

HPTLC - Based Quality Assessment and Standardization of Baljeevan Syrup

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Background: Baljeevan Syrup is a classical polyherbal Ayurvedic formulation containing Piper longum, Asparagus racemosus, Rosa centifolia, Emblica officinalis, Withania somnifera, and Acorus calamus known for their immunomodulatory, adaptogenic, and nutritive properties. With the increasing global acceptance of herbal medicines, quality control and standardization of such formulations have become essential.

Aim: To develop and validate a High-Performance Thin-Layer Chromatography (HPTLC) method for the phytochemical standardization of Baljeevan Syrup.

Materials and Methods: An optimized HPTLC protocol was established for the simultaneous qualitative and quantitative estimation of six phytoconstituents, mainly piperine, gallic acid, quercetin, kaempferol, rutin, and ellagic acid. Chromatographic fingerprints, retention factor (R_f) values, and densitometric evaluation were used for analysis.

Results: The developed method demonstrated high specificity, resolution, and reproducibility. Well-resolved chromatographic bands with consistent R_f values confirmed the presence of marker compounds. The validated method enabled accurate estimation of all six markers, ensuring reliability for routine analysis.

Conclusion: The study establishes a reproducible and validated HPTLC protocol for the quality control of Baljeevan Syrup. This method supports batch-to-batch consistency, scientific validation, and regulatory compliance, thereby strengthening the evidence base for Ayurvedic formulations.

Keywords: HPTLC, Baljeevan Syrup, polyherbal formulation, Ayurvedic medicine, Standardization

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Introduction

Ayurveda, the ancient medical system is founded on the principle of homeogenesis of the body, mind, and soul. Most of the formulations in *Ayurveda* are herbal or herbomineral. The *Ayurvedic* pharmacopeia includes diverse dosage forms such as decoctions, powders, oils & syrups. Post covid awareness of *Ayurveda* has increased. The global demand of *Ayurvedic* medicines has increased.[1] However, a major challenge in *Ayurvedic* medicine is the lack of uniformity and reproducibility in polyherbal formulations due to variations in raw material sourcing, processing techniques, and storage conditions.[2] These inconsistencies can compromise on quality, safety, and efficacy, thus robust standardization is essential for batch-to-batch consistency.

Baljeevan syrup is a polyherbal formulation manufactured by *Ayurveda Rasashala* Foundation. The major constituents of *Baljeevan* are *Piper longum*, *Asparagus racemosus*, *Rosa centifolia*, *Embllica officinalis*, *Withania somnifera*, and *Acorus calamus*. Almost all these herbs exhibit immunomodulatory, adaptogenic, and nutritive properties. With the growing demand of herbal medicines ensuring safety and efficacy is crucial for standardization may lead to undesired effects.[3] Historically, *Ayurvedic* formulations followed strict protocols for collection, processing, and storage of medicinal plants, but modern large-scale production faces challenges such as adulteration, contamination, & inconsistent composition.[4]

The World Health Organization (WHO) stresses importance of qualitative & quantitative characterization of herbal products, recommending biomarker quantification or use of specific marker compounds for analytical purposes.[5] Chromatographic fingerprinting combined with quantification of phytoconstituents provides a reliable method to authenticate and standardize formulations.[6] Pharmacopoeial monographs strengthen credibility by offering botanical descriptions, analytical profiles, therapeutic uses, and quality standards.[7]

Among available analytical tools, High-Performance Thin Layer Chromatography (HPTLC) has emerged as a powerful, rapid, and cost-effective method for evaluating complex herbal mixtures.[8] It enables simultaneous qualitative and quantitative analysis of multiple samples with minimal preparation.

Technological advancements such as automated sample application, controlled chromatographic development, and densitometric scanning have enhanced HPTLC's precision, reproducibility, and resolution.

The technique also allows fingerprint profiling for identity verification and quantification of marker compounds, making it invaluable for detecting adulteration, ensuring consistency, and validating label claims.

HPTLC plays a crucial role in the standardization and quality control of herbal formulations by enabling authentication, fingerprint profiling & quantification of key phytochemicals.

It offers flexibility in detection, including ultraviolet (UV)[9], fluorescence[10], and densitometry[11], and can be further enhanced by coupling with advanced methods like mass spectrometry (MS) and high-performance liquid chromatography (HPLC). [12]

Given global demand for safe & effective herbal therapeutics, the present study aims to develop and validate HPTLC method for simultaneous estimation of gallic acid, piperine, quercetin, kaempferol, rutin, and ellagic acid in *Baljeevan* syrup.

By integrating traditional *Ayurvedic* wisdom with modern analytical science, this research seeks to create a reproducible quality control protocol, ensuring the safety, efficacy, and credibility of the formulation for wider acceptance in domestic and international markets.

