"HPTLC Fingerprint Profiling and Phytochemical Characterization of Haridradi Churna"

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ABSTRACT
Background: Standardization of polyherbal formulations is crucial to maintain their quality, safety, and therapeutic efficacy. Haridradi Churna is a traditional Ayurvedic preparation commonly prescribed for conditions involving inflammation, infections, and delayed wound healing. H is widely recognized as an effective method for phytochemical analysis and quality assessment of such formulations. **Objective:** To develop an HPTLC fingerprint profile of Haridradi Churna and to identify its active phytochemical compounds. **Materials and Methods:** The methanolic extract of Haridradi Churna was prepared using maceration followed by sonication. HPTLC(High-Performance Thin-Layer Chromatography) analysis was carried out on silica gel 60 F254 plates, employing a mobile phase consisting of Toluene: Ethyl acetate: Formic acid (2:3:0.5 volume/volume/volume). The developed plates were scanned at 254 nm and 366 nm using a CAMAG TLC(Thin Layer Chromatography) scanner. **Results:** The chromatographic analysis revealed several well-resolved bands at different Rf(Retention factor) values under both wavelengths. Active phytoconstituents such as curcumin, demethoxycurcumin, berberine, flavonoids, phenolic compounds, and steroidal alkaloids were identified. Among these, curcuminoids exhibited comparatively higher intensity, suggesting their active presence. Fluorescent bands observed at 366 nm further supported the presence of curcuminoids and berberine. **Conclusion:** The HPTLC(High-Performance Thin-Layer Chromatography) fingerprint profile confirmed the presence of multiple biologically active compounds in Haridradi Churna that may contribute to its antimicrobial, anti-inflammatory, and antioxidant properties. The findings provide a scientific basis for its standardization and validate its traditional therapeutic applications.

Keywords
Haridradi Churna, High-Performance Thin-Layer Chromatography,herbal formulation,phytochemical analysis,Retention factor values standardization
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Graphical Abstract:
Herbal Drugs → Extraction → HPTLC → Fingerprint Bands → Phytochemicals → Therapeutic Effects → Standardization

Introduction

Standardization of polyherbal formulations is essential in contemporary Ayurvedic research to maintain uniformity in quality, safety, and therapeutic effectiveness. Among various analytical methods, HPTLC (High-Performance Thin-Layer Chromatography**)** is widely accepted as a reliable and consistent method for detecting phytoconstituents and generating characteristic fingerprint profiles of complex herbal preparations.

Haridradi Churna is a well-known classical Ayurvedic formulation used in the management of conditions involving inflammation, microbial infections, and delayed wound healing. Its therapeutic efficacy is primarily attributed to bioactive compounds derived from its herbal ingredients. The active components, such as *Curcuma longa*, *Berberis aristata*, and *Solanum indicum*, have been extensively investigated for their phytochemical constituents and pharmacological properties. Curcuminoids obtained from *Curcuma longa* are known to show significant anti-inflammatory and antioxidant activities, mainly through the regulation of inflammatory pathways. Likewise, berberine, a main isoquinoline alkaloid found in *Berberis aristata*, shows strong antimicrobial and anti-inflammatory effects, supporting its role infection-related disorders. (Kaphaj yoni vyapad).

Furthermore, flavonoids and phenolic compounds present in plants like *Solanum indicum* contribute to antioxidant defense, cellular protection, and tissue repair processes. HPTLC(High-Performance Thin-Layer Chromatography**)** fingerprinting has proven to be an efficient approach for the simultaneous identification of multiple phytoconstituents and plays an important role in ensuring the quality control and standardization of herbal formulations.

Therefore, the present study was undertaken to develop the HPTLC(High-Performance Thin-Layer Chromatography**)** fingerprint profile of Haridradi Churna and to identify its main phytochemical

markers, thereby providing scientific support for its traditional therapeutic applications.

Materials and Methods:

The HPTLC (High-Performance Thin-Layer Chromatography) fingerprint analysis for methanol extract of Haridradi Churna on was carried out at concentration 10.0 microliter under Visualizations: 254 nm (Ultraviolet-absorbing compounds) and 366 nm (fluorescent compounds). The test solution the sample was prepared by weighing 5 g of sample in a beaker and to it 100 mL of Methanol was added. The solution was sonicated for 15 minutes. It was then cooled and filtered with a simple filter paper. The filtrate thus obtained was used for HPTLC(High-Performance Thin-Layer Chromatography) fingerprinting. The Chromatography was performed on 10 × 10 cm thin layer chromatography (TLC) plates coated with 0.2 mm layers of silica gel 60 F254 (Merck). The samples were applied to the plate as 6 mm wide bands by means of a Linomat 5 (Serial Number:280008) sample applicator (CAMAG, Switzerland). The plate was developed to a distance of 8.0 cm with Toluene:Ethyl acetate: Formic acid (2:3:0.5 volume/volume/volume) as mobile phase in a CAMAG twin-trough chamber saturated with mobile phase vapor. The plate was then derivatized in CAMAG-Dip Tank for about 1 minute followed by drying using a TLC (thin layer chromatography) Plate Heater which was preheated at 100± 5°C FOR 3 minutes. The plate was later scanned at 254 nm and 366 nm by the use of a CAMAG TLC scanner 3 using winCATS 4 software (CAMAG, Switzerland).The area percentage values are approximate and based on visual densitometric interpretation of band intensity, as the instrument-generated peak area data were not available in the HPTLC(High-Performance Thin-Layer Chromatography) report.

