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ORIGINAL ARTICLE

Analytical Standardization and In-Vitro Antimicrobial Assessment of Easy V Gel: A Polyherbal Formulation

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ABSTRACT
Herbal Gel Easy V Gel is a polyherbal formulation traditionally used for treating various dermatological conditions. Standardization through chromatographic profiling and biological testing is critical for evaluating its therapeutic potential. To establish a reproducible HPTLC fingerprint of Herbal Gel Easy V Gel and assess its in-vitro antibacterial and antifungal activity against Pseudomonas aeruginosa and Candida albicans. HPTLC analysis was performed using silica gel 60 F254 plates with Ethyl acetate : Methanol : Water (8:2:1 v/v/v) as the mobile phase. Detection was carried out at 254 nm and 366 nm. The antibacterial activity was tested using the Cylinder Plate Method (Indian Pharmacopoeia 2014) against P. aeruginosa and C. albicans. Distinct phytochemical bands were observed at both wavelengths. A dominant peak at ~0.92 Rf was detected at 254 nm. The formulation showed a 7 mm zone of inhibition against P. aeruginosa and an 11 mm zone against C. albicans. The study successfully establishes a reproducible chromatographic fingerprint and demonstrates the antibacterial and antifungal potential of the formulation, encouraging further investigation into its therapeutic efficacy.
Keywords: *Herbal Gel Easy V Gel, HPTLC fingerprinting, Polyherbal formulation, Antibacterial activity, Pseudomonas aeruginosa, Candida albicans*

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INTRODUCTION

Polyherbal formulations are widely described in Ayurveda for the management of dermatological and infectious conditions [1]. Herbal gels prepared from classical dravyas are increasingly being evaluated for their antimicrobial and antifungal potential in modern scientific frameworks. Standardization of such formulations is essential to ensure reproducibility, safety, and therapeutic reliability [2]. Easy V Gel is a polyherbal topical formulation prepared using classical medicinal plants traditionally indicated for inflammatory, infectious, and dermatological disorders. The formulation contains Karanja, Lodhra, Lajjalu, Arishtaka, Gambhari, and Kumari. These herbs are known in Ayurvedic literature for their antimicrobial, wound-healing, anti-inflammatory, and antifungal properties [3][4][5][6][7][8]. Chromatographic fingerprinting using High Performance Thin Layer Chromatography provides a reliable analytical approach for evaluating phytochemical distribution and ensuring batch-to-batch consistency in polyherbal preparations [9]. Simultaneously, in-vitro antimicrobial testing offers preliminary biological validation of therapeutic claims. The present study was therefore undertaken to establish the HPTLC fingerprint profile of Easy V Gel and to assess its antibacterial and antifungal activity against *Pseudomonas aeruginosa* and *Candida albicans*.

MATERIAL AND METHODS

Raw drugs including Karanja, Lodhra, Lajjalu, Arishtaka, Gambhari, and Kumari were procured from standard pharmaceutical suppliers. Botanical identification and authentication of the raw drugs were carried out in the Department of Dravyaguna, Parul Institute of Ayurved and Research, Vadodara. The formulation was prepared in the Central Research Laboratory of Parul Institute of Ayurved and Research. The antimicrobial and antifungal studies were conducted in the Research and Development Cell of Parul University. Analytical studies including HPTLC were performed in accredited laboratories.

Batch details of the prepared formulation are as follows:

Batch Number: September 2025

Manufacturing Date: 18/09/2025

Expiry: 18 months from date of manufacturing

Drug Preparation

Coarse powder of all authenticated raw drugs was prepared. Sixteen parts of water were added to the combined coarse powder. The mixture was boiled over mild fire until one-fourth of the initial volume remained, following classical kwatha preparation principles.

After filtration, 2.8 percent Carbopol and 1 ml of PEG 400 were added to the concentrated decoction. The mixture was kept in a dark room for 24 hours to allow proper hydration and stabilization. The final gel was then prepared with uniform consistency and stored in appropriate containers for further analytical and microbiological evaluation.