Aim and Objectives

Aim

To standardize and evaluate the quality of a formulated herbal syrup through High-Performance Thin Layer Chromatography (HPTLC) profiling and quantification of bioactive constituents.

Objectives

1. To develop and validate an HPTLC method for fingerprint profiling of the herbal syrup.
2. To identify and quantify key phytochemical markers in the formulation using HPTLC.
3. To contribute towards standardization guidelines for ensuring safety, efficacy, and quality of herbal syrup preparations.

Materials and Methods

Chemicals and Reagents

The phytoconstituents standards, namely piperine, gallic acid, and ellagic acid, quercetin, kaempferol, and rutin, were procured from Yucca Enterprises, Mumbai. All the reagents and chemicals were of analytical grade and procured from Merck India Pvt, Ltd., Mumbai, India.

Apparatus

Linomat V Automatic Sample Spotter (CAMAG, Switzerland), 100 mL syringe (Hamilton, Switzerland), glass twin trough chamber (20 cm × 10 cm × 4 cm) (CAMAG), TLC Scanner 3 connected to Win Cats software (CAMAG), and 60 F254 (Merck India Pvt, Ltd., Mumbai, India) with a 0.2 mm thickness pre-coated with silica gel were used in this investigation. TLC plates were cleaned with methanol and activated for 10 minutes at 110°C in a dry heat oven before being applied.

Simultaneous estimation of Phytoconstituents

Two separate methods were used for simultaneous estimation of Gallic Acid, Piperine, Quercetin, Kaempferol and Rutin, ellagic acid from *Baljeevan* Syrup.

Sample Preparation

A total of 100 mg of *Baljeevan* Syrup was placed into a 10 mL volumetric flask. Five milliliters of methanol were introduced, and the solution was sonicated for 10 minutes. After this, the total volume was adjusted to 10 mL, and the mixture was filtered using a 0.22 µm syringe filter. The resulting concentration was 10 mg/mL.

Preparation of Standard Solutions

The standard solutions were prepared by incorporating 10 mg each of Gallic Acid, Piperine, Quercetin, Kaempferol, Rutin, and Ellagic Acid in 10 mL of methanol. Subsequently, known concentrations of 0.1 mg/mL for all standards were attained by diluting 1 mL of the corresponding stock solutions with 10 mL of methanol.

Procedure

Instrumentation and Method of Analysis:

The High-Performance Thin Layer Chromatography (HPTLC) analysis of the *Baljeevan* syrup was conducted on precoated silica gel plates 60F254 measuring 10 × 10 cm.

Before application, TLC plates were washed with methanol and heated at 110°C in a dry heat oven for 10 minutes. The samples (10 µL bands of 6 mm each) were applied using CAMAG Linomat V sample applicator fitted with a Hamilton micro syringe (100 µL). Chromatographic development was performed in CAMAG Automated Development Chamber (ADC 2) under controlled conditions. After development, the plates were dried in a hot air oven to ensure even drying. The plates were visualized with a CAMAG TLC Visualizer 2. Densitometric scanning was carried out using a CAMAG TLC Scanner 3 equipped with a UV detector. Data acquisition, peak integration, and chromatographic analysis were done using Vision CATS software (version 3.2 SP2, CAMAG, Switzerland).

TLC Development and Scanning of Gallic Acid, Piperine, Quercetin, and Kaempferol:

Thin-layer chromatographic (TLC) development and densitometric analysis were carried out to identify and quantify the key phytoconstituents, mainly Gallic acid, Piperine, Quercetin, Kaempferol, Rutin, & Quercetin, in polyherbal syrup. The simultaneous estimation of Gallic acid, Piperine, Quercetin, Kaempferol was performed using mobile phase Toluene : Ethylacetate : Formic acid (6.0:3.2:0.8v/v/v). Moreover, simultaneous estimation of Rutin and ellagic acid was achieved using a mobile phase, Toluene: Ethyl acetate : Methanol : Water : Formic acid (6.0:12:1.5:1.2v/v/v/v). The chamber was saturated for 20 minutes. The solvent front was allowed to migrate up to 80 mm (~80% of the plate length). The developed plates were scanned at an isosbestic wavelength of 299 nm and 306 nm.

Observations and Results

A quantitative simultaneous estimation of biologically active phytoconstituents, namely gallic acid, piperine, quercetin, kaempferol, rutin, and ellagic acid was successfully carried out in the polyherbal formulation *Baljeevan* Syrup using High-Performance Thin Layer Chromatography (HPTLC).

