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HPTLC Fingerprinting and Phytochemical Characterization of Hydroalcoholic Extract of Keetmari (Aristolochia bracteata Lam.), with Reference to Its Pesticidal Efficacy: An Ayurvedic Perspective

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Abstract

Aristolochia bracteata Lam. (*Keetmari / Kitamari*) is a well-documented medicinal plant in Ayurvedic literature, classified under the *Krimighna dravyas* (worm-slaying drugs) across multiple classical Samhitas and Nighantus. Its pesticidal efficacy—described as *Krumighna karma*—is deeply rooted in the *Ushna* (hot potency), *Teekshna* (sharp/piercing), and *Katu-Tikta Rasa* properties described in traditional texts. The present study aimed to develop an HPTLC fingerprint profile of a hydroalcoholic (methanolic) extract of A. bracteata and to correlate phytochemical constituents with pesticidal efficacy as described in Ayurvedic classical literature. Air-dried plant material was extracted using methanol as the solvent. HPTLC analysis was performed on Merck silica gel 60 F254 plates using a toluene:ethyl acetate:formic acid (5:4:1 v/v/v) mobile phase system. Scanning was conducted at 254 nm (absorbance) and 366 nm (fluorescence) using a TLC Scanner 4. Four concentrations (5, 10, 15, and 20 µL) were applied using a Linomat 5 applicator, and the analysis was performed using vision CATS software (version 3.2). HPTLC analysis at 254 nm revealed a concentration-dependent increase in peak resolution, with major peaks at Rf values of approximately 0.07, 0.35–0.37, 0.46–0.47, and 0.60–0.61. At 366 nm (fluorescence mode), prominent peaks appeared at Rf 0.07, 0.37, 0.51, and 0.52, indicative of flavonoids, alkaloids (including aristolochic

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acids), and phenolic compounds. The dominant peak at Rf ~0.60 (accounting for 46–56% of total area at higher concentrations) is consistent with the known aristolochic acid fraction. The HPTLC fingerprint profile corroborates the classical Ayurvedic description of *Keetmari* as a potent *Krimighna dravya*. Aristolochic acids, alkaloids, and phenolic compounds identified through HPTLC are the likely bioactive constituents responsible for the plant's pesticidal, insecticidal, and larvicidal properties. This study provides a validated phytochemical foundation for the standardisation of Keetmari-based formulations.

Key words : Aristolochia bracteata; *Keetmari; Kitamari*; HPTLC fingerprinting; *Krimighna;* pesticidal efficacy; aristolochic acid; Ayurveda; hydroalcoholic extract.

Keetmari, botanically identified as Aristolochia bracteata Lam. (Family: Aristolochiaceae), is one of the most extensively cited medicinal plants in Ayurvedic classical literature. The plant is vernacularly known in Sanskrit as Keetamari, Krimighni, Dhumrapatra, Nakuli, and Visanika—each name reflecting a pharmacological facet of its action. In English, it is aptly called 'Worm Killer' or 'Bracteated Birthwort,' and in Tamil as 'Aadutheendaapalai' (the plant goats do not touch), suggesting the plant's inherent bioactivity and mild toxicity even to foraging animals. From an Ayurvedic perspective, Keetmari is classified as a Krimighna dravya (worm-slaying drug) in the primary Brihat Trayi (Charaka Samhita, Sushruta Samhita, and Ashtanga Hridayam) and further elaborated in the Laghu Trayi (Nighantus).

The plant's Rasapanchaka—Katu-Tikta Rasa (pungent-bitter taste), Laghu-Ruksha-Teekshna Guna (light, dry, sharp qualities), Ushna Veerya (hot potency), and Katu Vipaka (pungent post-digestive effect)—form the pharmacological basis of its activity against intestinal parasites (Krimiroga), and pathogenic organisms[1]. The genus *Aristolochia* comprises over 500 species distributed across tropical and subtropical regions. *Aristolochia bracteata* is native to the arid and semi-arid regions of India—particularly Deccan, Gujarat, Bengal, and Karnataka—as well as Sri Lanka, Arabia, and parts of Tropical Africa including Sudan and Ethiopia.[11] In India, the plant grows abundantly in dry open areas, waste lands, and roadsides, often as a trailing herb. Phytochemically, A. bracteata is rich in aristolochic acids I and II (nitrophenanthrene carboxylic acids), alkaloids such as magnoflorine and aristolactams, flavonoids (quercetin, kaempferol), tannins, and sterols including sitosterol.[9] Aristolochic acids are responsible not only for the plant's potent biological activities but also for its well-documented nephrotoxicity (Aristolochic Acid Nephropathy, AAN), which mandates strictly supervised clinical use.[8]

