**Advances in Bioresearch**
Adv. Biores., Vol 17 (2) February 2026: 119-127
©2026 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.17.2.119127

Advances in Bioresearch

## ORIGINAL ARTICLE

# Phytochemical Characterization and *In-Vitro* Antibacterial Efficacy of Ervarubeejadi Yoga Elixir

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**ABSTRACT**
*Ervarubeejadi Yoga Elixir is a classical Ayurvedic polyherbal formulation traditionally indicated in urinary disorders and inflammatory conditions. Scientific validation through chromatographic profiling and biological evaluation is essential for quality standardization and pharmacological substantiation. To establish a reproducible HPTLC fingerprint profile of Ervarubeejadi Yoga Elixir and evaluate its in-vitro antibacterial activity against Escherichia coli. HPTLC analysis was performed using silica gel 60 F254 plates with Ethyl acetate : Methanol: Water (7:2:1 v/v/v) as the mobile phase. Detection was carried out at 254 nm and 366 nm using a CAMAG TLC Scanner 4. Antibacterial activity was assessed by the Cylinder Plate Method as per Indian Pharmacopoeia (2014) against E. coli ATCC 8739 using Mueller Hinton Agar. Distinct phytochemical bands were observed at both wavelengths. A dominant peak in the high-Rf region (~0.88–0.92) contributed approximately 57–62% of the total peak area at 254 nm. Fluorescence detection at 366 nm revealed additional characteristic bands. The formulation exhibited a 7 mm zone of inhibition against E. coli, indicating mild antibacterial activity under the tested conditions. The study establishes a reproducible chromatographic fingerprint and demonstrates measurable antibacterial activity. These findings provide preliminary analytical and biological validation and warrant further quantitative and multi-strain investigations.*
***Keywords:*** *Ervarubeejadi Yoga Elixir; HPTLC fingerprinting; Polyherbal formulation; Antibacterial activity; Escherichia coli; Cylinder Plate Method*

Received 30.11.2025 Revised 23.01.2026 Accepted 09.02.2026

**How to cite this article:**
Rachana M, Manjusha K and Prasanna M. Phytochemical Characterization and In-Vitro Antibacterial Efficacy of Ervarubeejadi Yoga Elixir Adv. Biores. Vol 17 [2] February 2026. 119-127

## INTRODUCTION

Ervarubeejadi Yoga is a classical Ayurvedic formulation traditionally indicated in urinary disorders and inflammatory conditions [1]. In Ayurvedic therapeutics, Mutrala (diuretic), Basti Shodhana (urinary tract cleansing), and Pittahara (pitta-pacifying) formulations are frequently prescribed for managing dysuria, urinary tract infections, and inflammatory disorders of the urinary system [2]. The formulation consists of Ervarubeeja (*Cucumis sativus*), Yasthimadhu (*Glycyrrhiza glabra*), and Daruharidra (*Berberis aristata*), each possessing distinct pharmacological properties described in classical Ayurvedic texts. Ervarubeeja is described as having Madhura rasa, Laghu and Ruksha guna, Sheeta veerya, and Madhura vipaka. It is primarily Mutrala and Pittahara in action and is traditionally indicated in urinary burning and inflammatory conditions [3]. Yasthimadhu possesses Madhura rasa, Guru and Snigdha guna, Sheeta veerya, and Madhura vipaka. It is Tridoshahara, Rasayana, and Vrishya, and is widely recognized for its anti-inflammatory, demulcent, and antimicrobial properties. Chemically, it contains glycyrrhizin, asparagine, phosphoric acid, malic acid, flavonoids, and saponins [4]. Daruharidra is characterized by Tikta and Kashaya rasa, Laghu and Ruksha guna, Ushna veerya, and Katu vipaka. It is Shophahara and indicated in inflammatory and infective disorders. It contains alkaloids such as berberine along with

 

flavonoids and phenolic acids, which are known for antimicrobial and anti-inflammatory activity [5]. Modern pharmacological studies report the presence of flavonoids, tannins, saponins, alkaloids, phenolic acids, and glycosides in these herbs, supporting their antimicrobial potential. Urinary tract infections are commonly associated with Escherichia coli, making it an appropriate organism for preliminary in-vitro antibacterial screening. Standardization of such polyherbal formulations requires reproducible chromatographic profiling. High Performance Thin Layer Chromatography provides a cost-effective and reliable method for establishing qualitative phytochemical fingerprints, ensuring batch-to-batch consistency and quality control.
The present study was therefore undertaken to establish a reproducible HPTLC fingerprint of Ervarubeejadi Yoga Elixir and to evaluate its in-vitro antibacterial activity against Escherichia coli.

