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# HPTLC Fingerprint Profiling of Methanolic Extract of Haridra (Curcuma longa Linn.) and its Relevance to Pesticidal Phytochemical Constituents

**Sariput N. Bhosikar[1], Rekha Parmar[2] and Mukesh Laichattiwar[3]**

[1]Department of Agadatantra, Faculty of Ayurved, Parul Institute of Ayurved & Research, Parul University, Waghodia, Vadodara, Gujarat - 391760 (India)
[2]Professor, Dept. of Dravyaguna, Faculty of Ayurved, Parul Institute of Ayurved, Parul University, Waghodia, Vadodara, Gujarat - 391760 (India)
[3]Assistant Professor, Dept. of Entomology, College of Agriculture, Parul University, Waghodia, Vadodara, Gujarat - 391760 (India)
**Corresponding Author:** [1]Sariput N. Bhosikar — drbsariput@gmail.com

## Abstract

*Curcuma longa* Linn. (Haridra), classified under *Vishaghna dashemani* in Ayurvedic texts, possesses phytochemical constituents—curcuminoids and sesquiterpene ketones—with broad-spectrum bioactivities. This study aimed to develop a comprehensive HPTLC fingerprint of its methanolic rhizome extract and correlate detected bands with documented pesticidal constituents.

Methanolic extract was applied in four volumes (5, 10, 15, 20 µL) on Merck Silica Gel 60 $F_{254}$ HPTLC plates; mobile phase Chloroform: Ethanol: Glacial Acetic Acid (9.5:0.5:0.1 v/v/v); scanning at 254 nm and 366 nm using TLC Scanner 4 with visionCATS software.

At 254 nm, 5–7 reproducible peaks appeared at $R_F$ 0.51–0.66, 0.75–0.83, and 0.88–0.97, corresponding to curcuminoids and sesquiterpenes. At 366 nm, 7–8 fluorescent bands dominated at $R_F$ 0.63, 0.68, 0.80, and 0.88–0.91. 3D overlays confirmed band reproducibility across all concentrations.

The HPTLC profile of *C. longa* methanolic extract demonstrates a characteristic multi-band pattern attributable to pesticidally active curcuminoids and sesquiterpenes, validating the Ayurvedic *Vishaghna* designation and supporting botanical pesticide development.

**Key words:** HPTLC; *Curcuma longa*; curcuminoids; botanical pesticide; phytochemical fingerprinting; Vishaghna; Agadatantra.

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[1]Ph.D. Scholar & Professor, [2]Ph.D. Guide, [3]Ph.D. Co-Guide

*Curcuma longa* Linn. (Zingiberaceae), known as Haridra in Ayurveda, is classified under *Vishaghna dashemani* (ten antitoxic drugs), *Krimighna*, and *Kusthaghna* Ganas in classical texts including *Charaka Samhita* and *Sushruta Samhita*[1,14]. These *Krimighna* (insecticidal/antiparasitic) and *Vishaghna* (antitoxic) designations reflect an ancient recognition of its capacity to neutralize toxins and pest organisms—now receiving mechanistic validation through modern phytochemical research.

The misuse of synthetic pesticides—particularly neonicotinoids such as imidacloprid—has resulted in residue concentrations well above the WHO acceptable daily intake (ADI) limits in Indian food crops[6,16]. Long-term sub-lethal exposure causes immune suppression, hormonal disruption, and carcinogenesis[3]. Swathi and Bharathi[15] demonstrated that *Haridra kashaya* achieves 46.64% inhibition of imidacloprid residue on baby corn surfaces through alkaline hydrolysis (pH 7.4–9.2) and curcumin's adsorption of non-metallic toxicants[15], while curcumin and ar-turmerone exhibit direct contact insecticidal, fumigant, nematicidal, and anti-feedant activities[5,12]. HPTLC fingerprinting, accepted by the Ayurvedic Pharmacopoeia of India (API) for herbal standardization, provides the systematic phytochemical evidence needed to link *C. longa*'s classical *Vishaghna* property to specific pesticidally active constituents—an integration not previously reported.