Ethical Approval

This study involved only laboratory-based phytochemical and HPTLC analysis of Haridradi Churna. No human or animal subjects were included; hence, ethical approval was not required. Standard laboratory and research ethics guidelines were followed.

Statistical Analysis

The study is qualitative in nature. Rf values were recorded, and band intensities were interpreted visually without applying inferential statistical methods.

Result

Table 1(given below) shows the Rf values of Haridradi Churna at 254 nm and 366 nm, indicating multiple well-resolved phytochemical constituents. The consistent band pattern across both wavelengths reflects the chemical complexity and reproducibility of the formulation. These findings support its suitability for standardization.

Table 1: Rf (Retention factor) values

254 nm	366 nm
Rf	**Rf**
0.35	0.40
0.47	0.53
0.55	0.58
0.60	0.65
0.72	0.70
0.83	0.78
0.90	0.85
0.98	0.96

Interpretation

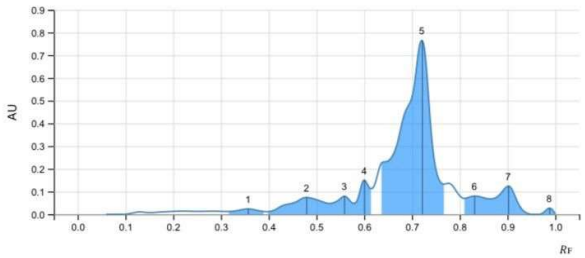

Figure1:HPTLC CHROMATOGRAPH@254nm
Figure 2: HPTLC Plate@254nm

Table 2 (given below) represents the probable phytochemical constituents identified in Haridradi Churna (given in figure 1 and figure 2) at 254 nm. The observed Rf values correspond to key compounds such as phenolics, berberine, flavonoids, curcumin, and steroidal alkaloids. Among these, curcumin showed higher relative area percentage, indicating its predominant presence. The identified constituents collectively contribute to antioxidant, antimicrobial, and anti-inflammatory activities of the formulation.

Table 2: Probable phytochemicals present in Haridradi Churna under 254 nm visualisation;

Rf	Phytochemical	Source	Relative Are	Pharmacological	References

			a %	Activity	
0.35	Phenolics	*Berberis aristata*	Low (5–8%)	Antioxidant	(1,2,7,9,13)
0.47	Berberine	*Berberis aristata*	Moderate (10–15%)	Antimicrobial	(1,2,6,10,13)
0.55	Flavonoids	*Solanum indicum*	Moderate (10–12%)	Anti-inflammatory	(1,2,3,5,12)
0.60	Phenolic glycosides	All	Low–moderate (8–10%)	Mucosal protection	(1,2,7,9,13)
0.72	Curcumin	*Curcuma longa*	High (20–25%)	Anti-inflammatory	(1,2,4,8,13)
0.83	Demethoxycurcumin	*Curcuma longa*	Moderate (12–15%)	Tissue repair	(1,2,4,8,13)
0.90	Steroidal alkaloids	*Solanum indicum*	Low (5–8%)	Anti-inflammatory	(1,2,3,5,11)
0.98	Volatile oils	*Curcuma longa*	Low (5%)	Antimicrobial	(1,2,4,13)

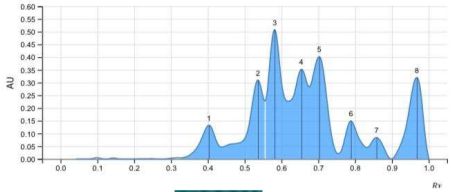

Figure3: HPTLC CHROMATOGRAPH@ 366 nm **Figure 4: HPTLC Plate@ 366 nm**

Table 3(given below) shows the probable phytochemicals observed at 366 nm, highlighting fluorescent compounds. Prominent bands corresponding to berberine and curcuminoids exhibited strong fluorescence, confirming their presence. Curcumin showed the highest relative area percentage, indicating its major contribution(show in figure 3 and figure 4). These compounds are associated with antimicrobial, anti-inflammatory, and healing properties, supporting the therapeutic potential of the formulation.