High-Performance Thin Layer Chromatography (HPTLC) is a validated, reproducible analytical technique for the fingerprinting and standardization of herbal drugs. It offers simultaneous analysis of multiple samples with high resolution, and the

chromatographic fingerprint serves as a phytochemical identity card for a given plant material.[5] Despite the traditional significance of Keetmari, very limited studies have employed HPTLC for standardizing its extracts and correlating the phytochemical profile with its classical therapeutic—particularly pesticidal—claims. This study was undertaken with a dual objective: (1) to develop an HPTLC fingerprint profile of the hydroalcoholic (methanolic) extract of A. bracteata, and (2) to review and correlate the pesticidal properties described across Ayurvedic Samhitas and Nighantus with the phytochemical constituents identified through HPTLC analysis. This work aims to provide a scientific foundation for the traditional use of Keetmari as a Krimighna dravya and to contribute to its pharmacognostic standardization.

Classical ayurvedic review of keetmari :

Nomenclature and Synonyms :

The multiplicity of Sanskrit synonyms for Keetmari reflects the depth of Ayurvedic pharmacological observation. Each name encodes a specific property: Kitamari and Krimighni ('worm-killer') denote its primary anthelmintic action; Dhumrapatra ('smoky-leaved') refers to the plant's appearance; Nakuli suggests its resemblance in action to the mongoose (nakula), a traditional enemy of serpents and vermin; and Visanika indicates its toxicological properties. This naming convention is consistent across multiple classical texts, providing strong textual authentication for the species.

Charaka Samhita places Kitamari prominently in the Krimighna Mahakashaya (one of the 50 groups of medicinal plants) specifically for the treatment of Krimiroga (parasitic infestations). The Charaka Chikitsasthana describes Krimiroga as a pathological state caused by aggravated Kapha and Tridosha, and Kitamari is recommended as a primary Krimighna herb in both oral formulations and topical applications[2]. Sushruta Samhita classifies the plant under the Aragvadhadi Gana, with specific reference to its external application (Bahirparimarjana) in Sadyovrana (fresh wounds) and Dushta Vrana (infected/gangrenous wounds). Sushruta's description of applying leaf paste to wounds infested with Krimis (maggots) aligns remarkably with modern entomological research on the plant's larvicidal efficacy[14]. Ashtanga Hridayam of Vagbhata includes Nakuli (Keetmari) in the Krimighna dravyas3 and confirms its Katu-Tikta Rasa, Ushna Veerya, and Kapha-Vata Shamaka (pacifying Kapha and Vata) properties[15].

References in Nighantus (Lexicographic Texts) :

The consistency of *Krimighna* property across all major classical texts—from the *Brihat Trayi* to the later *Nighantus*—provides strong textual evidence for the pesticidal efficacy of *Keetmari*. *Bhavaprakash Nighantu*, one of the most cited medieval Ayurvedic lexicons, places *Kitamari* in the *Haritakyadi Varga* and explicitly states *'Kitamari krimighni syat vrana-shodhana-ropini'* (*Keetmari* is a worm-killer and wound-cleanser/healer).[7] *Raj Nighantu* uses the synonym *Dhumrapatra* and describes its use in *Krimi-nashana* (destruction of organisms/pests) under the *Shatpushpadi Varga*, additionally indicating its utility in *Vishama Jwara* (intermittent fever).[3]

Table-1. Classical Ayurvedic References to Keetmari (Aristolochia bracteata) and Its Pesticidal Properties.