## MATERIAL AND METHODS

Raw drugs including Ervarubeeja (*Cucumis sativus*), Yasthimadhu (*Glycyrrhiza glabra*), and Daruharidra (*Berberis aristata*) required for the study were procured from standard pharmaceutical suppliers. Botanical identification and authentication were carried out in the Pharmacognosy Laboratory and Department of Dravyaguna, Parul Institute of Ayurveda and Research, Vadodara. The formulation was prepared under controlled laboratory conditions and subjected to analytical evaluation in accredited laboratories. The in-vitro antibacterial study was conducted at Vasu Research Centre, an AYUSH-approved Ayurvedic Testing Laboratory (License No. GATL/08), Vadodara, Gujarat.
AR No.: VARS/RS/25/08/042
Report Date: 22/08/2025

### Pharmacological Properties of Ingredients

**Ervarubeeja (*Cucumis sativus*)**
Rasa: Madhura
Guna: Laghu, Ruksha
Veerya: Sheeta
Vipaka: Madhura
Karma: Mutrala, Basti Shodhana, Pittahara
Chemical constituents include flavonoids, tannins, saponins, and steroids.

**Yasthimadhu (*Glycyrrhiza glabra*)**
Rasa: Madhura
Guna: Guru, Snigdha
Veerya: Sheeta
Vipaka: Madhura
Karma: Tridoshahara, Rasayana, Vrishya
Chemical constituents include glycyrrhizin, asparagine, phosphoric acid, malic acid, flavonoids, and saponins.

**Daruharidra (*Berberis aristata*)**
Rasa: Tikta, Kashaya
Guna: Laghu, Ruksha
Veerya: Ushna
Vipaka: Katu
Karma: Shophahara
Chemical constituents include alkaloids (berberine), flavonoids, and phenolic acids.

### Drug Preparation – Standard Operating Procedure

Hydro-ethanolic extracts of individual drugs were prepared in the following ratios:
- Ervarubeeja extract in the ratio 1:11
- Yasthimadhu extract in the ratio 1:8
- Daruharidra extract in the ratio 1:10

The extracts were prepared using hydro-ethanolic solvent systems and filtered appropriately. The individual extracts were then mixed in equal proportions in a conical flask.
One part of Tandulodaka was added to the mixture. Fizer’s reagent was added dropwise until the mixture turned pale milky white, indicating suspension formation.
The prepared suspension was further processed by adding:
- Mint soda – 0.2 percent
- Citric acid – 10 drops
- Acin – 0.2 percent

The mixture was homogenized thoroughly to ensure uniform dispersion and kept in a dark room for three days for stabilization and maturation of the formulation. The final elixir was stored in appropriate containers and used for analytical and antibacterial evaluation.

**HPTLC Analysis**

Chromatography was performed using:

- Stationary phase: Merck Silica Gel 60 F254 (100 × 100 mm)
- Application: CAMAG Linomat 5
- Mobile phase: Ethyl acetate : Methanol : Water (7:2:1 v/v/v)
- Chamber saturation: 20 minutes
- Development distance: 80 mm
- Scanner: CAMAG TLC Scanner 4
- Detection: 254 nm & 366 nm
- Integration: Savitzky–Golay smoothing

**In-Vitro Antibacterial Activity**

The antibacterial study was conducted at an AYUSH-approved laboratory:

Vasu Research Centre
(A Division of Vasu Healthcare Pvt. Ltd.)
Vadodara, Gujarat
AR No.: VARS/RS/25/08/042
Report Date: 22/08/2025

- Test organism: ***Escherichia coli*** ATCC 8739
- Method: Cylinder Plate Method (Indian Pharmacopoeia 2014, Chapter 2.2.10)
- Media: Mueller Hinton Agar (Hi-Media)
- Incubation: 35°C for 24 hours
- Sample: Directly used formulation
- Organism: *E. coli* ATCC 8739

**RESULTS**

**HPTLC Fingerprinting at 254 nm**

Multiple distinct phytochemical zones were observed across application volumes. A dominant peak was consistently observed in the high-Rf region (~0.88–0.92), contributing approximately 57–62% of total peak area, suggesting the presence of a major phytochemical constituent.

