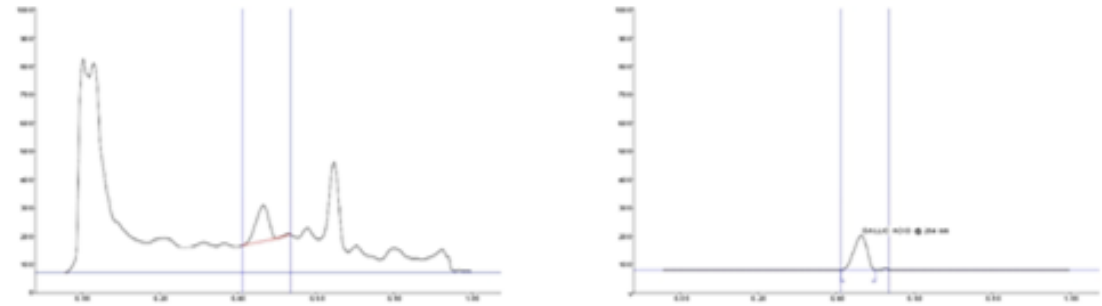
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.41	0.2	0.47	125.7	100.00	0.50	0.1	3183.0	100.00	GALLIC ACID

Track 4, ID: SZ10



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	1.2	0.47	111.1	100.00	0.50	0.2	2739.8	100.00	GALLIC ACID

Track 3, ID: SZ9



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.41	0.2	0.47	125.7	100.00	0.50	0.1	3183.0	100.00	GALLIC ACID

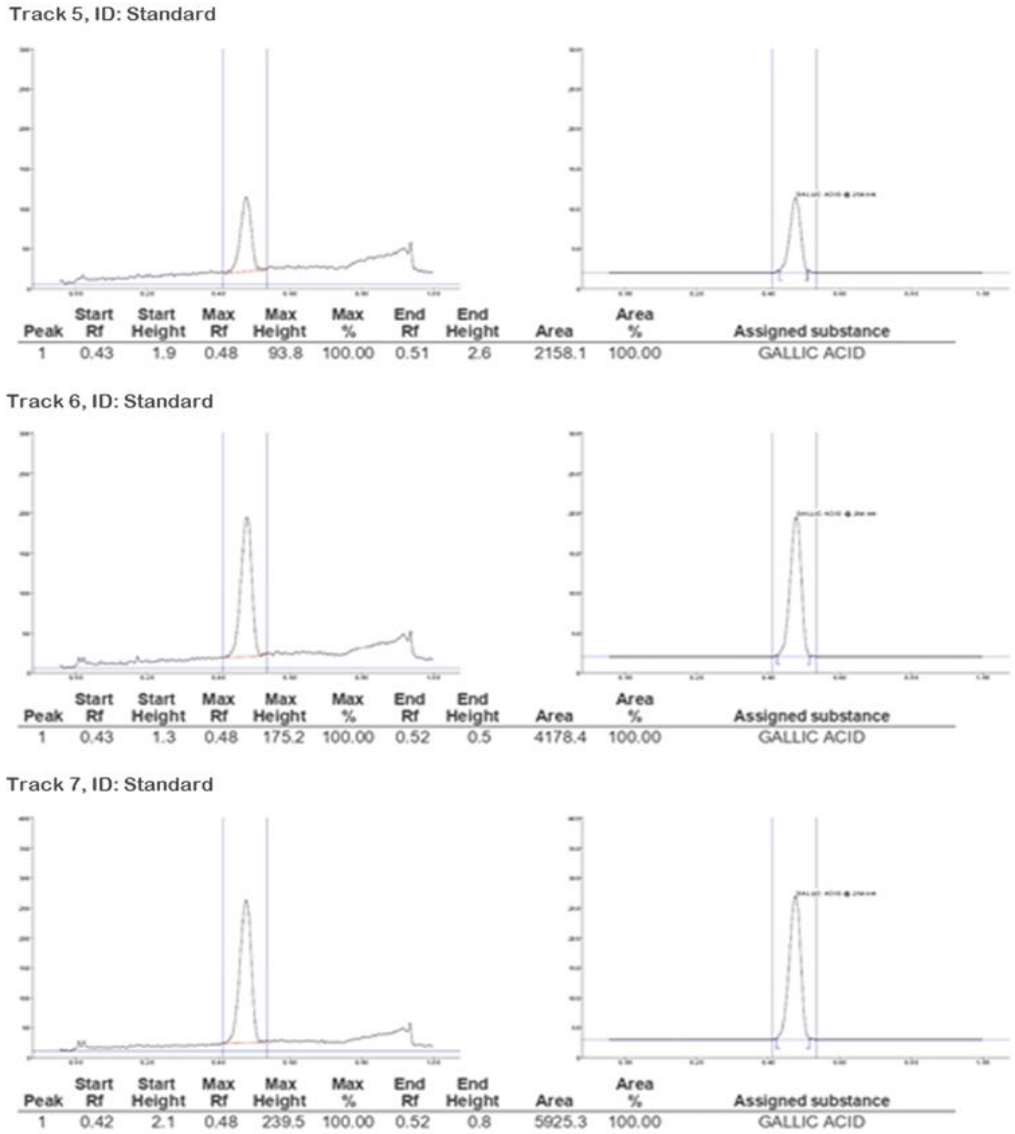


Figure 2. Densitometric chromatogram comparing gallic acid standard with *R. serpentina* root sample extracts.

DISCUSSION

The qualitative confirmation of gallic acid in all *R. serpentina* root samples highlights the phytochemical uniformity of this medicinal plant across diverse agro-climatic zones. Gallic acid is known to contribute significantly to antioxidant activity, which may synergistically enhance the therapeutic effects of R. serpentine (Kumar and Pandey, 2013). The use of HPTLC as a fingerprinting technique offers a reliable method for the authentication and quality control of herbal raw materials. Previous studies have reported the presence of phenolic compounds, including gallic acid, in several medicinal plants and have emphasized their role in mitigating oxidative stress and improving pharmacological efficacy (Wagner and Bladt, 2009). The present findings align with earlier reports and further support the inclusion of gallic acid as a potential phytochemical marker for the standardization of *R. serpentina* roots (Patel *et al*., 2012Joshi and Uniyal, 2008; Reich and Schibli, 2007; Harborne, 1998). Such marker-based approaches are essential for ensuring batch-to-batch consistency, safety, and efficacy of herbal formulations.

CONCLUSION

The present HPTLC-based qualitative study successfully confirmed the presence of gallic acid in Rauvolfia serpentina root samples collected from different agro-climatic regions of India. The results underscore the reliability of HPTLC as a rapid and effective tool for phytochemical identification and support the use of gallic acid as a marker compound in quality control protocols of *R. serpentina*.

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