Formulation Details

Sample Used: Herbal Gel Easy V Gel

Culture Used:

- *Pseudomonas aeruginosa* ATCC 9027
- *Candida albicans* ATCC 10231

HPTLC Analysis

Chromatography was performed using:

Stationary Phase: Merck Silica Gel 60 F254

Mobile Phase: Ethyl acetate : Methanol : Water (8:2:1 v/v/v)

Chamber Saturation: 20 minutes

Development Distance: 80 mm

Scanner: CAMAG TLC Scanner 4

Wavelengths: 254 nm and 366 nm

Integration Parameters: Savitzky–Golay smoothing

Sample Application Volume: 5 µL, 10 µL, 15 µL, 20 µL

In-Vitro Antibacterial and Antifungal Activity

The antibacterial and antifungal activity was evaluated using:

Organism: *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231

Method: Cylinder Plate Method (Indian Pharmacopoeia 2014, Chapter 2.2.10)

Media:

MHA (for P. aeruginosa)

SDA (for C. albicans)

Incubation:

35°C for 24 hours for bacterial plates

25°C for 48 hours for fungal plates

Sample Preparation: Approximately 2 gm of sample was refluxed with Dimethyl sulfoxide, Methanol, and water to prepare the test sample for activity.

RESULTS

HPTLC Fingerprinting at 254 nm

Multiple distinct phytochemical zones were observed across application volumes. A dominant peak (~0.92 Rf) was consistently observed, contributing approximately 57–62% of the total peak area.

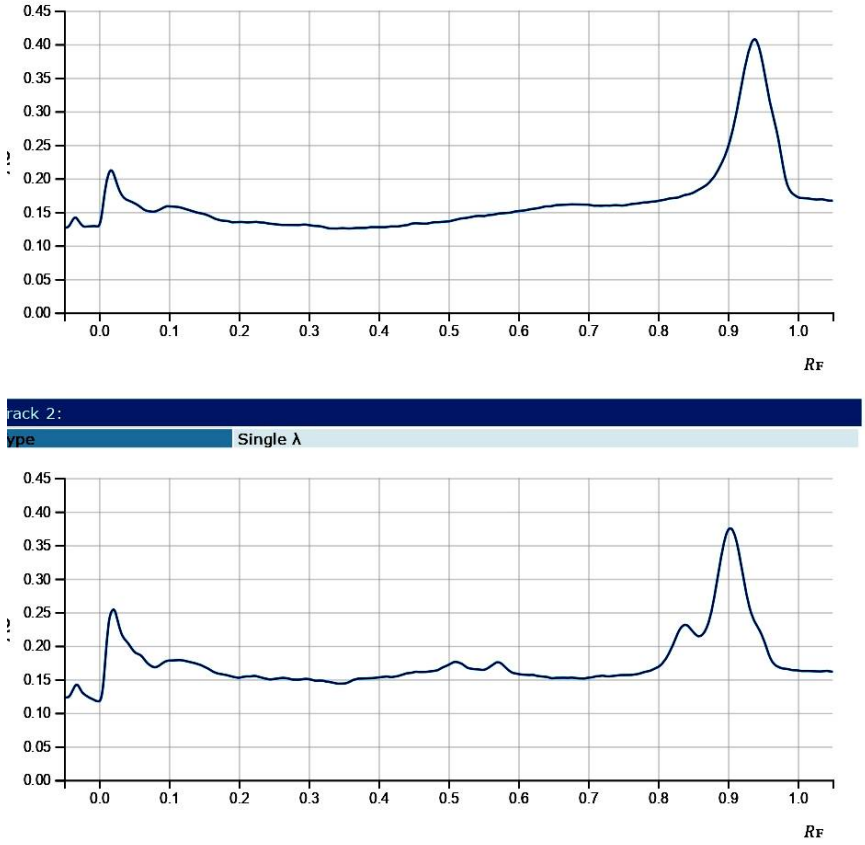

Figure 1. Densitometric chromatogram of Herbal Gel Easy V Gel (5 µL application) scanned at 254 nm.