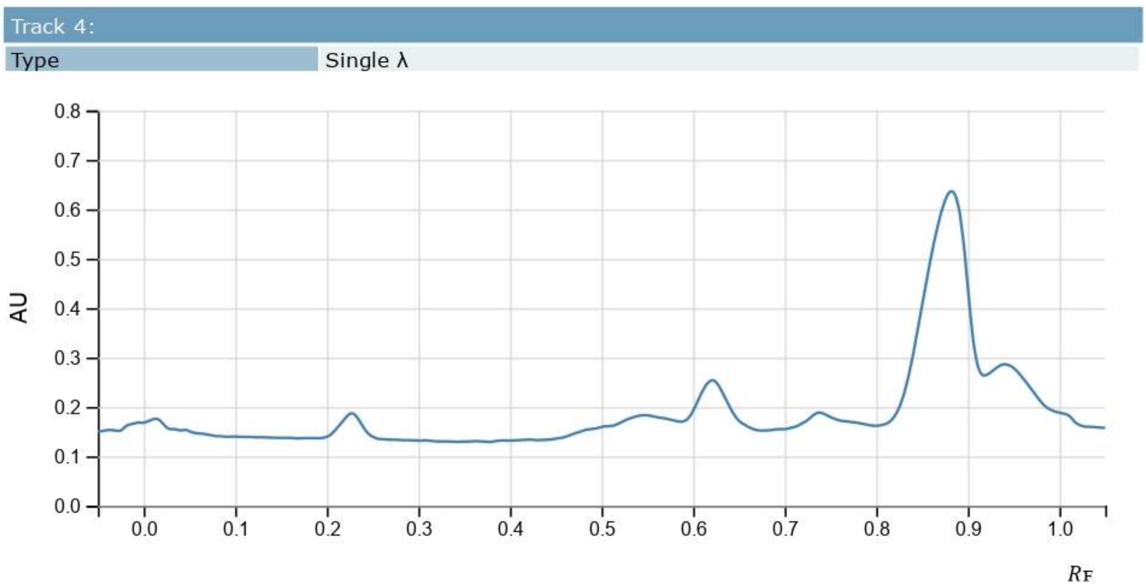


**Figure 1.** Densitometric chromatogram of Ervarubeejadi Yoga Elixir (20 μL application) scanned at 254 nm.

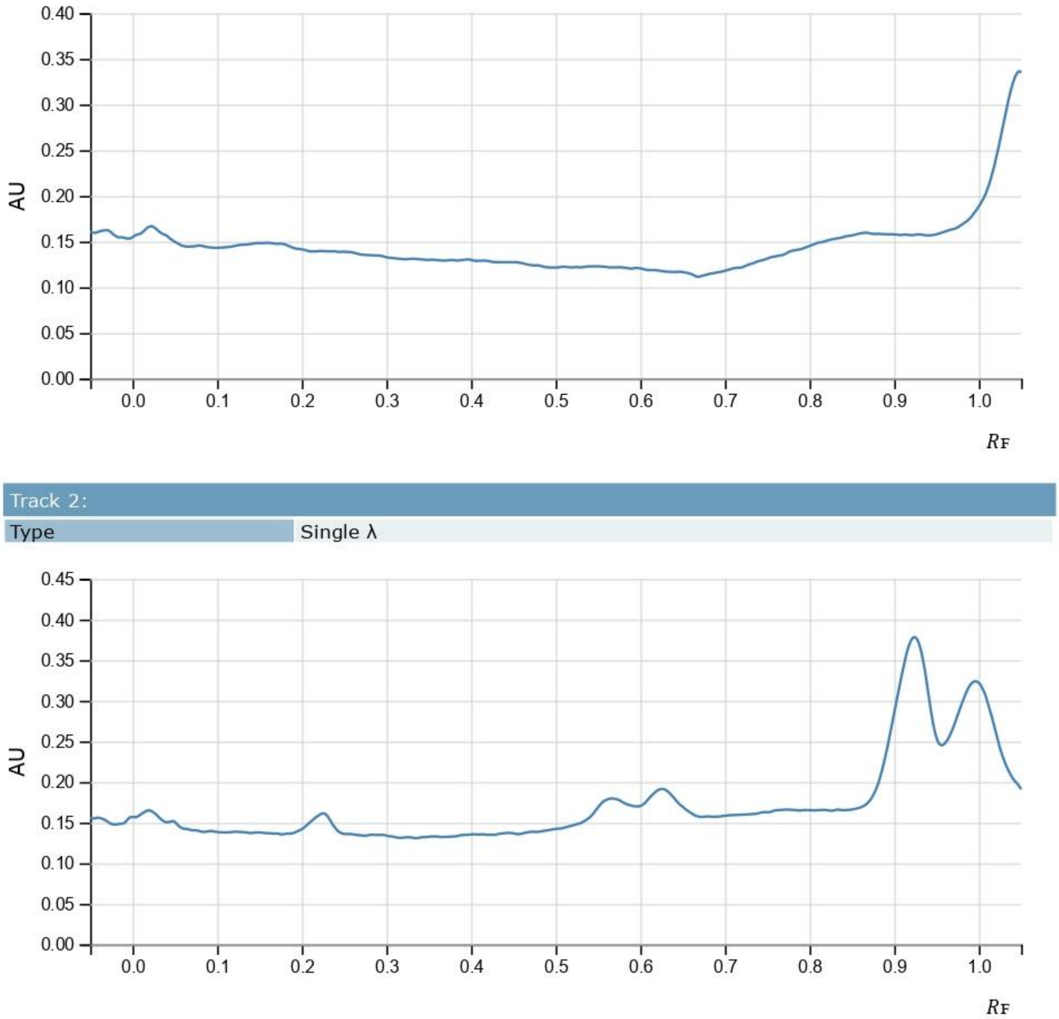


**Figure 2.** Densitometric chromatograms of Ervarubeejadi Yoga Elixir at lower application volumes (5 µL and 10 µL) scanned at 254 nm demonstrating concentration-dependent peak enhancement.

