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HPTLC FINGERPRINTING OF METHANOLIC EXTRACT OF *KOKILAKSHA (Astercantha longifolia NEES, Syn. Hygrophila spinosa T. ANDERS)* IN CRUSHED AND WATER EXTRACT FORM: A COMPARATIVE STUDY

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ABSTRACT

Kokilāksha (Astercantha Longifolia Nees. Syn., Hygrophila Spinosa T. Anders), an annual herb of the Acanthaceae family, is widely used in Ayurveda and other traditional Indian medical systems for its diverse therapeutic applications. The whole plant of *Kokilaksha* has been traditionally used in Ayurveda to manage a variety of conditions, including inflammatory disorders, urinary problems, and sexual health issues. *Āchāryas* have recommended it both as a *Kashaya* (water-based decoction) for its medicinal benefits and as a *Śāka Bhojana* (vegetable) for its nutritional value in daily diet. The present research is undertaken to evaluate the phytochemical profile of the methanolic extract of crushed whole plant and its *Kashaya* (aqueous decoction/aqueous extract) using High-Performance Thin-Layer Chromatography (HPTLC) at 254 nm and 366 nm. Results reveal that the methanolic extract of the crushed herb displays a richer and more diverse chromatographic profile than the methanolic extract of its water extract /decoction *(Kashaya).*This distinction underscores a fundamental Ayurvedic principle: therapeutic efficacy is not reliant on the complete phytochemical profile of a plant, but rather on the targeted extraction of specific bioactive constituents according to the disease. Thereby, *Ayurvedic* medicines are formulated in alignment with the pathogenesis of a disease with careful selection of ingredients and preparation methods that precisely target the disease process.

Key words : *kokilaksha*, HPTLC,*kashaya*, phytochemicals,methanolic extract

I. INTRODUCTION

Traditional medicine often employs both raw powdered herbs and their decoctions for therapeutic use. While the crude form represents the whole phytochemical spectrum, decoctions involve water-soluble constituents, possibly altering the phytochemical profile. HPTLC provides a visual and quantitative comparison of these two preparations.

Pharmacological studies on *Asteracantha longifolia Nees. Syn. Hygrophila Spinosa T. Anders* highlight its potential in treating conditions like diarrhoea, liver and kidney disorders, infections, and cancer. Its strong antioxidant activity is attributed to the presence of water-soluble compounds such as flavonoids, terpenoids, alkaloids, steroids, and tannins, which may contribute to reducing the risk of degenerative diseases.[1] *Asteracantha longifolia* is a widely

distributed aquatic shrub commonly found across India, Sri Lanka, and other parts of South Asia.[2] *Hygrophila auriculata* has been documented to exhibit a wide range of pharmacological activities, including analgesic, antitumor, antioxidant, hepatoprotective, hypoglycemic, haematinic, diuretic, free radical scavenging, anthelmintic, anti-inflammatory, antipyretic, and anabolic effects. Additionally, it shows androgenic, antimicrobial, antibiotic, insecticidal, and hormonal properties, highlighting its significant therapeutic and pharmaceutical potential.[3] Several previous studies have documented the pharmacological potential of *kokilaksha* reporting activities such as antitumor[4], hypoglycaemic[5], antibacterial[6] and hepatoprotective effects[7] thereby validating its traditional therapeutic applications. The plant is utilized in various forms, including hot water, methanol, and ethanol extracts.[8] Additionally, in certain eastern regions of India, it is consumed daily as a leafy vegetable during the course of medical treatment.[9]

MATERIALS AND METHODS

Collection of the plant

The whole *Asteracantha longifolia Nees (Kokilaksha)* plants were collected from field side of Tripunithura, Ernakulam, Kerala India. The plant specimen was taxonomically identified and authenticated by a botanist from the Department of Botany, St. Albert's College (Autonomous), Ernakulam and the whole dried plant was preserved with the voucher specimen SAC/BOT/HER/02/2025 in the Department's herbarium (Fig 1.)using the standard method (Jain and Rao,1977).