*Plant Material and Extract Preparation :*

Dried rhizomes of *Curcuma longa* Linn. were procured from an authenticated commercial source and botanical identity was confirmed by the Department of Dravyaguna, PIA. The rhizomes were cleaned, shade-dried, and coarsely powdered using a mechanical pulverizer, sieved through mesh No. 40. Methanolic extraction was performed using a Soxhlet apparatus: accurately weighed powdered drug (50 g) was packed into a thimble and extracted with analytical-grade methanol (Merck) for approximately 6–8 hours until the siphon solvent became colorless, indicating exhaustive extraction. The filtrate was filtered (Whatmann No. 1), concentrated under reduced pressure at 40°C using a rotary evaporator, reconstituted in methanol at a standardized concentration, and used for HPTLC analysis.

*HPTLC Chromatographic Conditions :*

HPTLC analysis was performed at the Centre of Research for Development (CR4D), Parul University, Vadodara, using visionCATS (Server HPTLC v3.2.23095.1). Merck Silica Gel 60 $F_{254}$ pre-coated plates (100 × 100 mm) were used as the stationary phase. Mobile phase: Chloroform:Ethanol:Glacial Acetic Acid (9.5:0.5:0.1 v/v/v). Sample volumes of 5.0, 10.0, 15.0, and 20.0 µL (Tracks 1–4) were applied using Camag Linomat 5 (150 nL/s). Plates were developed in a TTC 10×10 twin-trough chamber (20 min saturation; 80 mm development). Scanning was performed with TLC Scanner 4 at 254 nm (absorbance, deuterium lamp) and 366 nm (fluorescence, mercury lamp, K400 filter). Peak integration used Gaussian detection (sensitivity 0.1, threshold 0.1) with Savitzky–Golay smoothing (window 7) and lowest-slope baseline correction. Chromatographic parameters are summarized in Table-1.

Table-1. HPTLC Chromatographic Parameters

| Parameter | Details |
|---|---|
| Stationary Phase | Merck HPTLC Silica Gel 60 F254, 100 × 100 mm |
| Mobile Phase | Chloroform : Ethanol : Glacial Acetic Acid (9.5 : 0.5 : 0.1 v/v/v) |
| Application Volumes | 5.0, 10.0, 15.0, 20.0 µL (Tracks 1–4); Linomat 5 |
| Development | TTC 10×10 chamber; saturation 20 min; distance 80 mm |
| Scanning | 254 nm (Absorbance, D2 lamp) and 366 nm (Fluorescence, Hg lamp, K400) |
| Scanner | TLC Scanner 4; speed 100 mm/s; slit 6 × 0.45 mm (micro) |
| Software | visionCATS Server HPTLC v3.2.23095.1 |

*HPTLC Fingerprint at 254 nm :*

The absorbance scan at 254 nm revealed distinct multi-peak fingerprints across all four tracks (Figure 1; Table-2). Representative Track 1 (5 µL) resolved 6 peaks with major absorptions at $R_F$ 0.661 (31.39%), 0.767 (22.78%), 0.825 (32.64%), and 0.978 (5.91%). Track 4 (20 µL) resolved 5 peaks, with dominant areas at $R_F$ 0.626 (25.68%), 0.796 (34.60%), and 0.956 (26.98%). The band positions remained consistent across all concentrations; the 3D overlay (inset, Figure 1) confirmed proportional peak growth and positional reproducibility across Tracks 1–4.