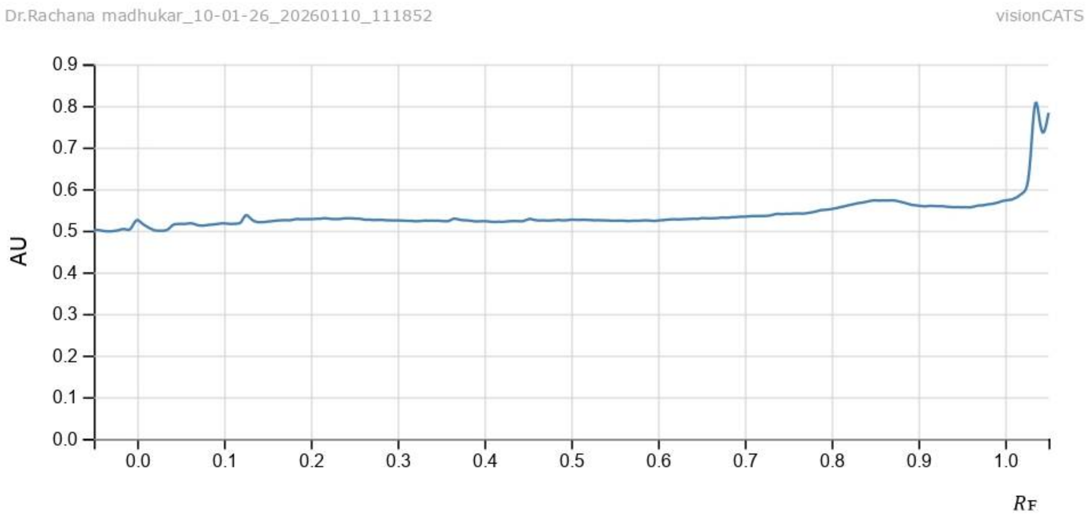


**Figure 3.** Densitometric chromatogram of Ervarubeejadi Yoga Elixir scanned at 366 nm (fluorescence detection)

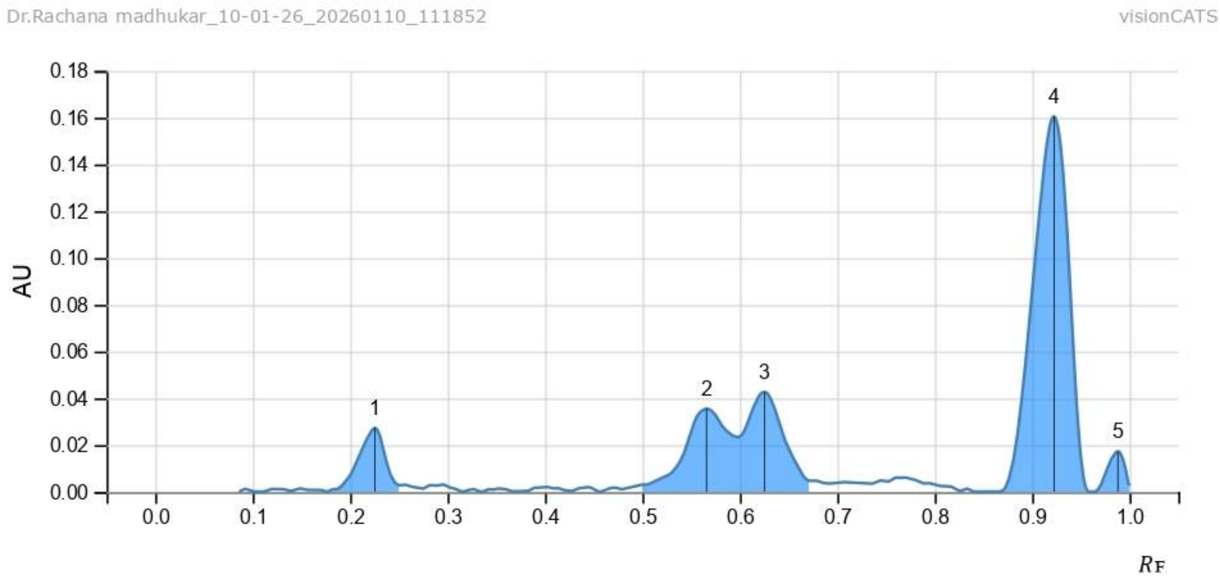


**Figure 4.** Densitometric chromatogram of Ervarubeejadi Yoga Elixir at 254 nm (15 µL