Fig 1. Herbarium of *Kokilaksha*

Preparation of the water extract or *kashaya*

The whole plant of *Kokilaksha* was then washed thoroughly, chopped and dried in sunlight. 50 gms of the crushed plant was boiled in medium flame with 800ml (16times) water and reduced to 200ml (1/4th) as per *Sarangdhara Samhita*.[10] The aqueous extract was transferred to an airtight container for storage.

Sample preparation

A total of 50 mL of the prepared *kashayam* (decoction) was evaporated to dryness using the oven drying method at 105°C, and the residue was collected. Separately, 1 g each of the dried decoction residue and the crushed raw herb were weighed accurately. Each sample was then mixed with 5 mL of methanol, followed by sonication in an ultrasonic bath for 20 minutes to enhance extraction efficiency. The mixtures were subsequently kept undisturbed overnight at room temperature to allow for thorough extraction. The following day, the supernatant methanolic extracts were carefully decanted and filtered and named as Sample 1 and Sample 2 respectively.These were then directly used for HPTLC analysis at Care keralam Ltd Koratty Thrissur, Kerala India

HPTLC analysis

Instrumentation

A CAMAG HPTLC system equipped with LINOMAT 5 applicator fitted with 100 µl syringe, CAMAG TLC scanner, and winCATS software was used.

Solvent system

Chloroform: Ethyl acetate: Formic acid (5:4:1)

Scanning

At both 254nm, 366nm

Visualisation

The developed plate was observed under UV light at wavelengths of 254 nm and 366 nm. The Rf values and the corresponding colours of the resolved bands were recorded.

Tracks representing sample position and volume

Track no.	Appl. Position	Appl. Volume	Vial#	Sample ID	Active
1	30.0mm	90.0 µL	1	SAMPLE 1	YES
2	76.6mm	90.0 µL	2	SAMPLE2	YES

Parameters used for hptlc; calibration parameters

PARAMETERS	VALUE
Calibration mode	Single level
Statistics mode	CV
Evaluation mode	Peak height

Linomat 5 application parameters

Spray gas	Inert gas
Sample solvent type	Methanol
Dosage speed	150 nl/s
Predosage volume	0.2 ul

Syringe size	100 µl
Number of tracks	2
Application position Y	15.0 mm
Band length	30.0 mm

Development - glass tank

Chamber type	Twin Trough Chamber 20x20cm
Executed on	Thursday, August 29, 2024 12:01:38 PM
Pre-conditioning	30mins
Mobile phase	Chloroform: ethylacetate: formic acid (5:4:1)
Solvent front position	120.0 mm
Volume	10.0 ml
Drying device	Oven
Temperature	60 °C
Time	5 Minutes

Detection - CAMAG TLC scanner instrument : CAMAG TLC scanner "scanner_171019" s/n 171019 (2.01.02)

Application position	15.0 mm
Solvent front position	120.0 mm
Number of tracks	2
Position of first track X	30.0 mm
Distance between tracks	46.6 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	145.0 mm
Slit dimensions	4.00 x 0.30 mm, Micro
Optimize optical system	Light
Scanning speed	20 mm/s
Data resolution	100 µm/step

Integration properties

Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	5.0 mm
Track end position	132.6 mm
Display scaling	Automatic

Measurement table

Wavelength	254
Lamp	D2 & W
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	189 V

Detector properties

Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (160)

RESULTS

HPTLC analysis of *Kokilaksha* confirmed the presence of various phytochemicals, as shown in the accompanying figures and tables. The chromatograms (FIGS. 2–4) were recorded under UV light at wavelengths of 254 nm and 366 nm, and corresponding peak tables were generated.