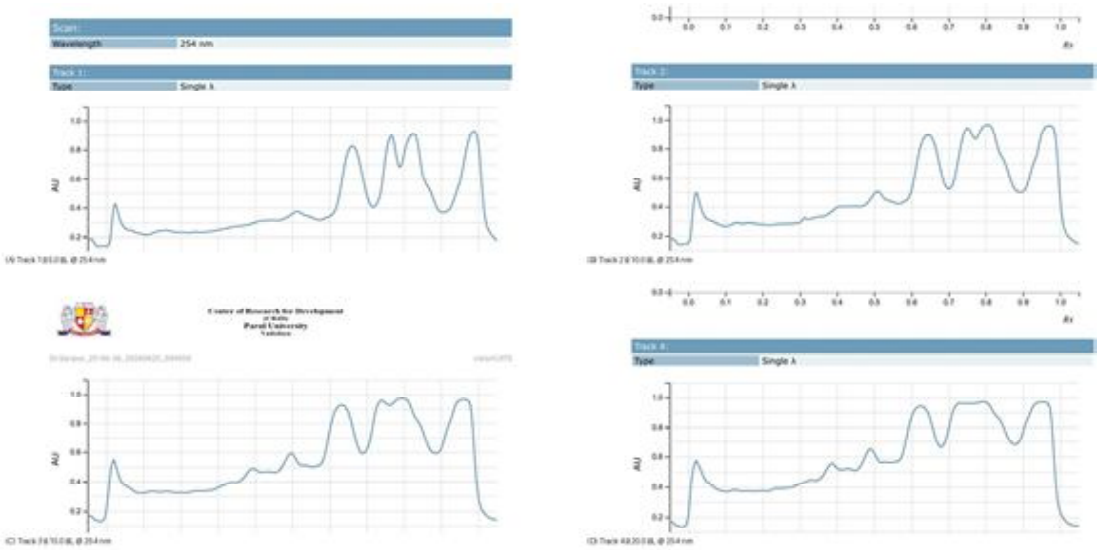

Figure 1. HPTLC densitograms of Haridra (C. longa) methanolic extract at 254 nm. (A) Track 1 – 5.0 µL; (B) Track 2 – 10.0 µL; (C) Track 3 – 15.0 µL; (D) Track 4 – 20.0 µL. Mobile phase: Chloroform:Ethanol:GAA (9.5:0.5:0.1 v/v/v); Stationary phase: Merck HPTLC Silica Gel 60 F254.

Table-2. Representative HPTLC Peak Data at 254 nm (Tracks 1 and 4) – Haridra Methanolic Extract

| Track | Peak # | Start RF | Max RF | End RF | Area % |
|---|---|---|---|---|---|
| 1 (5 µL) | 3 | 0.575 | 0.661 | 0.718 | 31.39 |
| | 4 | 0.718 | 0.767 | 0.789 | 22.78 |
| | 5 | 0.790 | 0.825 | 0.907 | 32.64 |
| | 6 | 0.931 | 0.978 | 1.000 | 5.91 |
| 4 (20 µL) | 3 | 0.543 | 0.626 | 0.679 | 25.68 |
| | 4 | 0.739 | 0.796 | 0.878 | 34.60 |
| | 5 | 0.878 | 0.956 | 1.000 | 26.98 |

*HPTLC Fingerprint at 366 nm and Phytochemical Band Assignment :*

The fluorescence scan at 366 nm resolved 7–8 bands per track, with dominant zones at $R_F$ 0.63, 0.68, 0.80, and 0.88–0.91 (Figure 2). Track 4 (20 µL) showed the highest area contributions at $R_F$ 0.879 (42.99%) and 0.911 (20.40%). Based on comparison with published HPTLC profiles of *C. longa* in chloroform-based systems[9,10], bands at $R_F$ 0.30–0.57 were assigned to bisdemethoxycurcumin, demethoxycurcumin, and curcumin respectively, while bands at $R_F$ 0.63–0.96 were attributed to sesquiterpene ketones (ar-turmerone, α/β-turmerone) and hydrocarbons (zingiberene, bisabolene). The band assignment and corresponding pesticidal activities are summarized in Table-3.

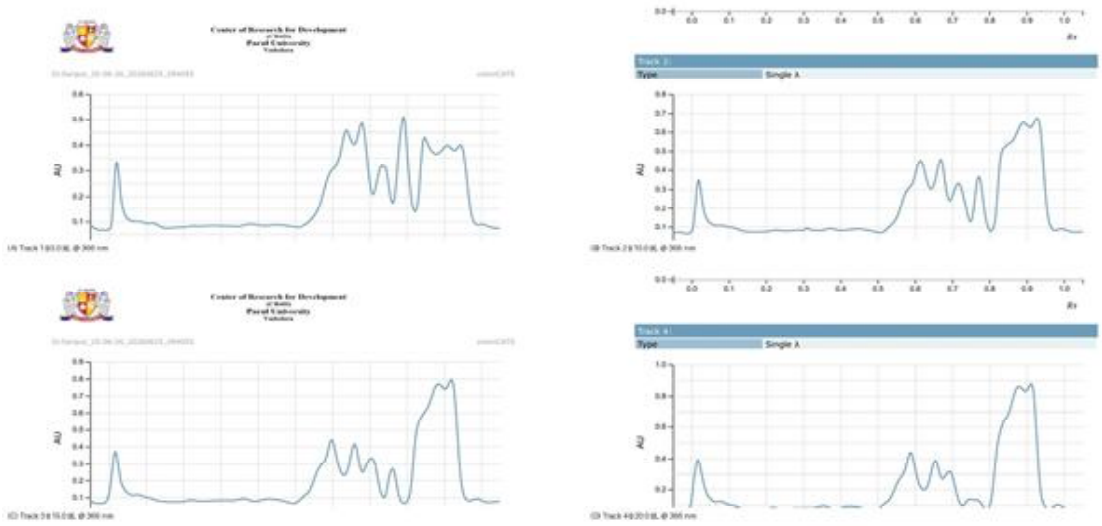

Figure 2. HPTLC densitograms of Haridra (C. longa) methanolic extract at 366 nm (fluorescence, K400 filter). (A) Track 1 – 5.0 µL; (B) Track 2 – 10.0 µL; (C) Track 3 – 15.0 µL; (D) Track 4 – 20.0 µL. Mercury lamp excitation.

