EJPMR, 2026, 13(5), 478-487 **Research Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

HPTLC METHOD DEVELOPMENT FOR THE ESTIMATION OF ASTERACANTHA LONGIFOLIA IN RASNAIRANDADI KASHAYAM – AN AYURVEDIC POLYHERBAL FORMULATION

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DOI: https://doi.org/10.5281/zenodo.20023529

How to cite this Article: Revathy A Kumar*, Rakesh Kumar Jat, J. Jaslin Edward. (2026). Hptlc Method Development For The Estimation Of Asteracantha Longifolia In Rasnairandadi Kashayam – An Ayurvedic Polyherbal Formulation. European Journal of Pharmaceutical and Medical Research, 13(5), 478–487.

Article Received on 28/03/2026 **Article Revised on 18/04/2026** **Article Published on 01/05/2026**

ABSTRACT

The herbal products market in India is aggressively expanding. The standardisation of these herbal medications is necessary for the safety and effectiveness of herbal goods. There is a need for more sophisticated standardisation approaches because the conventional methodology is insufficient for the contemporary herbal industry. With this view the present study was undertaken to develop a standardisation procedure viz., Q-HPTLC profiling for a polyherbal formulation-rasnairandadi kashayam. Initially, the ingredients of rasnairandadi kashayam was procured. Among all the ingredients necessary for the preparation of rasnairandadi kashayam, the present study focussed on quantitative estimation of an ingredient, *Asteracantha longifolia* root composition. The selected ingredient was standardized according to WHO guidelines 1992 and IP, 1996. Simultaneously, extraction and phytochemical screening of the selected ingredient was done. Next, by using all the purchased ingredients, different concentration of rasnairandadi kashayam formulation was prepared as per the procedure in Sahasrayoga Kashaya Prakarna. 428. Meanwhile, two brands of rasnairandadi kashayam (Brand A & B) available in the market of Kerala was purchased. The samples from all the four different concentrations of rasnairandadi kashayam prepared and two brands (Brand A & B) purchased were analysed for the phytoconstituents. Finally, QHPTLC evaluation were done to confirm the presence of ingredient in the formulations as per the guidelines of Ayurvedic Pharmacopoeia of India (API). From this study it was concluded that the sample from purchased brand A rasnairandadi kashayam contains *A. longifolia* as specified in API. But the Brand B contains only nearly half the amount of *A. longifolia*. Summarily, through this present study, it was able to develop a sophisticated method for quantification of individual ingredient present in poly-herbal Ayurvedic preparations.

KEYWORDS: Rasnairandadi kashayam, *Asteracantha longifolia* root, Q-HPTLC profiling

INTRODUCTION

The application of herbs for medicinal purposes represents the most ancient method of healthcare recognized by humanity, utilized across all cultures throughout history. Primitive humans acknowledged their reliance on nature for maintaining health, and since that era, humanity has relied on the variety of plant resources for sustenance, clothing, shelter, and medicinal remedies to cure numerous ailments. The understanding of plant-derived medications evolved over time and was transmitted through generations, thereby establishing the foundation for numerous traditional medicine systems globally. In certain communities, herbal medicine remains a vital component of their healthcare practices. Medicinal plants are found extensively across the globe, with the highest concentration in tropical regions.[1]

India has a lengthy, secure, and uninterrupted history of utilizing numerous herbal medicines within the officially acknowledged alternative health systems, namely Ayurveda, Yoga, Unani, Siddha, Homeopathy, and Naturopathy. These systems have justifiably coexisted alongside allopathy and are not in "the domain of obscurity". A millions of Indians consistently utilize herbal medications in various forms, including spices, home remedies, health foods, and over-the-counter

options for self-medication, as well as medications prescribed within non-allopathic systems. Over 500,000 non-allopathic practitioners have received training in the medical colleges (more than 400) associated with their respective health systems and are officially registered with the councils that oversee professionalism. Therefore, these systems should not be regarded as mere folklore or traditional herbal practices. They are founded on fundamental principles that contribute to a logical and systematic framework for understanding pathogenesis and diagnosis, which also serves as a basis for therapeutic interventions.[2]

Though herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control profile. At present no official standards are available for herbal preparations. Those manufacturers, who are currently doing some testing for their formulations, have their own parameters, many of which are very preliminary in nature. Presently it is very difficult to identify the presences of all the ingredients as claimed in a formulation. Hence the first important task is to evolve such parameter by which the presence of the entire ingredient can be identified, various chromatographic and spectrophotometric methods and evaluation of physicochemical properties can be tried to evolve pattern for identifying the presence of different ingredient. Wherever possible these methods can be applied for quantitative estimation of bioactive group of compounds like alkaloids, flavonoids, poly phenolic components or estimation of particular compound.[3-5] With this background, the present work aims at the standardisation of an ayurvedic polyherbal formulation, *Rasnairandadi kashayam*. Hopefully, this study stands as a strong proof for the standardisation of selected poly-herbal formulation.

MATERIALS AND METHODS

Procurement of ingredients of Rasnairandadi kashayam

Ingredients of Rasnairandadi kashayam (Table 1) was procured from Hema Ayurvedic Centre located at No. AP X/1391, Thazhamel, Anchal, Kollam District of Kerala.

Standardization of procured ingredients

One among the procured ingredients, *Asteracantha longifolia* root was standardized in the present study according to WHO guidelines 1992 and IP, 1996. The procedures recommended in IP, 1996 and WHO guidelines 1992 were followed to assess the physicochemical constants such as ash values viz., total ash, water soluble ash, acid insoluble ash and sulphated ash and extractive values viz., ethanol soluble extractive, water soluble extractive and ether soluble extractive values. Volatile oil content and loss on drying assessment were also done.