application) showing five resolved peaks with corresponding Rf values.

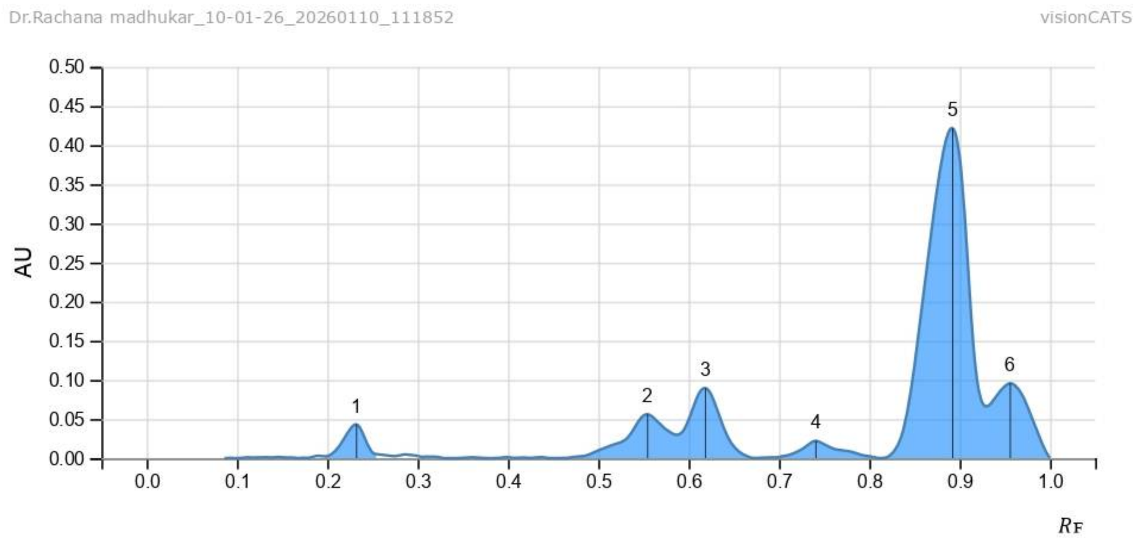


**Figure 5.** Densitometric chromatogram of Ervarubeejadi Yoga Elixir at 254 nm (20 µL application) demonstrating enhanced peak intensity and additional minor constituents**.**