Table-3. Tentative Phytochemical Band Assignment (RF at 254 nm) and Documented Pesticidal Activity

| Approx. RF | Tentative Compound | Phytochemical Class | Documented Pesticidal Activity |
|---|---|---|---|
| 0.30–0.35 | Bisdemethoxycurcumin | Curcuminoid | Insecticidal; Anti-feedant[8] |
| 0.45–0.50 | Demethoxycurcumin | Curcuminoid | Insecticidal; Nematicidal[8,9] |
| 0.50–0.57 | Curcumin | Curcuminoid | Insecticidal; Nematicidal; Antifungal[7,8,9] |
| 0.63–0.70 | Ar-Turmerone | Sesquiterpene ketone | Contact & fumigant insecticidal[4] |
| 0.75–0.85 | α/β-Turmerone | Sesquiterpene ketone | Repellent; Anti-feedant[4] |
| 0.88–0.96 | Zingiberene / Bisabolene | Sesquiterpene hydrocarbon | Nematicidal; Antifungal[13] |

The HPTLC fingerprint of *C. longa* methanolic extract is consistent with published curcuminoid profiles. Application across four concentrations (5–20 µL) demonstrated a linear range, and band positions at 254 nm and 366 nm remained reproducible across all tracks, satisfying key validation criteria under ICH Q2(R1). The dual-wavelength approach captured both UV-absorbing curcuminoids and fluorescent sesquiterpene conjugates in a single run, providing a more complete fingerprint than single-wavelength studies. The $R_F$ values and area percentages accord with API quality markers[2], supporting use of this fingerprint for batch standardization of *C. longa*-based botanical pesticide formulations.

Curcumin ($R_F \approx 0.50–0.57$) and its congeners exhibit insecticidal activity via acetylcholinesterase (AChE) inhibition; Kim et al. reported $LC_{50}$ values against *Tribolium castaneum* and *Sitophilus oryzae* comparable to synthetic pyrethroids, while curcumin achieves >80% nematicidal mortality against *Meloidogyne incognita* at 500 ppm within 72 hours[8,9]. Bisdemethoxycurcumin reduces *Spodoptera litura* feeding by ~ 60% [7]. The sesquiterpene fraction ($R_F$ 0.63–0.96)—ar-turmerone, α/β-turmerone, zingiberene—adds contact fumigant, repellent, and antifungal dimensions against stored-grain pests and phytopathogens[4,13]. Collectively, these activities provide the phytochemical basis for the Ayurvedic *Vishaghna* (antitoxic) and *Krimighna* (insecticidal) designations of Haridra. *Haridra kashaya* achieved 46.64% inhibition of imidacloprid residue on vegetable surfaces—surpassing tap water (30.48%) and salt water (30.79%)—through alkaline hydrolysis (pH 7.4–9.2) and curcumin's adsorption of the neonicotinoid. The HPTLC fingerprint established here serves as the analytical identity map underpinning this translational activity, and as a practical quality control tool for bioassay-directed isolation of pesticidally active fractions.

The present study establishes a reproducible, dual-wavelength HPTLC fingerprint of *Curcuma longa* methanolic extract revealing 5–8 characteristic bands attributable to curcumin, demethoxycurcumin, bisdemethoxycurcumin, ar-turmerone, α/β-turmerone, and zingiberene—each with documented insecticidal, nematicidal, anti-feedant, or pesticide-detoxifying activities. The fingerprint validates the Ayurvedic *Vishaghna* and *Krimighna* classification of Haridra at the phytochemical level and provides an analytical foundation for quality standardization of *C. longa*-based botanical pesticide formulations. Future work should include bioassay-directed isolation of the identified active fractions and field efficacy evaluation against target agricultural pest species.

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**Conflict of interest**

The authors declare no conflict of interest.

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