Extraction and preliminary phytochemical evaluation

Powdering and extraction of selected ingredient, *A. longifolia* root (Soxhlet extraction using the solvents such as petroleum ether, chloroform, ethyl acetate, methanol and water) and the preliminary phytochemical evaluation of prepared extracts (test for alkaloids, glycosides, phenolic compounds and tannins, flavonones and flavonoids, carbohydrates, proteins and aminoacids, terpenoids, saponins, and sterol) were done in reference with the standard procedure.[6-13]

Preparation of Rasnairandadi kashayam

Four different concentrations (N-Std; Q-Std; H-Std; D-Std) of rasnairandadi kashayam were prepared. For the preparation, all the ingredients mentioned in the Table 1 was cleaned and crushed to form a coarse powder and add it to 16 times water and boil it till it reduces to 1/4th its actual amount and get it off the flame and let it cool. Filter this decoction and keep the filtrate in tinted glass bottles.

Table 1: Ingredients of Rasnairandadi Kashayam.

No	Ingredients	Scientific name	Family	Plant part used
1	Rasna	*Alpina galanga*	Zingiberaceae	Root/leaf
2	Ikshura	*Asteracantha longifolia*	Acanthaceae	Root
3	Vasa	*Adhatoda vasica*	Acanthaceae	Root
4	Sahachara	*Barleria prionitis*	Acanthaceae	Whole plant
5	Ghana	*Cyperus rotundus*	Cyperaceae	Rhizome
6	Shati	*Hedychium spicatum*	Zingiberaceae	Rhizome
7	Vishwa	*Zingiber officinale*	Zingiberaceae	Rhizome
8	Vari	*Asparagus racemosus*	Asparagaceae	Root
9	Dusparsha	*Tragia involucrata*	Euphorbiaceae	Whole plant
10	Bala	*Sida cordifolia*	Malvaceae	Root
11	Amruta	*Tinospora cordifolia*	Menispermaceae	Stem
12	Dehahva	*Cedrus deodara*	Pinaceae	Hard wood
13	Ativisha	*Aconitum heterophyllum*	Ranunculaceae	Root tuber
14	Eranda	*Ricinus communis*	Euphorbiaceae	Root

Preparation of N-Std was done as per the procedure mentioned in Sahasrayoga Kashaya Prakarna. 428. For the preparation of Q-Std, 1/4th of the standard quantity of the ingredients mentioned in the Sahasrayoga Kashaya Prakarna. 428 were taken and the preparation was done as per the procedure stated above. Likewise, the H-Std was prepared by using ½ of the standard quantity and D-Std was prepared by using double the amount of standard quantity of ingredients recommended by the Sahasrayoga. All the four prepared formulations were stored properly for further studies.

Procurement of marketed brands of rasnairandadi kashayam

Two brands (Brand A & B) of rasnairandadi kashayam available in the market of Kerala was purchased from the Kollam district of Kerala and preserved properly for further studies.

Phytochemical analysis of the formulations prepared and purchased

The samples from all the four different concentrations of rasnairandadi kashayam prepared and two brands (Brand A & B) purchased were analysed for the presence of phytoconstituents as stated above.

HPTLC evaluation

In the HPTLC evaluation, the ingredient, *A. longifolia* root (No. 2 in Table 1) was analyzed comparatively with the normal standard of rasnairandadi kashayam prepared to confirm the presence of that ingredient in the preparation. In the next level, the quantitative HPTLC evaluation was done in which the selected ingredient was subjected to comparative evaluation with four different concentrations of the prepared rasnairandadi kashayam and the two purchased (Brand A & B) formulations from the market. The instrument details such as system setup and chromatographic data are presented in Table 2. The mobile phase used in HPTLC analysis was Ethyl acetate: Methanol: Ammonia in the ratio of 8: 2: 1. The Rf value was found at 254nm.

Table 2: HPTLC and Chromatography specifications.

Parameter		**Specification**
System setup	Software	Server Desktop-60R112G, Version 3.2.23095.1
	Linomat 5	S/N: 241506
	TLC scanner 4; TLC visualizer 2	S/N: 241072; S/N: 241537
Plate layout	Stationary phase	Merck, HPTLC Silica gel 60 F_{254}
	Plate format; Application type	100 × 100mm; Band
	Application	PositionY:8.0mm;Length: 8.0mm; Width:0mm
	Track	Ist position X: 15.0mm Distance: 11.4mm (In quantitative HPTLC, Ist position X: 20.0mm)
	Solvent front position	70mm
Sample solvent type: Methanol; **Dosage speed**: 150nL/s; **Pre-dosage vol**.: 0.20µl		
Chamber	Tank; Saturation time	20 × 10; 20min
	Use saturation pad;	true
	Use smartALERT	False
	Vol. front through; Vol. rear through	10mL; 20mL
	Drying time & Temp.;	5min; Room temperature
Image developed (plate 1a)	Quantity; RT white; R254; R366	Std.; Auto, level 85% Band
Scan developed (plate 1b & 1c)	Scanner type & Speed	Single λ; 20mm/sec
	Optimization for; Measurement mode	Resolution; absorbance
	Detector mode; Data resolution	Automatic; 100µm/step
	Slit	5 × 0.2mm; micro
	Lamp	For plate 1b: Deuterium & Tungsten; For Plate 1c: Mercury
	Wavelength	For plate 1b: 254nm; For plate 1c: 366nm

RESULTS AND DISCUSSION

Among the total of thirteen ingredients necessary for the preparation of rasnairandadi kashayam, the present study focussed on quantitative estimation of an ingredient, *A. longifolia* root powder in the four different concentrations (N-Std; Q-Std; H-Std; D-Std) of rasnairandadi kashayam prepared and two brands (Brand A & B) of rasnairandadi kashayam available in the

market of Kerala. Initially, the physicochemical constants such as ash values, extractive values, volatile oil content and loss on drying score of the root powder of *A. longifolia* were assessed and their results are shown in Table 3.

