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Quantitative and Qualitative Estimation of Gallic Acid in Triphala by HPTLC

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KEYWORDS

Triphla;

Gallic acid; Antioxidant activity;

HPTLC; Phytochemical analysis;

Herbal medicine.

ABSTRACT:

The present study focuses on the quantitative and qualitative estimation of gallic acid in Triphala using High-Performance Thin Layer Chromatography (HPTLC). Standardized HPTLC methods were carefully developed and validated for the simultaneous identification and estimation of these phytochemical markers. Methanolic extracts of the samples were prepared, analysed, and compared against authentic standard compounds to ensure accuracy and reproducibility. The results revealed significant variations in the concentration of gallic acid, polyherbal formulation indicating the synergistic and balanced contribution of each fruit to the overall efficacy of Triphla.

This investigation clearly validates the application of HPTLC as a reliable, rapid, and cost-effective analytical tool for the quality control, authentication, and standardization of Ayurvedic formulations. In addition, the study highlights the critical role of marker-based standardization in addressing the challenges of variability in herbal medicines caused by environmental, seasonal, and post-harvest factors. By establishing gallic acid as chemical markers, the present work provides a scientific benchmark that can be adopted for regulatory purposes and industrial-scale production. Furthermore, the findings strengthen the evidence supporting the antioxidant potential, rejuvenating activity, and therapeutic relevance of Triphla, thereby bridging the gap between traditional wisdom and modern pharmacological validation. Ultimately, this study promotes the safe, effective, and globally acceptable use of Triphla as a standardized natural remedy in complementary and alternative medicine systems.

Introduction

Triphala, a well-known polyherbal formulation in Ayurveda. These fruits has been individually valued in traditional medicine for centuries, and their synergistic combination in Triphala provides a broader spectrum of therapeutic properties (Verma *et al*., 2021). The formulation has been traditionally recommended for maintaining overall health, improving digestion, detoxification, and promoting longevity. In Ayurvedic literature, Triphala is classified as a *Rasayana*, which refers to rejuvenating preparations that enhance vitality, immunity, and resistance to diseases (Baliga *et al*., 2012).

The pharmacological properties of Triphala are attributed to its rich phytoconstituents, particularly polyphenols, flavonoids, tannins, and vitamins. Among these, gallic acid are regarded as one of the most important bioactive markers. Gallic acid, a naturally occurring phenolic compound, is recognized for its strong antioxidant capacity, antimicrobial activity, anti-inflammatory effects, and potential anticancer properties (Baliga *et al*., 2012). It contributes significantly to the free radical scavenging ability of Triphala, thereby reducing oxidative stress and cellular damage. On the other hand, ascorbic acid (Vitamin C), predominantly found in Triphala, plays a crucial role as a water-soluble antioxidant. It is essential not only for neutralizing free radicals but also for collagen synthesis, wound healing, and enhancing the immune response. Together, these compounds add considerable therapeutic value to Triphala and contribute to its wide

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acceptance as a natural health supplement (John *et al*., 2022; Vijapura & Soni, 2023a).

However, the quality and efficacy of herbal formulations like Triphala are highly dependent on multiple factors such as the source of raw materials, methods of processing, geographical and seasonal variations, and storage conditions. For example, the concentration of gallic acid and ascorbic acid can vary due to differences in soil quality, climatic conditions where the plants are grown, or due to degradation during prolonged storage. Moreover, the drying and extraction methods employed also influence the final phytochemical profile. Such variations may affect the consistency, potency, and safety of Triphala preparations available in the market (Soni & Kapoor, 2019; Vijapura & Soni, 2023b). Therefore, the development of reliable analytical techniques for standardization and quality control is of paramount importance (Prajapati *et al*., 2022).

High-Performance Thin Layer Chromatography (HPTLC) has emerged as a powerful analytical tool for herbal medicine research. Unlike conventional thin-layer chromatography (TLC), HPTLC provides enhanced resolution, accuracy, and reproducibility, making it suitable for both qualitative fingerprinting and quantitative estimation of phytochemicals (Sethi, 2008). The technique allows the simultaneous analysis of multiple samples under identical conditions, which makes it efficient and cost-effective. With the help of specific derivatization reagents and densitometric scanning, HPTLC can detect and quantify bioactive compounds like gallic acid and ascorbic acid with high precision. Additionally, minimal sample preparation and the ability to handle complex plant matrices make it particularly advantageous for herbal formulations (Rana *et al*., 2022; Soni *et al*., 2024).