The solvent system Toluene: Ethyl acetate: Formic acid (6.0:3.2:0.8 v/v/v) provided effective resolution and allowed for the simultaneous estimation of gallic acid, piperine, quercetin, and kaempferol. The identification of these compounds was verified through the observation of clear, well-defined spots on the chromatogram as shown in Fig.1 and Fig.2.

However, solvent mixture of Toluene: Ethyl acetate: Methanol: Water: Formic acid (6.0:12:1.5:1.2 v/v/v/v) achieved effective separation of rutin and ellagic acid. Their presence was validated by the emergence of significant, dark brown spots that were dense and well-defined. The quantification of biomarkers in Baljeevan syrup was determined by comparing peak area of standard compounds to that of sample compound by referring the formula

$$(ASMP \times W_{STD} \times f \times \text{Dilution of Smp} \times \text{Application vol. of sample} \times P \times D \times 10) / (ASTD \times \text{Dilution of Std} \times WSMP \times \text{Application of vol. of standard} \times 100)$$

Where ASMP is average area of sample; ASTD is average area of standard; WSTD is weight of standard, mg; WSMP is weight of sample, g; Dilution of Smp is dilution of sample, mL; Dilution of Std is dilution of standard, mL; P is percent purity of standard; f is conversion factor; D is density of syrup, mg/mL.[13]

The chromatographic peaks obtained were symmetrical, sharp, and showed no evidence of tailing, indicating good separation and peak quality. The developed HPTLC method was successfully applied for the simultaneous detection and quantification of key phytomarkers present in Baljeevan Syrup. The separation of all components was distinct, with well-defined peaks corresponding to phytoconstituents, confirming the method's suitability for routine quality control and phytochemical standardization of the formulation. HPTLC analysis of *Baljeevan* syrup confirmed presence of six major phytoconstituents, as shown in Table 1.

Table 1: Rf Values and Percentage Composition of Phytoconstituents Identified in Baljeevan Syrup Formulation by HPTLC Analysis

SN	Compound	Mobile Phase	Rf Value	% Composition
1.	Gallic Acid	Toluene: Ethyl acetate: Formic acid (6.0:3.2.:0.8v/v/v)	0.172	0.607
2.	Kaempferol	Toluene: Ethyl acetate: Formic acid (6.0:3.2.:0.8v/v/v)	0.456	0.130
3.	Piperine	Toluene: Ethyl acetate: Formic acid (6.0:3.2.:0.8v/v/v)	0.522	0.041
4.	Quercetin	Toluene: Ethyl acetate: Formic acid (6.0:3.2.:0.8v/v/v)	0.333	0.010
5.	Rutin	Toluene: Ethyl acetate: Methanol: Water: Formic acid (6.0:12:1.5:1.2 v/v/v/v)	0.063	0.792
6.	Ellagic Acid	Toluene: Ethyl acetate: Methanol: Water: Formic acid (6.0:12:1.5:1.2 v/v/v/v)	0.464	2.344

Discussion

Standardization of *Ayurvedic* Products is essential to ensure consistency, quality, efficacy, and safety of formulations. *Ayurvedic* preparations often contain complex mixtures of phytoconstituents whose therapeutic efficacy depends on their quantity & stability. Standardization validates authenticity, prevents adulteration & ensures reproducible pharmacological activity, thereby meeting both regulatory and consumer safety requirements.[14]

High-Performance Thin Layer Chromatography (HPTLC) plays a pivotal role in the standardization of herbal products. It offers accuracy, reproducibility, and cost-effectiveness, enabling the creation of phytochemical fingerprints, quantification of bioactive compounds, and detection of adulterants. [15] Recommended by WHO, HPTLC ensures batch-to-batch consistency and is indispensable for linking phytoconstituent profiles with pharmacological activity.[16]

The method involves applying both sample and standard solutions onto precoated silica gel plates, developing them in optimized mobile phases, and scanning the separated spots densitometrically. Peak areas are compared against calibration curves prepared from known standards, enabling precise estimation of marker compounds. In *Baljeevan* Syrup, we developed a method and confirmed the presence and concentration of gallic acid, piperine, quercetin, kaempferol, rutin, and ellagic acid, validating its phytochemical profile. The structures of the listed phytoconstituents are pasted in Fig.3.

In the current investigation, the detection of antioxidant components including piperine[17-18], gallic acid[19-20], ellagic acid[21-22], quercetin[23-24], kaempferol[25,26], and rutin[27] in Baljeevan syrup were validated. These compounds can neutralize reactive oxygen species (ROS), boost the activity of endogenous antioxidant enzymes (such as SOD and GPx), and safeguard immune cells from oxidative harm.[28-29]

All six compounds exhibit anti-inflammatory effects by inhibiting the NF-κB pathway, decreasing COX-2/iNOS levels, and reducing pro-inflammatory cytokines (TNF-α, IL-6, IL-1β), thereby lowering systemic inflammation.[30-33] Furthermore, these phytochemicals play a crucial role in modulating the immune system through various mechanisms.