Table 3: Analysis of physicochemical constants of *Asteracantha longifolia* root powder – An ingredient of rasnairandadi kashayam

Physicochemical constants		*A. longifolia* root powder
Ash value (%w/w)	Total ash	8.60
	Water soluble ash	8.20
	Acid insoluble ash	1.75
	Sulphated ash	10.60
Extractive value (%w/w)	Ethanol soluble extractive	5.25
	Water soluble extractive	15.81
	Ether soluble extractive	3.25
Volatile oil (%w/w)	--	0.25
Loss on drying (%w/w)	--	1.87

In the preliminary phytochemical evaluation of *A. longifolia* root powder extracts, alkaloids and glycosides were found in ethyl acetate and methanol extracts of only. Tannins, carbohydrate, protein and amino acids were present all the tested extracts. Flavonoids and sterol were present in all the tested extracts except petroleum ether extract. Saponins were present in ethyl acetate, methanol and aqueous extracts (Table 4).

Table 4: Preliminary phytochemical evaluation of the *Asteracantha longifolia* root powder extracts.

Constituents	*A. longifolia* root powder extracts				
	1	2	3	4	5
Alkaloids	–	–	+	+	–
Glycosides	–	–	+	+	–
Tannins	+	+	+	+	+
Flavonoids	–	+	+	+	+
Terpenoids	–	–	+	+	+
Sterol	–	+	+	+	+
Saponin	–	–	+	+	+
Carbohydrate	+	+	+	+	+
Protein & Amino acids	+	+	+	+	+

1-Petroleum ether extract; 2-Chloroform extract; 3-Ethyl acetate extract; 4-Methanol extract; 5-Aqueous extract

In the preliminary phytochemical evaluation of all the four prepared concentrations of rasnairandadi kashayam and also the purchased rasnairandadi kashayam both brand A & B showed the presence of above stated phytoconstituents. The presence of *A. longifolia* and also the other ingredients present in the formulation might reasoned for the results obtained here.

A. longifolia one of the ingredients of rasnairandadi kashayam is extensively used in traditional system of medicine for various ailments and this plant finds mention in Ayurvedic treatise like "Sushruta Samhita" and "Charka Samhita" as Rasayan or rejuvenator. It is classified in ayurvedic system as Seethaveeryam, Mathuravipaka. It is used for the treatment of diabetes, dysentery, jaundice, dropsy, rheumatism, hepatic obstructions, dissolution of gallstones, kidney stones, liver dysfunction, edema, gout, diseases of urinogenital tracts, inflammation, pain, malaria, impotence and as aphrodisiac. The seeds are used as ingredients in various aphrodisiacs and tonic confections, and in the treatment of blood disorders, biliousness, gonorrhoea, spermatorrhoea and fever. The seeds are ground into a paste and given in buttermilk to cure diarrhoea. The ashes of the plant are also used against dropsy. A tincture of the whole plant is beneficial in urinary affections, dysuria, and painful micturition. A root decoction drunk to combat rheumatism, gonorrhoea, and hepatic obstruction. The leaves are diuretic, sweet, tonic, aphrodisiac, hypnotic and useful in the treatment of cough, diarrhoea, thirst, urinary calculi, urinary discharges, inflammations and spermatorrhoea.[14]

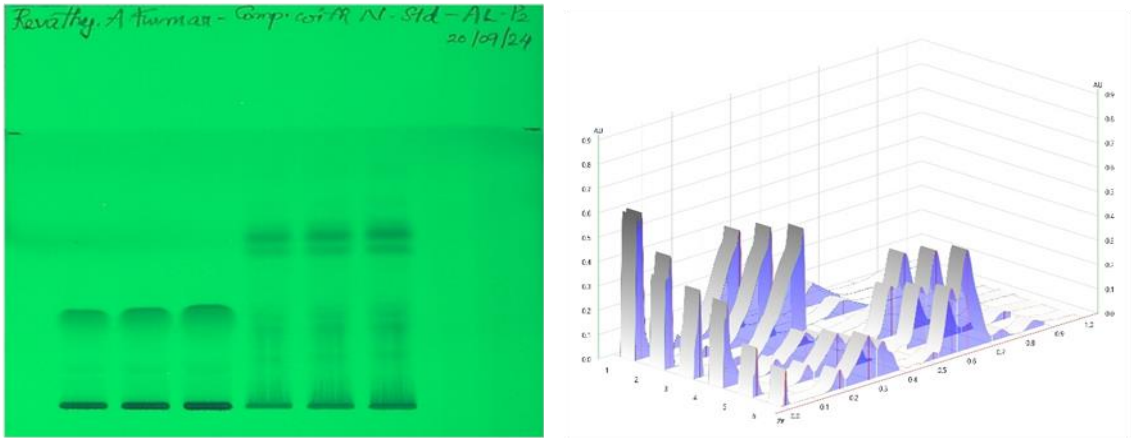
Literatures documented the presence of several phytochemicals, particularly, presence of alkaloids, fatty acids, and also lupeol, stigmasterol, β-sitosterol, butelin, n-Hexadecanoic acid, 9, 12-Octadecadienoic acid (Z, Z), Tetradecanoic acid, Ellipticine, 2-3 D ihydro benzofuran, β –carotene, Luteolin, Luteolin-7-rutinoside, Ascorbic acid, Apigenin, Hentriacontane, Apigenin 7-O-glucuronide, Apigenin-7 –O-glucoside, 2-propanone etc.[15]

In the HPTLC analysis of *A. longifolia*, initially, in the comparison of *A. longifolia* extract with normal standard

preparation of rasnairandadi kashayam was done by running the samples consecutively over six different tracks. Different concentration of normal standard was spotted in the first three tracks followed by different concentration of pure extract of *A. longifolia* on other three tracks as per the Table 5 and Figure 1. The 3D image of analysis was obtained by the wavelength scanning of all the six tracks. In this study, spot with Rf value 0.34 showed the presence of *A. longifolia* in all the six tracks.