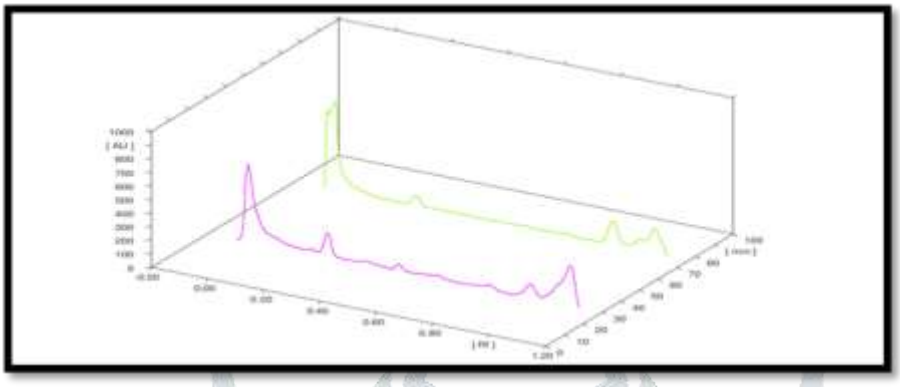


Fig 2. 3D densitogram of all tracks scanned at 254 nm

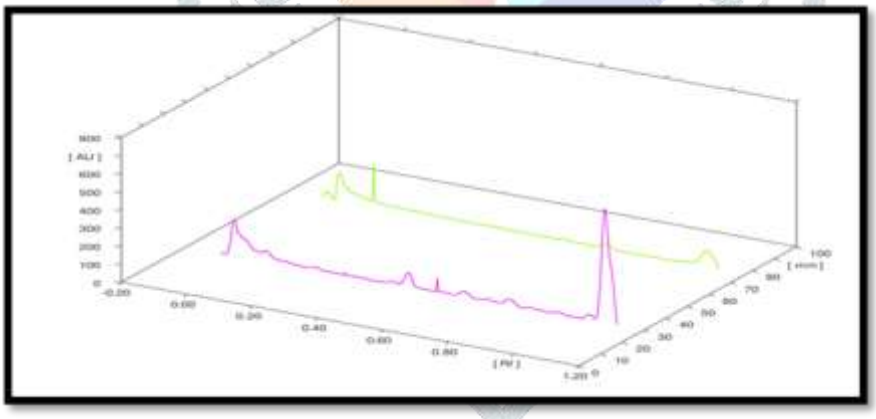
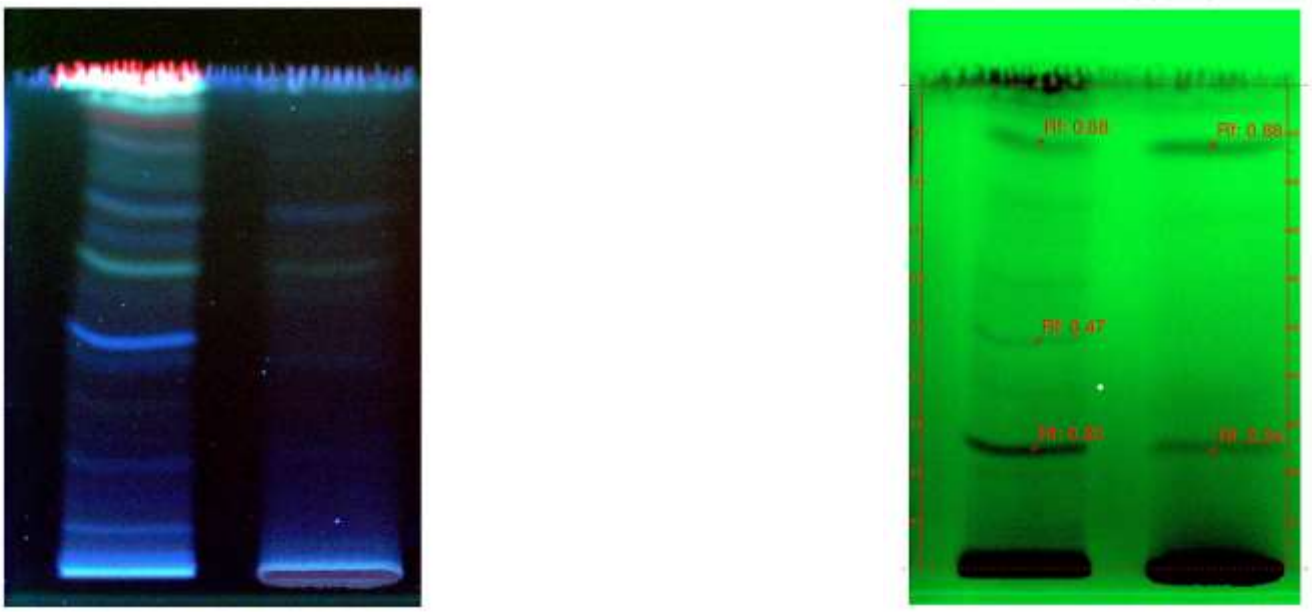