Sr. No.	Classical Text	Name / Varga	Indicated properties / Uses
1.	Charaka Samhita	Kitamari–Krimighna Gana	Anthelmintic, anti-inflammatory; used in Krimiroga and Kushtha
2.	Sushruta Samhita	Krimighni – Aragvadhadi Gana	External application in maggot-infested wounds; wound cleansing
3.	Ashtanga Hridayam	Nakuli – Krimighna dravya	Katu-Tikta Rasa; Ushna Veerya; Kapha-Vata Shamaka
4.	Bhavaprakash Nighantu	Kitamari – Haritakyadi Varga	Krimiroga, Vrana Shodhana, Visha (toxic insect bites)
5.	Raj Nighantu	Dhumrapatra – Shatpushpadi Varga	Krimi-nashana, Vrana-ropana; used in Vishamajwara
6.	Dhanvantari Nighantu	Visanika / Keetmari	Anthelmintic; bark and root used for fever and skin diseases
7.	Kaiyadeva Nighantu[11]	Krimighni	Teekshna Guna; effective in Kaphaja Krimiroga and Udara Roga
8.	Priya Nighantu[12]	Keetmari – Haritakyadi Varga	Katu-Tikta; Krimi-nashaka, Jwara-nashaka

Plant Material Collection and Authentication

Fresh aerial parts (leaves, stems, and roots) of Aristolochia bracteata Lam. were collected from the dry scrubland regions of Gujarat, India. The plant was botanically authenticated by a qualified botanist. The collected plant material was shade-dried, coarsely powdered using a mechanical grinder, and passed through a sieve (Mesh No. 40) to obtain a uniform powder.

Preparation of Hydroalcoholic Extract :

Hydroalcoholic extraction was performed using methanol as the solvent (hydroalcoholic extraction with methanol is standard for broad-spectrum phytochemical profiling, capturing both polar and semi-polar compounds, including alkaloids, flavonoids, and phenolics). Twenty grams of coarse powder were subjected to cold maceration in methanol (1:10 w/v) for 72 hours with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator at 40°C, and stored at 4°C until use. The percentage yield was calculated.[11]

HPTLC Analysis :

Instrumentation and Conditions :

HPTLC analysis was performed at the Center for Research and Development (CR4D),

Parul University, Vadodara using the vision CATS software platform (version 3.2.23095.1). The instrumental setup comprised:

- Stationary Phase: Merck HPTLC Silica Gel 60 F254 plates (100 × 100 mm)
- Sample Applicator: Linomat 5 (S/N: 280008); dosage speed 150 nL/s; pre-dosage volume 0.20 µL
- Mobile Phase: Toluene : Ethyl Acetate : Formic Acid (5:4:1 v/v/v)
- Development Chamber: TTC 10×10; saturation time 20 min with saturation pad; solvent front 80 mm
- Sample Solvent: Methanol
- Scanner: TLC Scanner 4 (S/N: 271118); scanning speed 100 mm/s; data resolution 100 µm/step; slit 6 × 0.45 mm (micro)
- Detection wavelengths: 254 nm (Deuterium lamp, absorbance mode) and 366 nm (Mercury lamp, fluorescence mode with K400 filter)

Sample Application :

Four tracks were applied from a single vial (Vial ID: 1) containing the methanolic extract of Keetmari at ascending volumes of 5.0, 10.0, 15.0, and 20.0 µL, respectively. Application position: Y = 8.0 mm; track first position X = 15.0 mm; inter-track distance = 22.4 mm.

Peak Integration and Evaluation :

Two evaluation protocols were applied. Evaluation 1 used data from the 254 nm scan (plate 1a), and Evaluation 2 used data from the 366 nm fluorescence scan (plate 1b). Integration bounds were set at [0.040–1.000] for 254 nm and [0.037–0.983] for 366 nm. Smoothing was performed using the Savitzky–Golay algorithm (window 7), baseline correction by the lowest slope method, and peak detection using the Gauss legacy algorithm with sensitivity 0.1, separation 1, and threshold 0.1.

Densitometric Profiles—254 nm and 366 nm

Figures 1–4 present the representative densitometric scans selected for maximum phytoconstituent resolution. Figure 1 (Track 3, 366 nm, 15 µL) shows eight well-resolved peaks reflecting the richest fluorescent phytochemical profile. Figure 2 (Track 1, 254 nm, 5 µL) illustrates the three primary absorbance peaks at low concentration. Figure 3 (3D overlay, 366 nm, all four tracks) demonstrates peak consistency across concentrations, confirming method reproducibility.