Table 5: Comparison of *A. longifolia* extract with prepared rasnairandadi kashayam (N-std.)

Track no.	Sample name	Volume (µl)	Rf
1	N-std.	2	0.20; 0.34
2	N-std.	2.5	0.20; 0.34
3	N-std.	3	0.20; 0.34
4	AL	2	0.20; 0.30; 0.34
5	AL	2.5	0.20; 0.30; 0.34
6	AL	3	0.20; 0.30; 0.34



A **B**

Figure 1: Comparative study of *A. longifolia* extract with N-Std. of prepared rasnairandadi kashayam – A) Chromatogram development; B) 3D image.

In case of quantitative HPTLC, the pure extract of *A. longifolia* followed by N-std., Q-std., H-Std., and D-Std., sample from brand A and B were spotted at different concentrations over 15 tracks as mentioned in the Table 6 and Figure 2-17. The results showed that the sample A contains *A. longifolia* as specified in API because the AUC is same as that of Rf value 0.26 obtained in normal standard. But the sample B contains only nearly half the amount of *A. longifolia* and the AUC is similar to that of prepared half standard.

Table 6: QHPTLC analysis of *A. longifolia* extract and different concentration of rasnairandadi kashayam prepared and purchased brand (Sample A & B).

Track no.	Sample name	Volume (µl)	Rf	Area
1	AL	3	0.26	0.00448
2	AL	3	0.26	0.00429
3	AL	3	0.26	0.00448
4	N-std	3	0.26	0.03036
5	N-std	3	0.26	0.03041
6	Q-std	3	0.26	0.00780
7	Q-std	3	0.26	0.00798
8	H-std	3	0.26	0.01548
9	H-std	3	0.26	0.01533
10	D-std	3	0.26	0.05870
11	D-std	3	0.26	0.05816
12	Sample A	3	0.26	0.03058
13	Sample A	3	0.26	0.03051
14	Sample B	3	0.26	0.01569
15	Sample B	3	0.26	0.01597

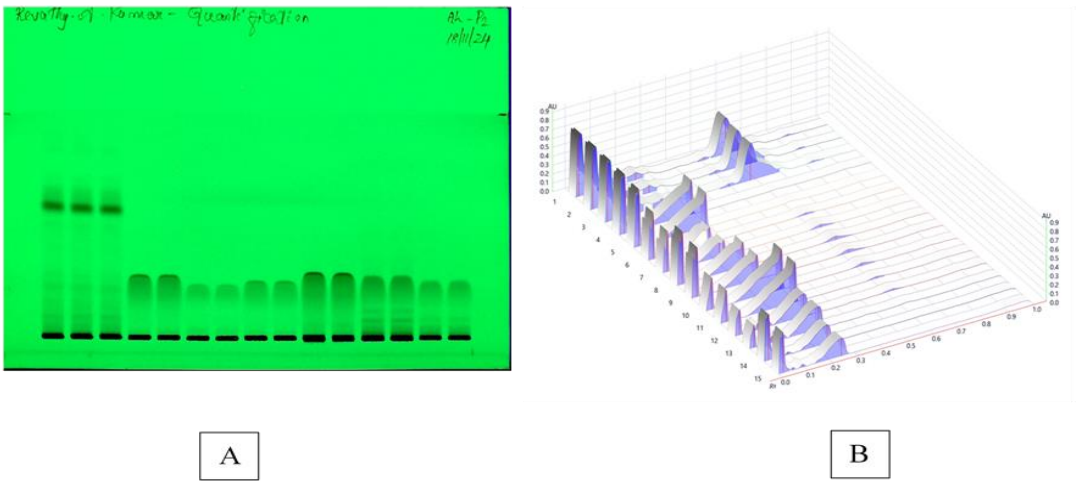


Figure 2: Quantitative HPTLC analysis of pure extract of *A. longifolia* with different concentration of rasnairandadi kashayam prepared and purchased brands - A) Chromatogram development; B) 3D image.

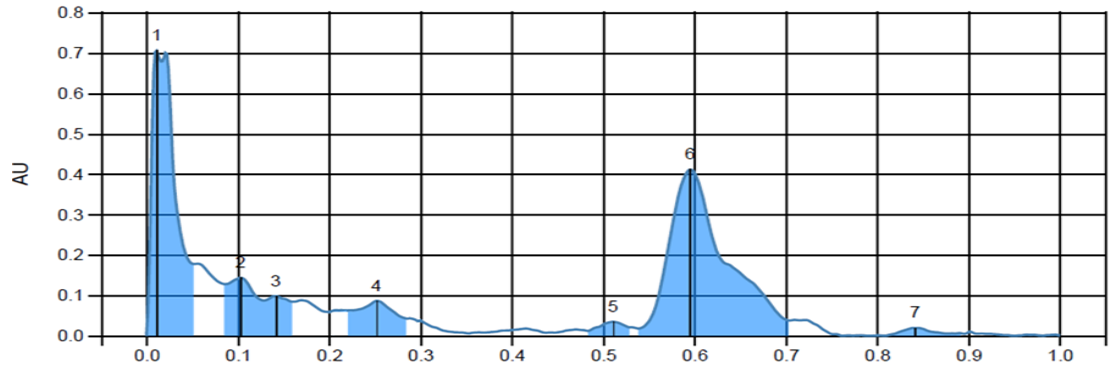


Figure 3: QHPTLC analysis of *A. longifolia* extract (Track 1).