Fig 3. 3D densitogram of all tracks scanned at 366 nm



(a) (b)

Fig 4 HPTLC CHROMATOGRAMS VISUALIZED UNDER (A) UV 254NM (B) UV 366NM

The retention factor (Rf) values, along with peak height, peak area, and percentage area of the unidentified phytochemicals, are detailed in the (TABLES 1–4). At 254 nm Sample 1 exhibited 10 distinct bands with significant peaks at Rf 0.99 (27.66% area) and Rf -0.06 (39.93% area), indicating the presence of diverse and abundant phytoconstituents - Sample 2 (*Kashaya*) showed 8 bands, with a dominant peak at Rf -0.06 (43.41%) and another at Rf 1.08 (15.53%). Overall, fewer bands and lower area percentages were observed (TABLE 5).

PEAK NO.	Rf VALUE	AREA(AU)	%AREA(AU)
1	-0.06	28069.5	39.93
2	0.04	2643.9	3.76
3	0.17	1701.4	2.42
4	0.23	6431.8	9.15
5	0.36	1473.3	2.10
6	0.48	2003.5	2.85
7	0.62	1551.9	2.21
8	0.80	1832.5	2.61
9	0.95	5149.4	7.32
10	1.09	19447.3	27.66

TOTAL PEAKS: 10 TOTAL AREA: 70304.5

TABLE 1: Rf value & % area of methanolic extract of dry *Kokilaksha* herb (sample 1) at 254nm

PEAK	Rf VALUE	AREA(AU)	%AREA
1	-0.09	8882.2	15.31
2	-0.06	25193.3	43.41
3	0.07	294.6	0.51
4	0.23	2752.8	4.74
5	0.76	865.4	1.49
6	0.93	7698.5	13.27
7	1.03	3327.7	5.73
8	1.08	9014.7	15.53

TOTAL PEAKS: 8 TOTAL AREA: 58029.2

TABLE 2: Rf value & % area of methanolic extract of *Kokilaksha kashaya* (sample 2) at 254nm

PEAK NO	Rf VALUE	AREA	%AREA
1	-0.05	9871.6	23.93
2	0.04	2391.6	5.80
3	0.19	645.0	1.56
4	0.28	73.1	0.18
5	0.43	448.4	1.09
6	0.48	2359.1	5.72
7	0.57	442.9	1.07

8	0.65	1145.6	2.78
9	0.72	541.1	1.31
10	0.79	1281.3	3.11
11	0.94	422.5	1.02
12	1.03	822.6	1.99
13	1.08	20815.2	50.45

TOTAL PEAKS: 13 TOTAL AREA: 41260

TABLE 3: Rf value & % area of methanolic extract of dry *Kokilaksha* herb (sample 1) at 366nm

PEAK	Rf VALUE	AREA	%AREA
1	**-0.08**	**587.6**	**3.53**
2	**-0.04**	**7661.9**	**45.99**
3	**0.06**	**2384.7**	**14.31**
4	**0.77**	**1214.0**	**7.29**
5	**1.08**	**4812.5**	**28.89**

TOTAL PEAKS: 5 TOTAL AREA: 16660.7

TABLE 4: Rf value & % area of methanolic extract

of Kokilaksha kashaya (sample 2) at 366nm

At 366 nm Sample 1 revealed 13 bands, with dominant peaks at Rf 1.08 (50.45%) and Rf -0.05 (23.93%). - Sample 2 had only 5 bands, prominently featuring Rf -0.04 (45.99%) and Rf 1.08 (28.89%). The reduced number of bands indicates that fewer UV-fluorescent phytoconstituents were present in the *Kashaya* (TABLE 6).