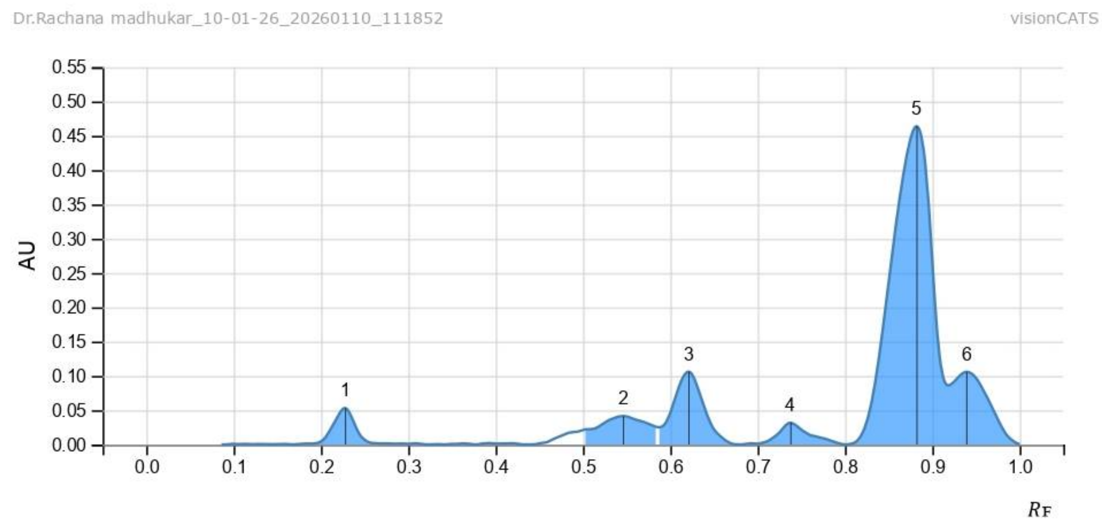


**Figure 6.** Validated densitometric profile of Ervarubeejadi Yoga Elixir at 254 nm showing six resolved peaks.

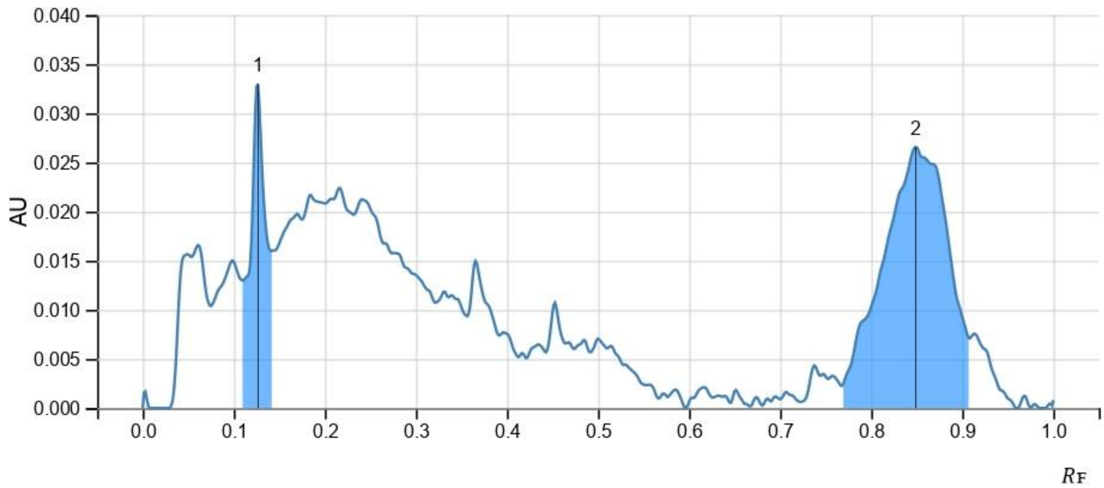


**Figure 7.** Densitometric chromatogram of Ervarubeejadi Yoga Elixir at 366 nm (5 µL application) showing two fluorescent peaks at Rf 0.110 and 0.768.