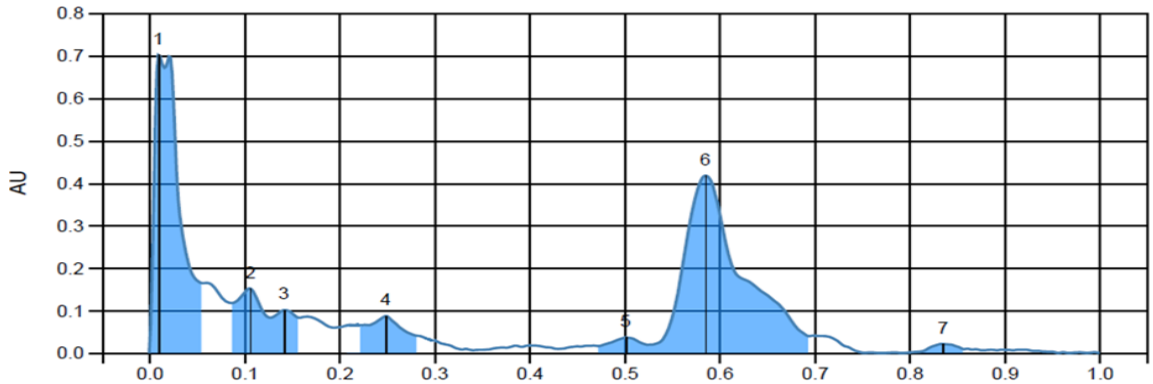


Figure 4: QHPTLC analysis of *A. longifolia* extract (Track 2).

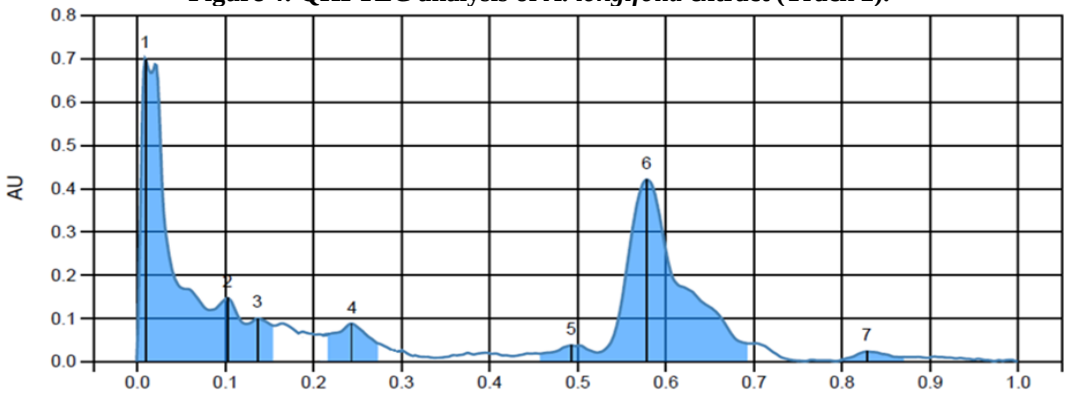


Figure 5: QHPTLC analysis of *A. longifolia* extract (Track 3).

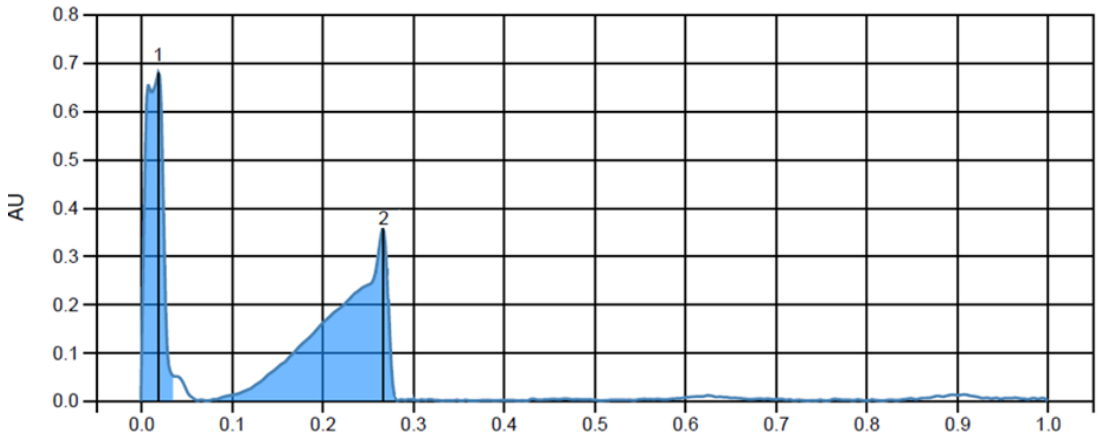


Figure 6: QHPTLC analysis of rasnairandadi kashayam (N-Std.; Track 4).

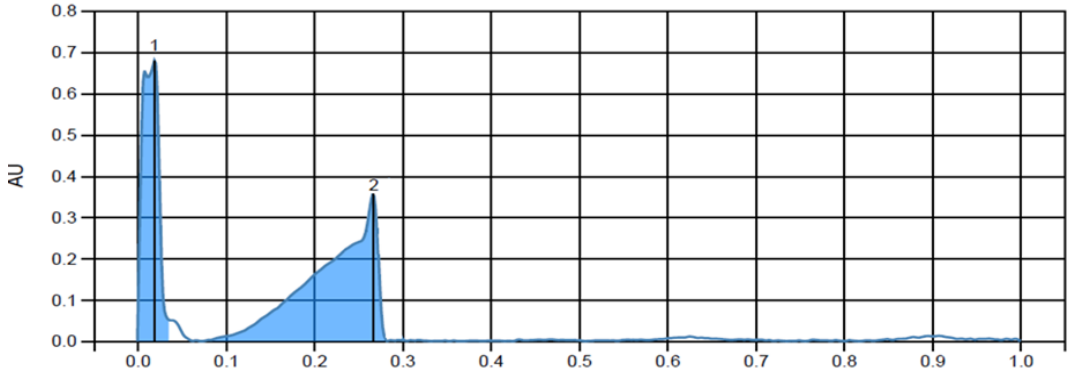


Figure 7: QHPTLC analysis of rasnairandadi kashayam (N-Std.; Track 5).