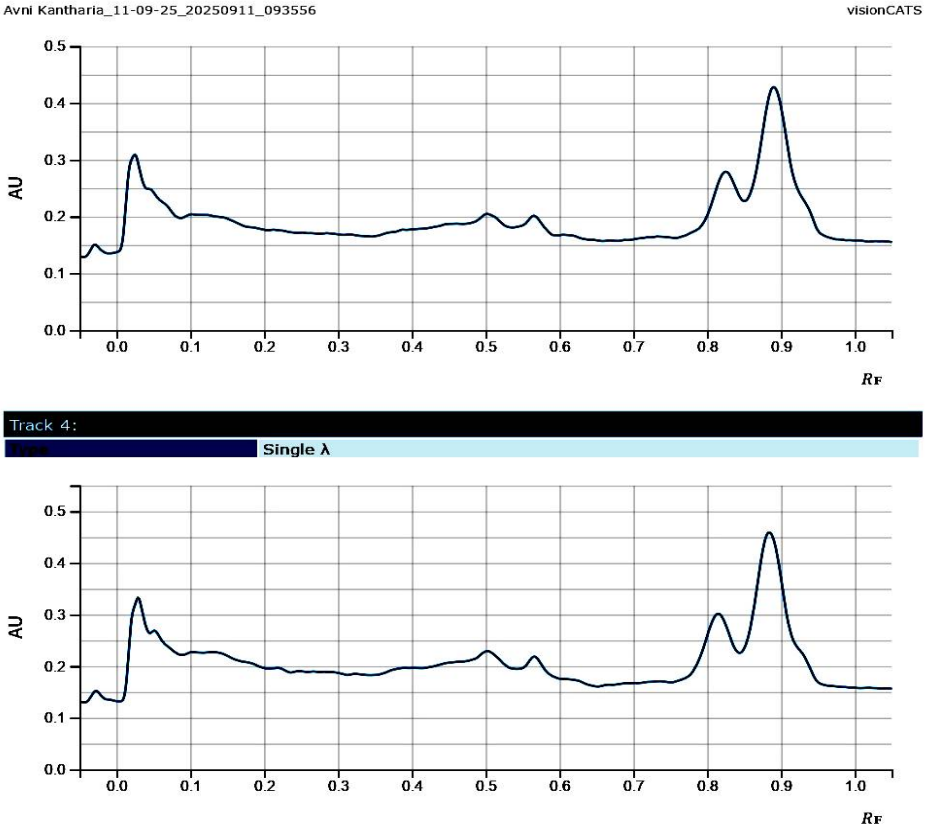

Figure 2. Densitometric chromatograms of Herbal Gel Easy V Gel at lower application volumes (10 µL, 15 µL) scanned at 254 nm demonstrating concentration-dependent peak enhancement.

HPTLC Fingerprinting at 366 nm

At 366 nm, fluorescence detection revealed additional bands, with a dominant band at ~0.75–0.80 Rf contributing to a significant portion of the sample's profile.

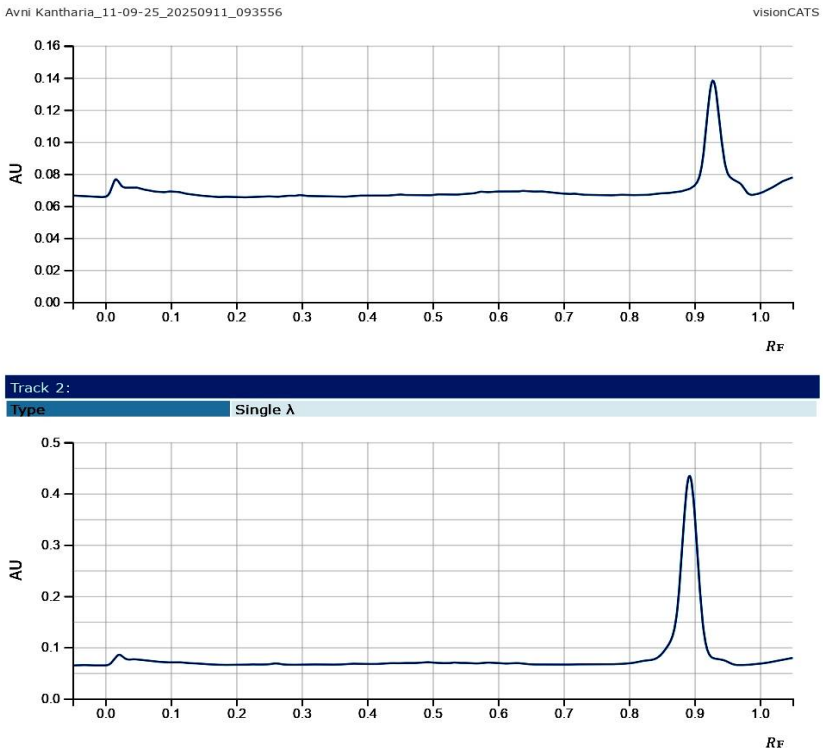

Figure 3. Densitometric chromatogram of Herbal Gel Easy V Gel scanned at 366 nm (fluorescence detection).