In the present investigation, HPTLC is employed to determine the content of gallic acid and ascorbic acid in Triphala. This study not only aims to provide a validated method for quantifying these active markers but also contributes to the establishment of a standardization protocol for Triphala. Standardization ensures batch-to-batch consistency, enhances therapeutic reliability, and minimizes the risks associated with adulteration or substandard preparations (Upadhyay *et al*., 2024). By focusing on gallic acid and ascorbic acid as chemical markers, the study addresses one of the critical aspects of quality assurance in herbal medicine research. Furthermore, the findings may help bridge the gap between traditional knowledge and modern scientific validation, thereby strengthening the credibility of Triphala in global healthcare systems (Vijapura & Soni, 2023a; John *et al*., 2022).

Ultimately, this investigation highlights the importance of phytochemical standardization as a means to ensure the safety, efficacy, and acceptance of herbal medicines like Triphala. The use of HPTLC as a validated method offers a practical and scientific approach to maintain quality control standards, paving the way for more reliable applications of Ayurvedic formulations in contemporary clinical practice (Baliga *et al*., 2012; Soni & Kapoor, 2019).

Materials and Methods

Chemicals and Reagents: Standard reference compounds Gallic Acid were obtained from Natural Remedies, while HPLC-grade solvents were sourced from Merck Ltd., New Delhi, India.

Instrumentation:
High-Performance Thin-Layer Chromatography (HPTLC) analysis was conducted using a CAMAG system equipped with a Linomat-5 applicator. Detection of chromatographic bands was carried out using the CAMAG Scanner III.

HPTLC Analysis: Samples were applied as 6 mm wide bands on precoated silica gel aluminium plates (Silica gel 60 F254, 10 × 10 cm, 0.2 mm thickness; E. Merck, Germany) using a CAMAG Linomat-V applicator. Application rate was maintained at 150 nL/s with a spacing of 5.5 mm between bands. The scanning was performed at a slit size of 6 mm × 0.3 mm with a scan speed of 20 mm/s. Linear ascending development was executed in a twin-trough chamber pre-saturated with a mobile phase composed of toluene, ethyl acetate, and methanol in a 4:4:5 (v/v) ratio. The chamber was saturated for 30 minutes at ambient temperature. The solvent front migration was 70 mm. After development, the plates were air-dried using a blower, and densitometric scanning was conducted with a CAMAG TLC Scanner III (Aneja, K. R. 2022).

Calibration of Marker Compounds: A stock solution (100 µg/mL) of Ascorbic Acid and Gallic Acid was