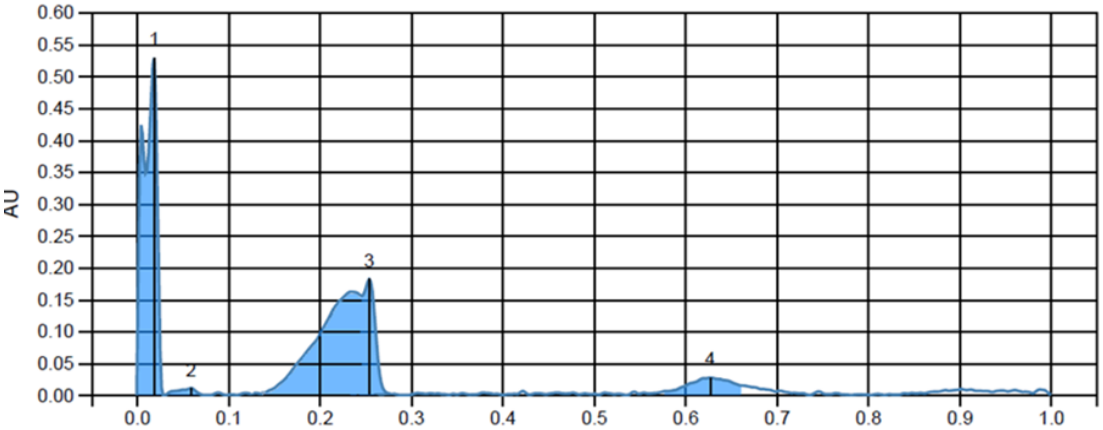


Figure 8: QHPTLC analysis of rasnairandadi kashayam (Q-Std., Track 6)

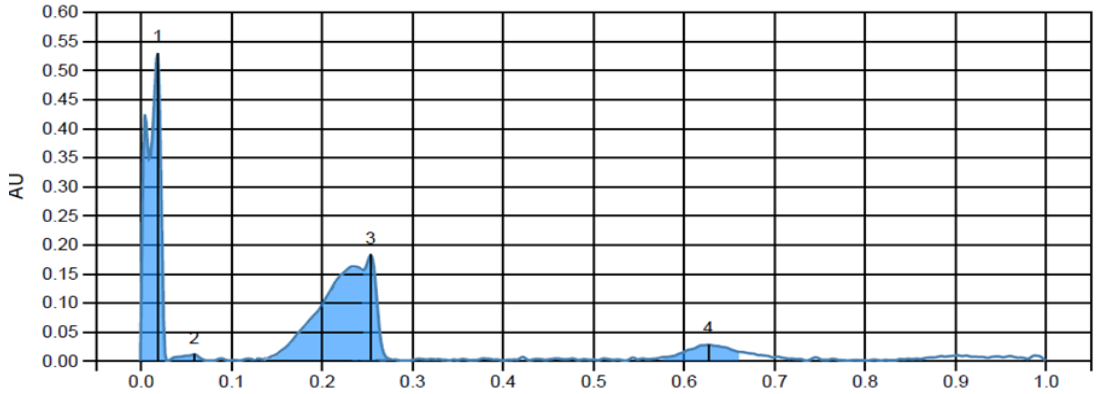


Figure 9: QHPTLC analysis of rasnairandadi kashayam (Q-Std., Track 7).

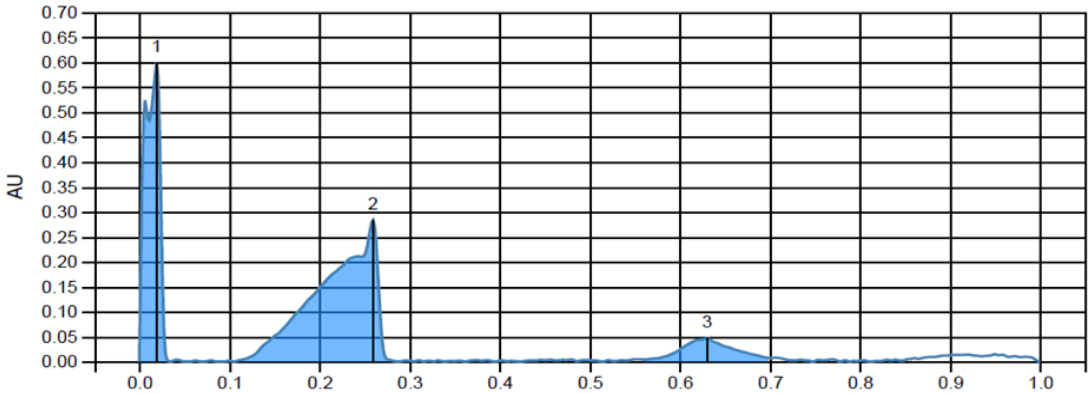


Figure 10: QHPTLC analysis of rasnairandadi kashayam (H-Std., Track 8).