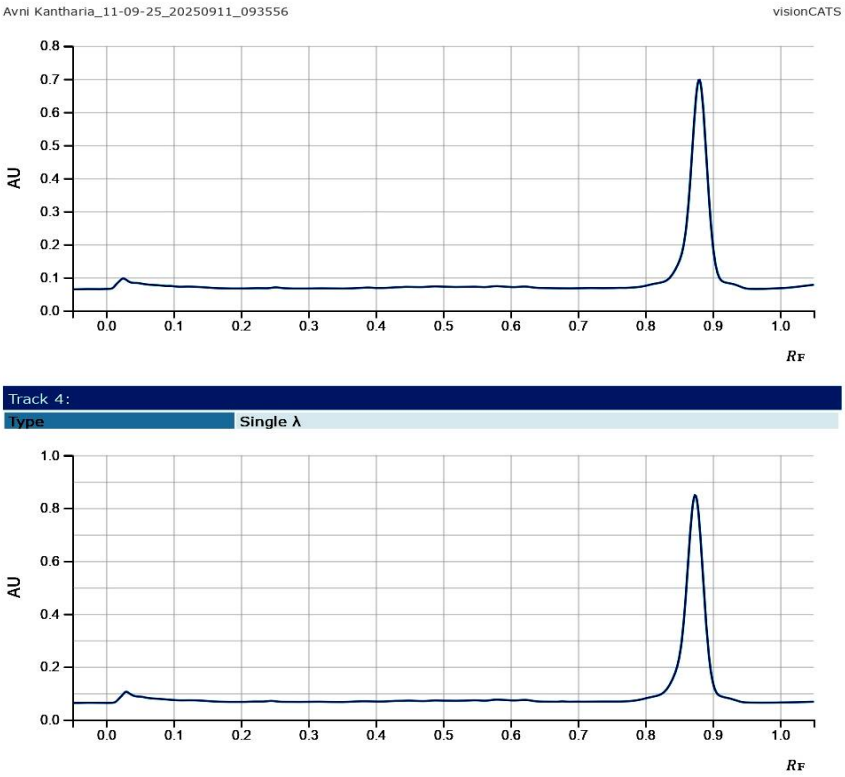

Figure 4. Densitometric chromatogram of Herbal Gel Easy V Gel at 366 nm (15 µL application) showing multiple resolved peaks.

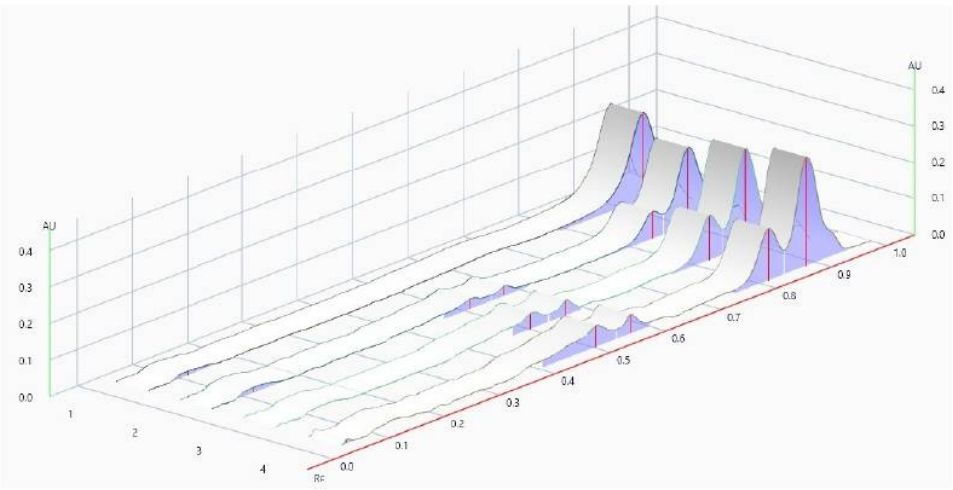

Figure 5. Three-dimensional densitometric overlay of Herbal Gel Easy V Gel at 254 nm

In-Vitro Antibacterial and Antifungal Activity

The formulation demonstrated antibacterial activity against *P. aeruginosa* (zone of inhibition: 8 mm) and antifungal activity against *C. albicans* (zone of inhibition: 11 mm).

Table 1. In-vitro antibacterial and antifungal activity of Herbal Gel Easy V Gel.

Organism	Method	Incubation	Zone of Inhibition
P. aeruginosa	Cylinder Plate (IP 2014)	35°C, 24 h	8 mm