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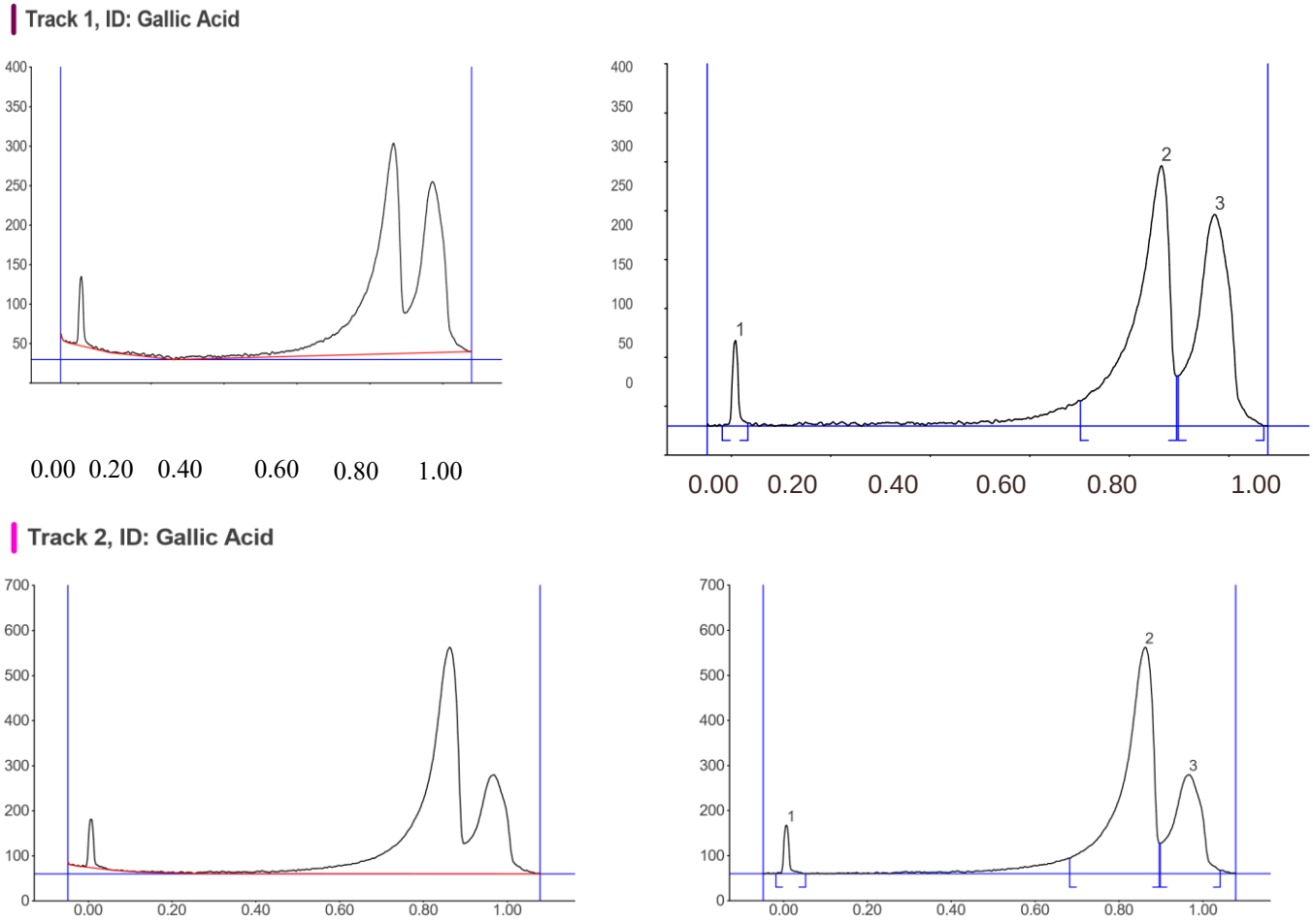
JCHR (2025) 15(6), 2580-2584 | ISSN:2251-6727

prepared in methanol. Aliquots ranging from 0.1 to 2.5 mL were transferred to 10 mL volumetric flasks and diluted with methanol to obtain concentrations between 1 and 25 ng/µL. Duplicate applications were made on TLC plates to yield final concentrations of 50–250 ng/spot. Calibration curves were generated by plotting peak area and height against concentrations, and the data were analyzed using linear least-squares regression.

Limit of Detection (LOD) and Limit of Quantification (LOQ): To evaluate LOD and LOQ, concentrations from the lower linear range of the calibration curve for Ascorbic Acid and Gallic Acid were analyzed.

Result

In Gallic acid first trace Presence of confirmed values was start Rf -0.02 and End Rf 1.07, start height was 0.1 to 0.97 where 266.4 is Maximum height on. End height 3.4 to 0.4 where 1.07, Observed in 3 peaks. area 756.5 to 10043.6 (41.95 %) on 10 Peaks. Rf value range of 0.96 confirmed the presence of flavonoids in the *Triphla* fruit extracts. While in second trace of Gallic acid values was found where, start Rf -0.02 and end Rf value was 0.90. start height 0.1 to 67.7 on 3 peaks. While stint maximum height 502.4 (60.58 %) and end of height was 67.6 on 2 peaks, and area is 25326.2 (68.89 %) The results from HPTLC finger print for Hydro alcoholic extract of *Triphala fruit* are given in graph no 1.