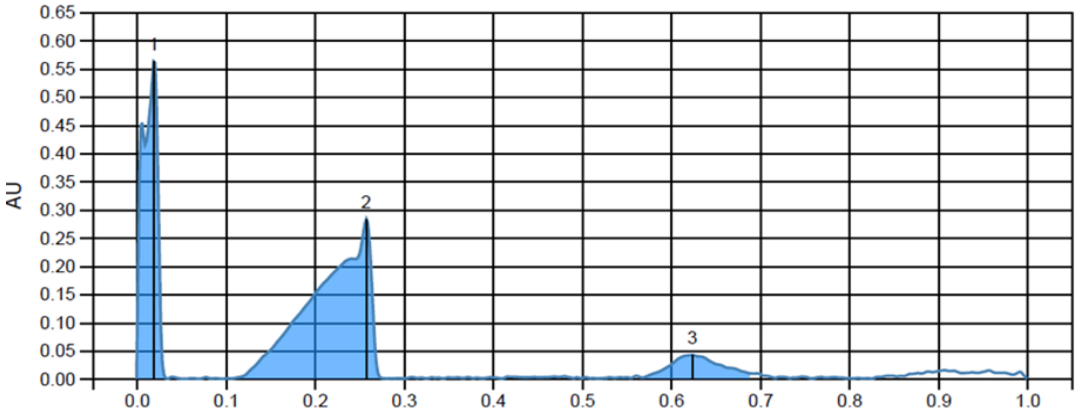


Figure 11: QHPTLC analysis of rasnairandadi kashayam (H-Std., Track 9).

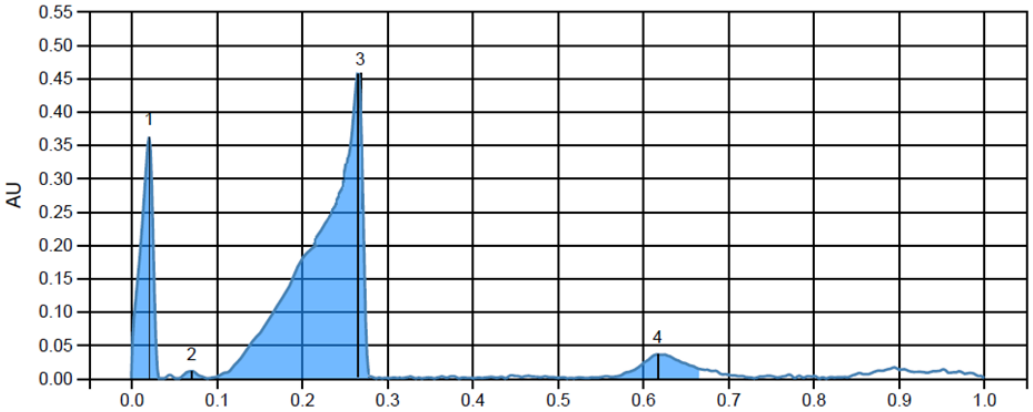


Figure 12: QHPTLC analysis of rasnairandadi kashayam (D-Std., Track 10).

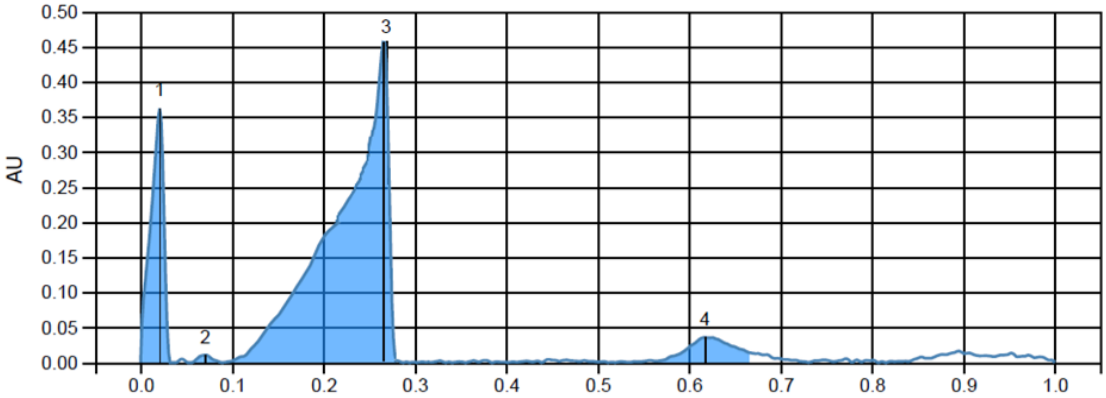


Figure 13: QHPTLC analysis of rasnairandadi kashayam (D-Std., Track 11).

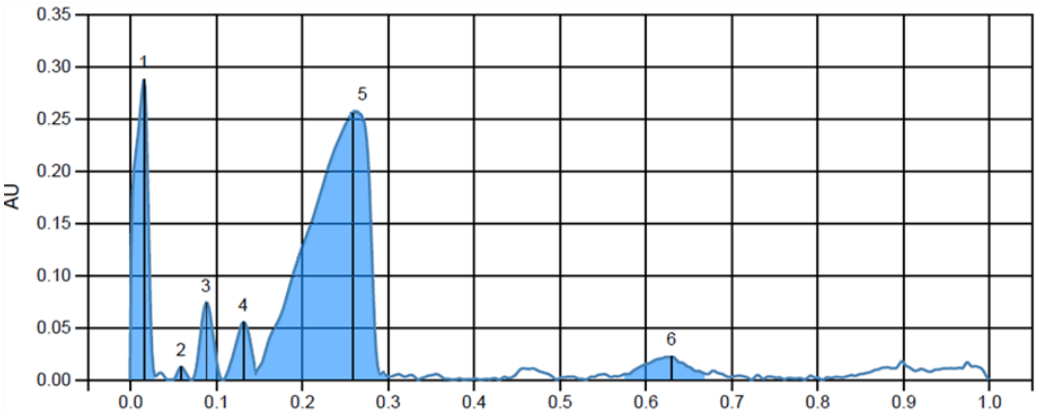


Figure 14: QHPTLC analysis of rasnairandadi kashayam brand A (Track 12).