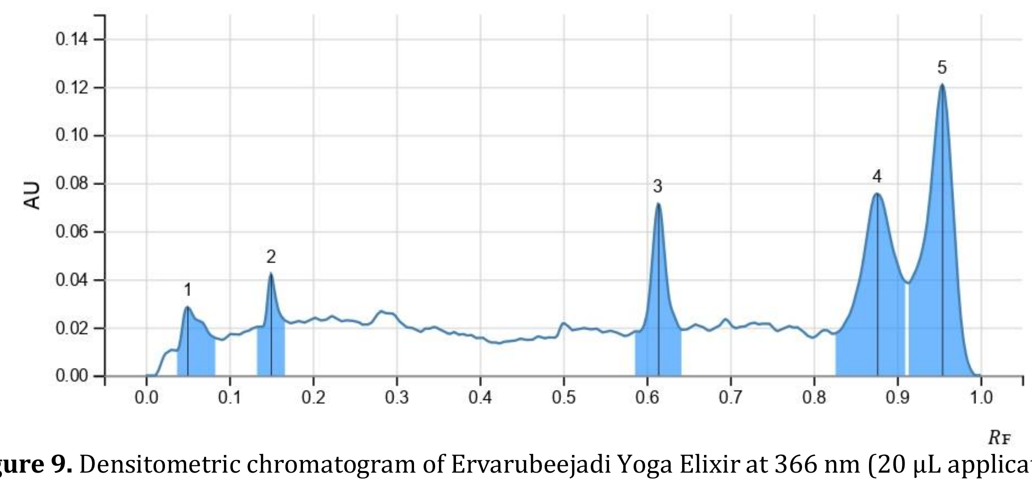


**Figure 9.** Densitometric chromatogram of Ervarubeejadi Yoga Elixir at 366 nm (20 µL application) demonstrating enhanced fluorescence intensity and additional minor peaks.

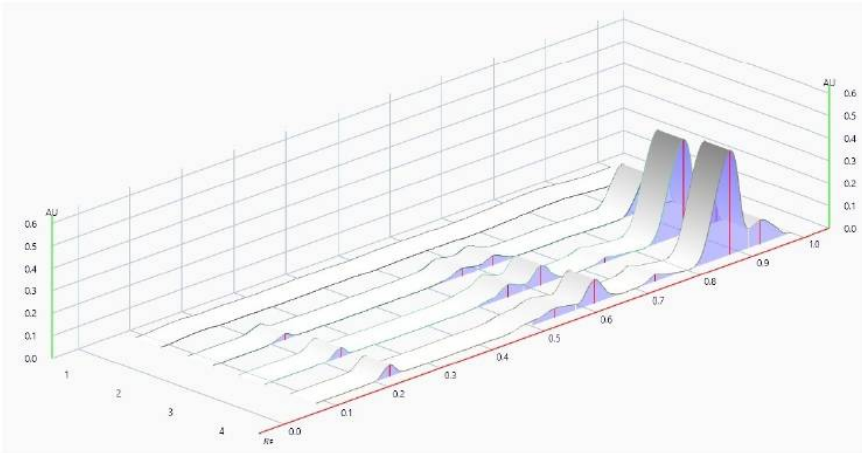


**Figure 10.** Three-dimensional densitometric profile of Ervarubeejadi Yoga Elixir at 254 nm illustrating peak distribution across different application volumes.

***In-Vitro* Antibacterial Activity**

The formulation demonstrated antibacterial activity against *E. coli* ATCC 8739 with a measurable zone of inhibition.

**Table 1. *In-vitro* antibacterial activity of Ervarubeejadi Yoga Elixir against *E. coli*.**

| Organism | Method | Incubation | Zone of Inhibition |
|---|---|---|---|
| *E. coli* ATCC 8739 | Cylinder Plate (IP 2014) | 35°C, 24 h | 7 mm |

The observed 7 mm zone indicates mild antibacterial activity under the experimental conditions.

## DISCUSSION

The present study established a reproducible HPTLC fingerprint profile for Ervarubeejadi Yoga Elixir and demonstrated measurable *in vitro* antibacterial activity against *E. coli* ATCC 8739. In polyherbal and herbo-mineral formulations, chromatographic fingerprinting is particularly important because therapeutic activity often arises from the combined contribution of multiple phytoconstituents rather than a single isolated marker. HPTLC has therefore become a widely accepted tool for herbal drug identification, standardization, and quality control, as it allows visualization of multiple resolved bands within a single analytical run and supports batch-to-batch comparison of complex formulations [6,7]. In the present study, the chromatographic profile showed multiple separated peaks distributed across low-, mid-, and high-*R*<sub>F</sub> regions, indicating a chemically heterogeneous formulation with more than one class of detectable phytoconstituent.

A notable feature of the chromatographic pattern was the consistent appearance of a dominant peak in the high-*R*<sub>F</sub> region across the tested application volumes. Such reproducibility suggests that this band may represent a major phytochemical fraction of the formulation and could be explored further as a characteristic reference zone for routine identity testing and quality evaluation. Similar HPTLC-based standardization approaches have been reported for herbal raw materials and polyherbal formulations, where fingerprint reproducibility and marker-oriented profiling were emphasized as practical tools for quality assurance [6,7]. The present findings are in line with that analytical concept, although definitive assignment of a specific chemical marker to the dominant band would require co-chromatography with standards or hyphenated methods such as HPTLC-MS or HPLC-based confirmation.