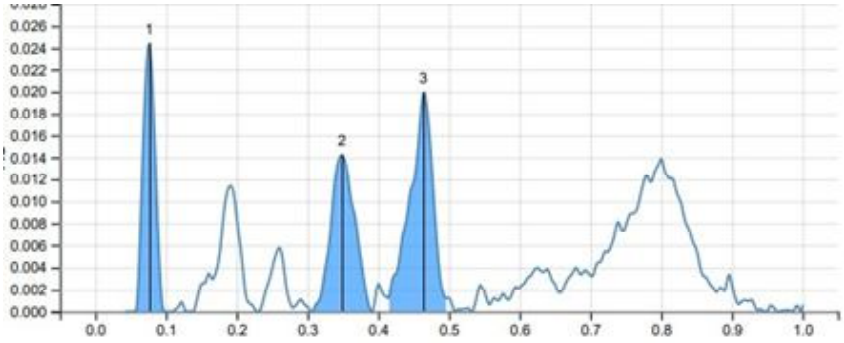

Figure 1. HPTLC densitogram — Track 3 (15 µL), 366 nm fluorescence. Eight peaks reveal flavonoids, aristolactams, and phenolic acids (dominant pesticidal constituents).

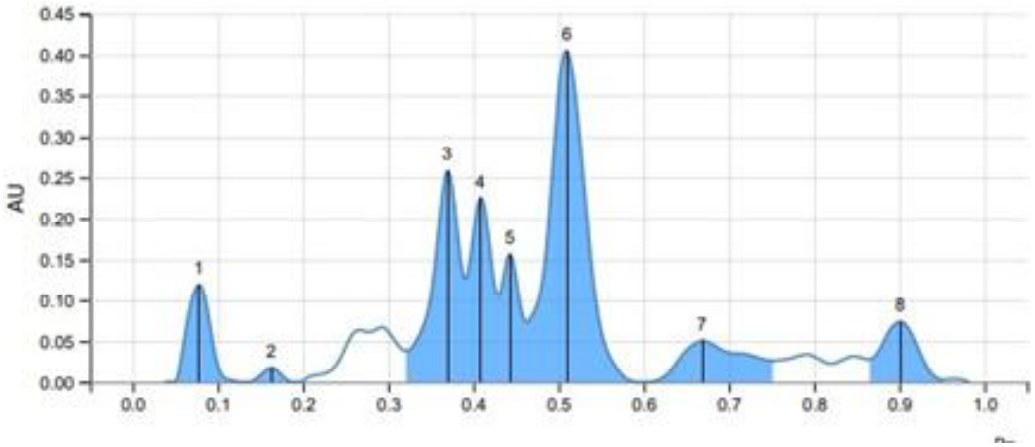

Figure 2. HPTLC densitogram — Track 1 (5 µL), 254 nm absorbance. Three peaks: Rf ~0.076 (magnoflorine), ~0.349 (aristolochic acid II), ~0.464 (aristolochic acid I).

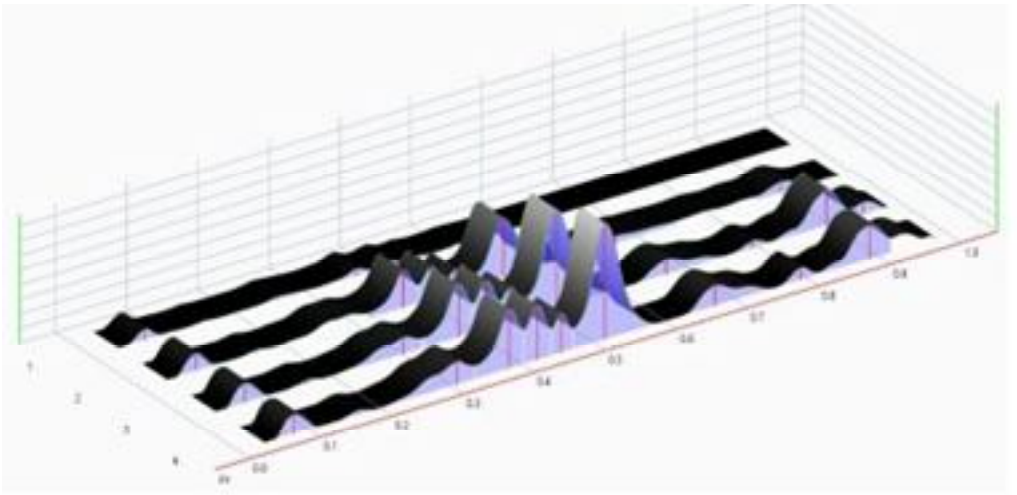

Figure 3. 3D overlay chromatogram — 366 nm (all four tracks). Consistent peak positions confirm method reproducibility; dominant bands at Rf ~0.37 and ~0.51.