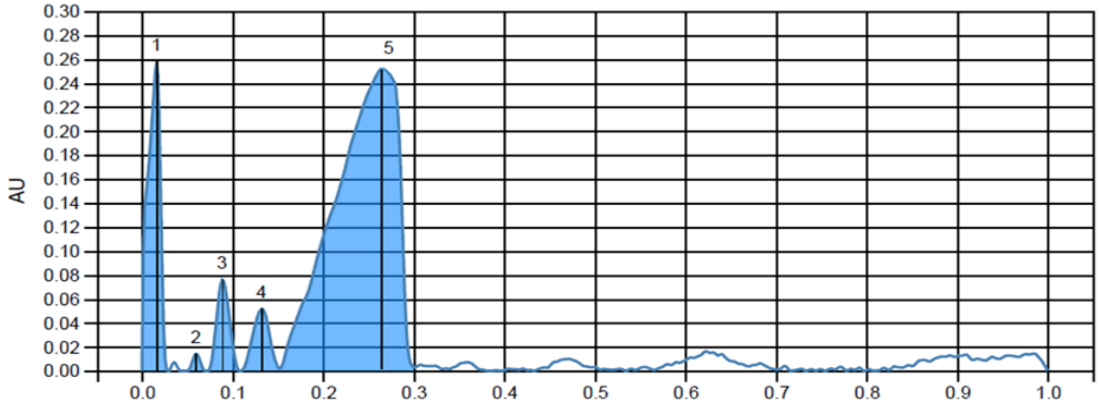


Figure 15: QHPTLC analysis of rasnairandadi kashayam brand A (Track 13).

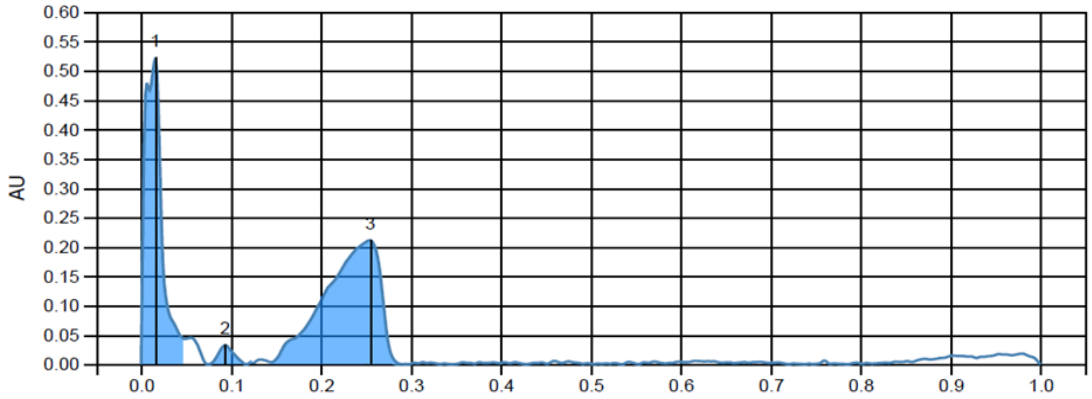


Figure 16: QHPTLC analysis of rasnairandadi kashayam brand B (Track 14)

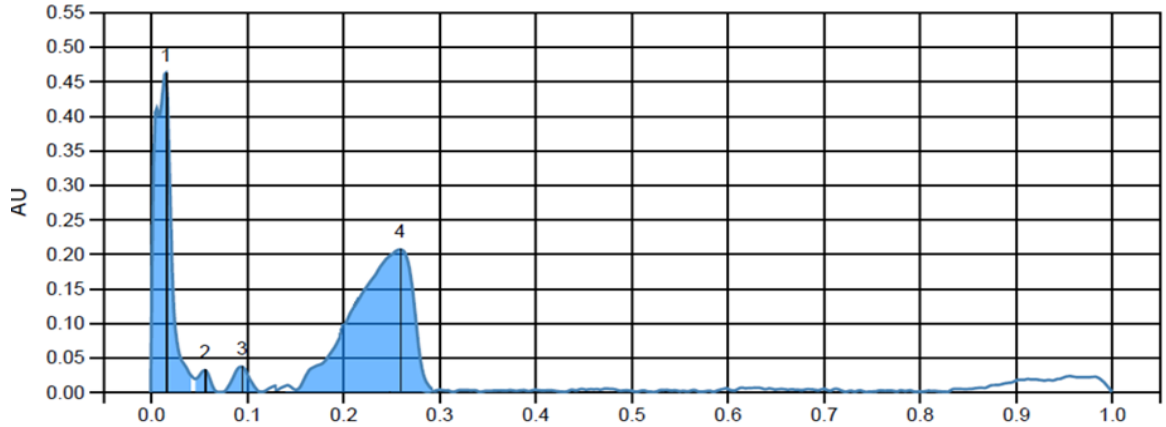


Figure 17: QHPTLC analysis of rasnairandadi kashayam brand B (Track 15).

CONCLUSION

In the present study, the quantitative estimation of *A. longifolia* in rasnairandadi kashayam**,** an ayurvedic polyherbal formulation was done successfully. From the results of present study, it was able to identify that the sample from brand A rasnairandadi kashayam contains *A. longifolia* as specified in Ayurvedic Pharmacopoeia of India because the AUC is same as that of Rf value 0.26 obtained in normal standard. But the sample from brand B showed the presence of only nearly half the amount of *A. longifolia* and the AUC is similar to that of prepared half standard. Of course, several factors may contribute to these minor variations in active constituent. Shortly, the results of the present study have strongly stressed the need of standardisation of Ayurvedic formulations and also laid a stone for the development of quantification procedures for the standardisation of Ayurvedic formulations in the future.

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