Table 3: Probable phytochemicals present in Haridradi Churna under 366 nm visualisation;

Rf	Phytochemical	Source	Relative Area %	Pharmacological Activity	Citations
0.40	Phenolics	*Berberis aristata*	Low (5–7%)	Antioxidant	(1,2,7,9,13)
0.53	Berberine (fluorescent)	*Berberis aristata*	Moderate (12–15%)	Antimicrobial	(1,2,6,10,13)
0.58	Flavonoids	*Solanum indicum*	Moderate (10–12%)	Antioxidant	(1,2,3,5,12)
0.65	Phenolic derivatives	All	Low–moderate (8–	Healing	(1,2,7,9,13)

			10%)		
0.70	Curcumin (bright fluorescence)	*Curcuma longa*	High (25–30%)	Anti-inflammatory	(1,2,4,8,13)
0.78	Demethoxycurcumin	*Curcuma longa*	Moderate (12–15%)	Repair	(1,2,4,8,13)
0.85	Steroidal alkaloids	*Solanum indicum*	Low (5–8%)	Anti-inflammatory	(1,2,3,5,11)
0.96	Volatile compounds	*Curcuma longa*	Low (5%)	Antimicrobial	(1,2,4,13)

Discussion

At 254 nm, prominent bands corresponding to Rf(Retention factor) values around 0.47, 0.72, and 0.83 suggest the presence of berberine and curcuminoids. The additional peaks in the range of 0.35–0.60 indicate phenolics and flavonoids. At 366 nm, enhanced fluorescence of bands at Rf (Retention factor) 0.53, 0.70, and 0.78 further confirms the presence of berberine and curcuminoids, along with Ultraviolet-active flavonoids. The presence of berberine, which is a predominant isoquinoline alkaloid from *Berberis aristata*, contributes significant antimicrobial and anti-inflammatory activity. This is particularly relevant in conditions associated with *Kapha* dominance and microbial involvement. Curcuminoids derived from *Curcuma longa*, identified at higher Rf(Retention factor) values, are known to modulate inflammatory pathways and promote tissue repair through antioxidant mechanisms. Flavonoids and steroidal alkaloids found in *Solanum indicum* further enhance the antioxidant and tissue-protective effects of Haridra, aiding in restoration of mucosal integrity. Additionally, the presence of non-polar volatile constituents at higher Rf(Retention factor) values supports their role in *Dhoopana* therapy, facilitating localized drug delivery through fumigation and exerting *Shoshana* (drying), *Lekhana* (scraping of excess *Kapha*), and *Krimighna* (antimicrobial) actions.

Conclusion:

The study successfully developed a characteristic HPTLC(High-Performance Thin-Layer Chromatography) fingerprint profile of Haridradi Churna, indicating the presence of important active phytoconstituents, including curcuminoids, berberine, flavonoids, phenolic compounds, and steroidal alkaloids. The well-defined Rf(Retention factor) values observed at 254 nm and 366 nm reflect good chemical consistency of the formulation, with curcuminoids contributing predominantly. These results substantiate the antimicrobial, anti-inflammatory, and antioxidant potential of the formulation and provide scientific support for its traditional use. HPTLC(High-Performance Thin-Layer Chromatography) was found to be an effective and reliable technique for quality assessment and standardization of the formulation. However, further studies involving quantitative estimation and advanced analytical methods are recommended for more comprehensive validation.

Abbreviations

HPTLC: High-Performance Thin-Layer Chromatography; Rf: Retention factor; TLC: Thin Layer Chromatography; UV: Ultraviolet; v/v/v: volume/volume/volume; AU: Absorbance Units; µL: microliter; S/N: Serial Number.

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Conflicts of interest: There are no conflicts of interest.

Summary:

This study aimed to standardize and evaluate the phytochemical composition of Haridradi Churna using High-Performance Thin-Layer Chromatography (HPTLC). The methanolic extract was examined at UV wavelengths of 254 nm and 366 nm to generate a characteristic fingerprint profile. The analysis revealed several well-defined bands with distinct Rf values, indicating the presence of diverse bioactive constituents.Major compounds identified included curcumin, demethoxycurcumin, berberine, flavonoids, phenolic compounds, and steroidal alkaloids. Among these, curcuminoids were predominant, as reflected by their higher intensity. Fluorescent bands at 366 nm further supported the presence of curcuminoids and berberine.Overall, the study highlights HPTLC as a dependable technique for qualitative assessment and standardization of polyherbal formulations. The identified phytochemicals contribute to the antimicrobial, anti-inflammatory, and antioxidant potential of Haridradi Churna, thereby supporting its traditional medicinal use.

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