DISCUSSION

The present investigation successfully establishes a reproducible HPTLC fingerprint profile for Herbal Gel Easy V Gel, confirming the presence of multiple phytochemical constituents within the formulation. The chromatographic pattern observed at 254 nm demonstrated a consistent and dominant peak in the high Rf region (~0.88–0.92) across varying application volumes (5–20 µL). The persistence of this peak with increasing concentration, along with proportional enhancement in peak area percentage, indicates the presence of a major phytochemical fraction that may serve as a potential analytical marker for quality control standardization. Such dominant high-Rf peaks typically correspond to relatively less polar constituents, suggesting that the formulation may contain significant amounts of lipophilic or semi-polar bioactive compounds. At 366 nm, fluorescence detection revealed additional resolved bands that were either less prominent or not distinctly visible at 254 nm. This variation in detection patterns between wavelengths highlights the chemical diversity of the formulation. Compounds that fluoresce at 366 nm are often associated with flavonoids, coumarins, phenolic acids, or other conjugated secondary metabolites. The appearance of a prominent band around Rf ~0.75–0.85 under fluorescence suggests the possible presence of such bioactive phytoconstituents. The dual-wavelength profiling therefore strengthens the reliability of the chromatographic fingerprint and supports the complexity expected in polyherbal formulations. The three-dimensional densitometric overlay further confirms the reproducibility and consistency of peak distribution across application volumes. The progressive amplification of peak intensity without significant peak shifting indicates good chromatographic resolution and methodological robustness. This reproducibility is essential for future batch-to-batch comparison and pharmacognostic authentication. The antimicrobial evaluation demonstrated measurable biological activity of the formulation. The observed 8 mm zone of inhibition against *Pseudomonas aeruginosa* suggests mild antibacterial potential. Given that *P. aeruginosa* is known for its intrinsic resistance mechanisms and ability to survive in diverse environments, even moderate inhibition may indicate the presence of active phytoconstituents with bacteriostatic properties. More notably, the formulation exhibited a comparatively larger zone of inhibition (11 mm) against *Candida albicans*, indicating stronger antifungal potential. This differential activity may be attributed to the presence of

phenolic compounds, flavonoids, or tannins, which are widely reported to possess antifungal mechanisms such as membrane disruption, enzyme inhibition, and oxidative stress induction. The correlation between chromatographic dominance in the high-Rf region and observed antimicrobial activity raises the possibility that the major phytochemical fraction may contribute significantly to the biological effect. However, in the absence of compound isolation or spectroscopic characterization, definitive attribution of activity to specific constituents cannot be established. Despite the promising findings, the antimicrobial results remain preliminary. The absence of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination limits quantitative interpretation of potency. Additionally, testing against a single bacterial and fungal strain does not provide comprehensive insight into spectrum of activity. Future studies should incorporate multi-strain screening, standardized antibiotic comparison controls, and quantitative phytochemical estimation to strengthen pharmacological validation. Overall, the study provides an integrated analytical and biological assessment of Herbal Gel Easy V Gel. The reproducible HPTLC fingerprint establishes a foundation for quality standardization, while the preliminary antimicrobial findings support its potential therapeutic relevance. Further phytochemical isolation, spectroscopic identification, and detailed pharmacological evaluation are warranted to substantiate these findings and translate them into clinical relevance.

LIMITATIONS AND FUTURE SCOPE

Although valuable insights into the potential of Easy V Gel have been provided, there are areas that could benefit from further investigation. One limitation of the current study is the absence of identification of specific compounds responsible for the observed effects. While biological activity has been demonstrated, the exact active ingredients have not been pinpointed. Future studies should aim to identify these key compounds, which would enhance the formulation's efficacy and improve its therapeutic applications. In addition, antibacterial testing was conducted using only one strain of Pseudomonas aeruginosa. Expanding the testing to include a wider variety of microbial strains, such as Gram-positive bacteria and multi-drug-resistant organisms, would offer a more comprehensive understanding of the formulation's antimicrobial spectrum. The inclusion of antifungal controls is also recommended to validate the formulation's effectiveness against a broader range of pathogens. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests were not conducted in this study, limiting the understanding of the formulation's potency. These tests should be performed in future research to determine the dose-dependent effectiveness and better define the therapeutic potential. Additionally, quantitative phytochemical estimation would provide valuable data on the concentration of active constituents, offering a clearer picture of how the formulation works at a chemical level. To strengthen the findings, future studies should include replicate-based statistical validation, as well as comparison with standard controls. Conducting clinical trials and multi-strain antimicrobial tests will further substantiate the therapeutic claims of the formulation and aid in its clinical application. Thus, while this study lays a solid foundation, further research is needed to confirm and expand upon these findings. The direction set by this initial work will contribute to the scientific validation of Easy V Gel and ensure its clinical relevance in future healthcare practices.

CONCLUSION

The study successfully established a reproducible HPTLC fingerprint profile for Herbal Gel Easy V Gel and demonstrated measurable antibacterial and anti-fungal activity. These findings provide preliminary validation of the formulation, encouraging further detailed microbiological and phytochemical research to substantiate its therapeutic claims.

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