The chromatographic complexity observed in the present work is also pharmacognostically reasonable considering the phytochemical diversity of the ingredients. *Glycyrrhiza glabra* is known to contain glycyrrhizin, flavonoids, saponins, chalcones, and other phenolic constituents with broad biological relevance [8]. *Berberis aristata* is well recognized as a rich source of protoberberine alkaloids, particularly berberine, along with related isoquinoline compounds that have documented antimicrobial and anti-inflammatory significance [9,10]. Likewise, *Cucumis sativus* has been reported to contain flavonoids and other phenolic constituents, and studies on peel extracts have demonstrated antibacterial potential against organisms including *E. coli* [11]. Therefore, the resolved peaks observed in the densitometric profile may plausibly reflect the combined presence of alkaloidal, phenolic, flavonoidal, and glycosidic fractions contributed by the individual ingredients.

The variation in the peak pattern and relative intensity across different observed zones also suggests that the formulation contains compounds with differing polarity and chromophoric properties. In herbal mixtures, such diversity is expected because structurally distinct secondary metabolites migrate differently on silica gel under the same mobile phase conditions. While HPTLC does not by itself provide structural identification, it offers an efficient first-line analytical map of the formulation. This becomes especially useful in classical formulations or newly developed dosage adaptations, where establishing a stable reference fingerprint is often the first requirement before more advanced quantitative or marker-specific studies are attempted [6,7].

The *in vitro* antibacterial study demonstrated a measurable zone of inhibition of 7 mm against *E. coli* ATCC 8739, indicating mild antibacterial activity under the experimental conditions employed. This observation is biologically plausible when interpreted in light of the known properties of the component herbs. *Glycyrrhiza glabra* has been reported to exhibit antibacterial activity against various bacterial pathogens, and its flavonoids, saponins, and related constituents are believed to contribute to membrane-related and enzyme-modulating effects [8]. *Berberis aristata* and berberine-containing extracts have also shown antimicrobial activity in experimental studies, with berberine being one of the best-studied plant alkaloids in this regard [9,10]. Berberine has been reported to interfere with bacterial cell function and may also enhance antibacterial efficacy in combination settings, which supports the possibility that it contributes meaningfully to the observed inhibition [10]. In addition, *Cucumis sativus* extracts have shown antibacterial effects in experimental models, including activity against *E. coli*, suggesting that even this component may contribute supportive activity within the composite formulation [11].

Since Ervarubeejadi Yoga Elixir is a multi-ingredient formulation, the antibacterial effect observed in this study is likely to reflect the combined or possibly synergistic action of several phytochemical classes rather than the effect of a single constituent alone. This is an important point in the evaluation of Ayurvedic formulations, where the therapeutic rationale is often based on the cooperative action of ingredients with overlapping and complementary properties. The present findings therefore provide preliminary support for the traditional use of the formulation in urinary disorders, especially because *E. coli* remains one of the most common organisms associated with urinary tract infections. However, the observed zone of inhibition was small, and therefore the result should be interpreted as indicative of mild activity rather than strong antibacterial potency.

At the same time, the present microbiological findings should be viewed within their methodological limits. The study used a single test organism and relied on zone of inhibition as the primary endpoint. Although this is appropriate for preliminary biological screening, it does not provide information on minimum inhibitory concentration, minimum bactericidal concentration, killing kinetics, or mechanism of action. The absence of a standard antibiotic comparator also limits direct assessment of relative potency. Therefore, while the current data are sufficient to suggest biological relevance, they do not yet establish therapeutic equivalence or strong antimicrobial efficacy. More detailed microbiological testing involving multiple uropathogens, positive controls, MIC/MBC determination, and replicate-based statistical validation would be necessary to define the true antibacterial potential of the formulation more clearly.

Taken together, the analytical and biological findings of the present study provide an initial scientific basis for the evaluation of Ervarubeejadi Yoga Elixir. The HPTLC fingerprint offers a reproducible platform for future standardization and quality control, while the observed *in vitro* activity against *E. coli* supports the possibility of antibacterial relevance in the context of urinary disorders. Because this is a newly studied formulation, the present work should be considered foundational rather than definitive. Future investigations integrating marker-based phytochemical confirmation, quantitative estimation of major constituents, and expanded antimicrobial profiling would help strengthen the pharmacological interpretation and improve the evidence base for its therapeutic application.

## CONCLUSION

The present study establishes a reproducible HPTLC fingerprint profile of Ervarubeejadi Yoga Elixir and demonstrates measurable in-vitro antibacterial activity against *E. coli*. The findings provide preliminary analytical and biological validation of the formulation and support further quantitative, multi-strain, and mechanistic investigations.

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