Track	No. of peaks	Major Peak Rf (Intensity %)	Total Area (%)
Sample 1	10 peaks	Rf 0.99 (20.88%), Rf -0.06 (39.94%)	100%
Sample 2	8 peaks	Rf -0.06 (36.74%), Rf 1.08 (10.09%)	100%

Table 5: At 254 nm (Absorbance Mode)

Track	No. of Peaks	Major Peak Rf (Intensity %)	Total Area (%)
Sample 1	13 peaks	Rf 1.08 (51.84%)	100%
Sample 2	5 peaks	Rf -0.04 (28.08%), Rf 1.08 (16.84%)	100%

TABLE 6: At 366 nm (Fluorescence Mode)

DISCUSSION

HPTLC analysis of the methanolic extracts from crushed *Kokilaksha* (Sample 1) and *Kokilaksha Kashaya* (Sample 2) revealed the presence of various phytoconstituents in differing concentrations, as illustrated in the accompanying figures and tables. Sample 1 (crushed herb) exhibited a more diverse distribution of peaks, indicating a broader phytochemical profile. In contrast, Sample 2 (decoction) showed fewer peaks, reflecting the presence of primarily water-soluble compounds. However, it retained some prominent constituents also observed in Sample 1—for instance, at Rf 1.08. Shared peaks in the Rf range of 0.88 to 1.08 suggest the presence of common bioactive compounds in both preparations.

Common peaks across samples

Rf ~1.08: Present in both samples at both 254 nm and 366 nm → indicates a shared major compound.

Rf ~0.23 & 0.93: Similar retention, but intensity varies — potentially indicating degradation or transformation during decoction.

Peak diversity

Crushed herb shows more peaks (13 at 366 nm) indicating greater phytochemical complexity. Decoction retains fewer but stronger peaks, especially water-soluble or thermally stable ones. While methanolic extraction yields a broader spectrum, the decoction exhibits a curated and selective profile, consistent with the Ayurvedic principle of targeted extraction.This is not indicative of inferiority but rather of intentional formulation.

Ayurvedic medicine emphasizes that not all phytochemicals are needed or beneficial in every disease condition. Decoctions are typically administered for acute conditions, where astringency, lightness, and rapid action are needed—qualities often associated with polar and water-soluble compounds. This selectivity may exclude lipid-soluble or volatile constituents that are not appropriate for the intended therapeutic action.

Supporting literature confirms that the method of extraction significantly impacts the phytochemical profile. Similar outcomes were observed in studies of Ginger[11], where aqueous formulations yielded fewer bands compared to methanolic counterparts but retained their therapeutic effects. This supports the Ayurvedic perspective that efficacy arises from the synergy and relevance of active principles, not their sheer quantity.This finding affirms the Ayurvedic concept of *Samskara Visheshat*, where the mode of preparation governs the pharmacological outcome by modifying the chemical behavior of the drug.

CONCLUSION

HPTLC profiling demonstrates that Ayurvedic *Kashaya* preparations, while chemically less complex than methanolic extracts, reflect a highly selective and purpose-driven extraction strategy rooted in classical Ayurvedic principles.Ayurvedic formulations like Kashaya are designed not for chemical abundance, but for therapeutic precision—extracting only those phytoconstituents necessary to correct the underlying doshic imbalance However, the limitations of HPTLC in compound identification highlight the need for advanced phytochemical analyses such as LC-MS QTOF, NMR, or HPLC-MS to elucidate the structural identity, synergistic interactions, and pharmacological potential of the bioactive compounds present. Such integrative analysis can bridge traditional Ayurvedic wisdom with modern pharmacognosy, supporting evidence-based standardization and therapeutic validation.This study, being primarily spectro-analytical, warrants follow-up investigations using techniques such as LC-MS QTOF, NMR, and in vitro pharmacological validation to substantiate compound identity and activity

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