Piperine enhances Th1 cytokines and boosts macrophage activity while diminishing pro-inflammatory mediators such as TNF- α and IL-1 β . [34-35] Gallic acid promotes phagocytosis, increases T-cell proliferation, and facilitates ROS production in macrophages.[36-37] Ellagic acid influences immune balance by inhibiting Th1/Th17 cytokines (IFN- γ , IL-17) & elevating IL-4 & GATA3, while also suppressing NF- κ B signaling.[38-39] Quercetin reduces the activity of the NF- κ B and MAPK pathways, decreases levels of IL-6 and TNF- α , and encourages a Th1-skewed immune response.[40-41] Rutin reinforces innate immunity through the modulation of nitric oxide & activation of macro-phages.[42] Kaempferol mitigates the responses of IL-6 and IL-33, balances Treg/Th17 activity, and inhibits the NF- κ B and JNK pathways.[43] These findings validate the phytochemical profile of Baljeevan syrup & reinforce its consistency in bio-active content, ensuring both quality & therapeutic reliability.

Conclusion

Thus, a simple, accurate, and precise HPTLC method was developed to estimate phytoconstituents of *Baljeevan* syrup. The approach enables the rapid simultaneous estimation of six phytochemicals, bridging the research gap in this area. The current standardization proposes a targeted and speedy tool for herbal research, enabling the establishment of quality standards for the identity, reproducibility, and transparency of biomarkers in the well-known *Ayurvedic* formulation *Baljeevan* syrup.

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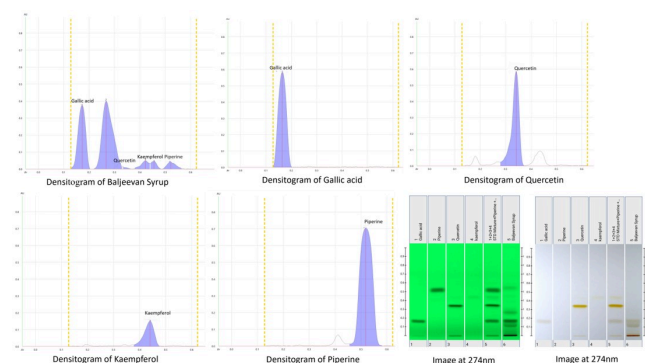


Figure 1: Densitogram of Baljeevan syrup; Standard gallic acid, kaempferol, piperine and quercetin; densitogram of standard mixture

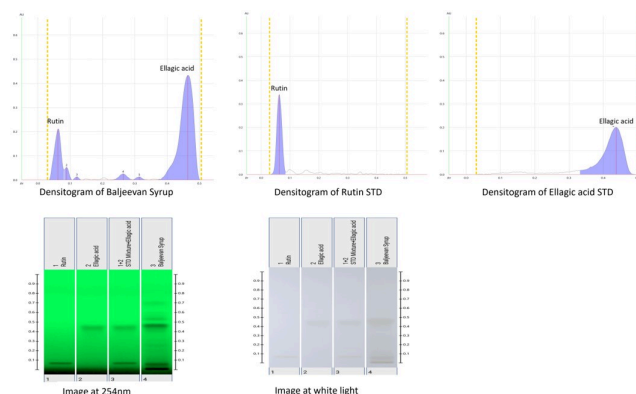


Figure 2: Densitogram of Baljeevan syrup sample; Standard ellagic acid, rutin; Standard mixture; Image at 254nm and white light.

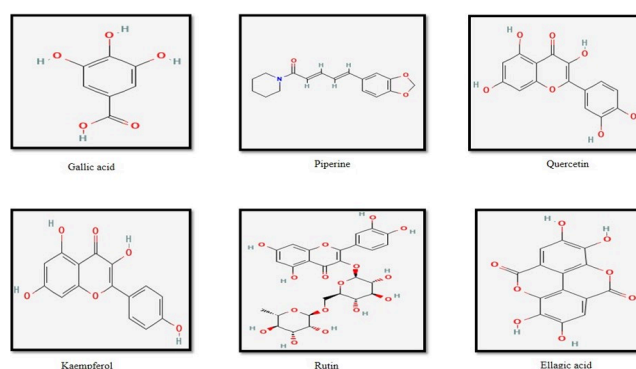


Figure 3: Structure of Gallic acid, Piperine, Quercetin, Kaempferol, Rutin and Ellagic acid. The chemical structures of phytochemicals were retrieved from the PubChem database (<http://www.ncbi.nlm.nih.gov/pccompound>)

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