Graph 1: HPTLC Chromatogram of Gallic Asid scanned at 270nm (All track)

In *Triphla* fruit extract first trace Presence of confirmed values was start Rf -0.05 and End Rf 0.93, start height was 3.4 to 327.5 where 759.7 is Maximum height on. End height 1.8, Observed in 7 peaks. area 91778.7 (29.67 %) on 2 Peaks. Rf value range of 0.90 confirmed the presence of flavonoids in the *Triphla fruit* extracts.

Journal of Chemical Health Risks

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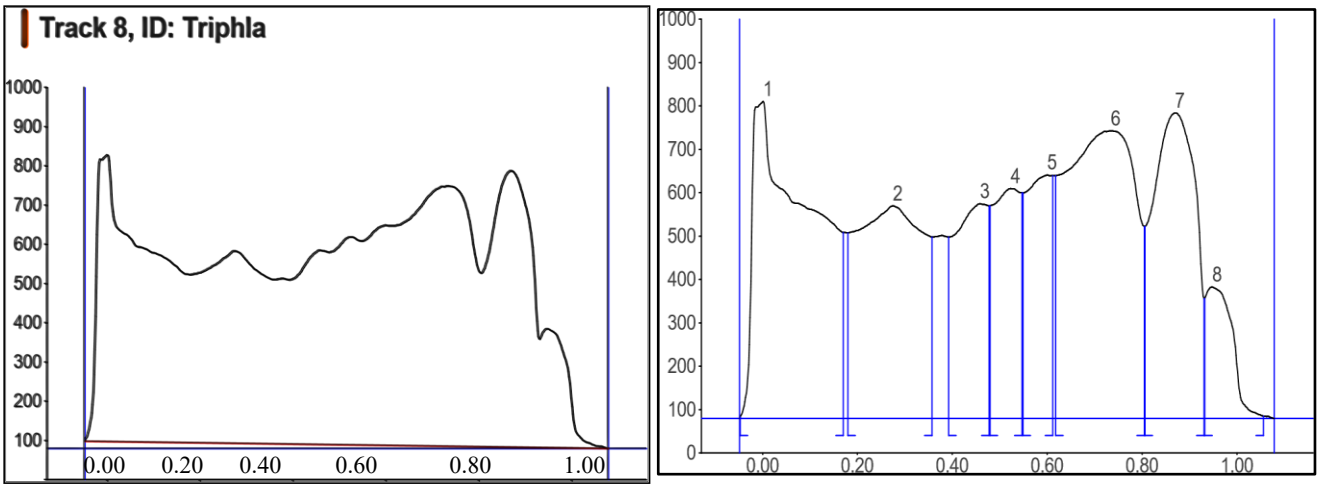
While in second trace of this fruit extract values was found where, start Rf -0.05 and end Rf value was 0.93. Where 731.1 is maximum height on 1 peaks. While stint maximum area is 70614.04 (22.69 %) The results from HPTLC finger print for Hydro alcoholic extract of *Triphala fruit* are given in graph number 2.



Graph 2: HPTLC chromatogram of Triphla fruit extract scanned at 270 nm (All track)

Discussion

The findings are consistent with previous literature, which reports that Triphala is a rich source of vitamin, phenolic compounds. The presence of gallic acid in Triphala enhances its antioxidant potential, explaining its role in reducing oxidative stress, improving digestion, and supporting immune function. The observed variations between individual components and the formulation highlight the synergistic effects of polyherbal combinations in Ayurveda. Standardization using HPTLC ensures reproducibility, authenticity, and quality assurance of herbal formulations.

Our data aligns with earlier reports (Baliga *et al*., 2012; Sethi, 2008) and recent advancements in phytochemical evaluation. Furthermore, the references provided from researchers and colleagues emphasize the growing importance of analytical validation, sustainable approaches, and phytopharmacological studies across multiple domains.

Conclusion

This study successfully standardized Triphala components for gallic acid using HPTLC. where Triphala exhibited higher gallic acid concentration, confirming synergistic enhancement. The developed method is simple, reproducible, and suitable for routine quality control. These results reinforce the therapeutic claims of Triphala as an antioxidant-rich formulation and provide a scientific basis for its inclusion in modern integrative medicine. Future research may expand into pharmacokinetic and in vivo studies to further validate its efficacy.

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