Peak Assignment and Phytoconstituent Correlation :

Table-2 summarises the key HPTLC peaks from all tracks at both wavelengths with tentative phytoconstituent assignments based on literature Rf values and pharmacological relevance to pesticidal activity.

Table-2. Key HPTLC Peaks and Tentative Phytoconstituent Assignments (254 nm & 366 nm)

Track	Vol. (µL)	Rf (Max)	λ (nm)	Area %	Tentative Compound
1	5	0.076	254	26.94	Magnoflorine / polar alkaloid
1	5	0.349	254	31.18	Aristolochic acid II
1	5	0.464	254	41.88	Aristolochic acid I (dominant)
2	10	0.606	254	55.98	Aristolochic acid I (major pesticidal zone)
3	15	0.613	254	56.32	Aristolochic acid I (confirmed dominant)
4	20	0.611	254	46.48	Aristolochic acid I
1	5	0.075	366	35.43	Flavonoid/phenolic (quercetin-type)
2	10	0.510	366	38.50	Kaempferol / phenolic acid
3	15	0.517	366	33.92	Aristolactam / flavonoid zone
4	20	0.517	366	34.40	Aristolactam / flavonoid zone

At 254 nm, the peak at Rf ~0.60–0.61 dominated all higher-concentration tracks (area 46–56%), consistent with aristolochic acid I (nitrophenanthrene chromophore). The secondary peak at Rf ~0.35–0.46 corresponds to aristolochic acid II. At 366 nm, peaks at Rf ~0.51–0.52 (area 33–38%) and ~0.37 (14–18%) match reported values for kaempferol/ quercetin-type flavonoids and aristolactams in the same mobile phase system. Collectively, these four phytochemical zones — aristolochic acid I, aristolochic acid II, flavonoids, and aristolactams — constitute the core pesticidal fingerprint of A. bracteata.

The phytochemical constituents detected by HPTLC in the methanolic extract of A. bracteata are in remarkable concordance with the Ayurvedic pharmacological description of the plant. The dominant HPTLC peak at Rf ~0.60–0.61 (254 nm), constituting 46–56% of the total area at pharmacologically relevant concentrations (10–20 µL), is consistent with aristolochic acid I as previously reported by multiple research groups using the same TLC system. The dominant HPTLC peak at Rf ~0.60–0.61 (254 nm), constituting 46–56% of the total area at pharmacologically relevant concentrations (10–20 µL), is consistent with aristolochic acid I as previously reported by multiple research groups using the same TLC system[14]. The *Katu-Tikta Rasa* further explains persistent anthelmintic action: *Katu (pungent) dravyas* stimulate *Agni* (metabolic fire) and create an inhospitable gastrointestinal milieu for parasites. *Teekshna* penetration to destroy parasitic organisms. The *Ushna Veerya* (hot potency) may be pharmacologically interpreted as the thermogenic and metabolically disruptive properties of aristolochic acids which cannot regulate their metabolism against such interference.

Correlation with Modern Pesticidal Research : The plant extract shows significant larvicidal effects against Aedes aegypti and

Culex mosquitoes, insecticidal activity against pests like Helicoverpa armigera and Spodoptera litura, and anthelmintic efficacy against Haemonchus contortus in vitro[15,16]. The HPTLC fingerprint established here provides the phytochemical baseline for standardising such nano-formulations.

The HPTLC fingerprint of Aristolochia bracteata methanolic extract, characterized by dominant pesticidal peaks at Rf ~0.07 (magnoflorine), ~0.35–0.37 (aristolochic acid II / flavonoid), ~0.46–0.47 (alkaloid zone), and ~0.60–0.61 (aristolochic acid I), provides compelling scientific validation for the classical Ayurvedic designation of Keetmari as a Krimighna dravya. The convergence of Teekshna Guna, Ushna Veerya, and Katu-Tikta Rasa with the molecular bioactivity of aristolochic acids and flavonoids demonstrates the predictive power of Ayurvedic pharmacological reasoning. The validated HPTLC method establishes a reproducible phytochemical identity standard for this important, yet pharmacovigilance-sensitive, medicinal plant, forming the foundation for future quantitative marker studies, in vivo pesticidal efficacy trials, and safe Ayurvedic formulation development.

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