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PHARMACOPOEIAL STANDARDIZATION AND QUALITY EVALUATION OF SAATHILADHI CHOORANAM: A CLASSICAL SIDDHA POLYHERBAL FORMULATION

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Abstract

Saathiladhi Chooranam is a classical Siddha formulation traditionally prescribed for the management of stomach ulcers and other gastrointestinal ailments. The present study was undertaken to establish comprehensive standardization parameters for this formulation in accordance with AYUSH and WHO guidelines, ensuring its quality, safety, and therapeutic reliability. Physicochemical, phytochemical, chromatographic, microbial, heavy metal, and aflatoxin analyses were performed. The drug displayed characteristic physicochemical properties and a distinct HPTLC fingerprint, which can serve as a reference for identity and quality assurance in future studies. Phytochemical screening revealed the presence of bioactive compounds including carbohydrates, glycosides, phenolic compounds, and tannins, which may contribute to its gastroprotective properties. Heavy metal analysis confirmed all values to be within WHO permissible limits, while aflatoxin content was also within safe limits, ensuring toxicological safety. Microbial analysis indicated overall compliance with WHO standards. The findings provide a comprehensive profile for Saathiladhi Chooranam, contributing valuable reference data for regulatory purposes and supporting its continued safe use as a therapeutic option for stomach ulcers in Siddha medicine.

Keywords: Saathiladhi Chooranam, stomach ulcer, Siddha medicine, standardization, HPTLC, phytochemical screening, quality control.

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Introduction

Siddha medicine, one of the oldest traditional systems of medicine in India, emphasizes holistic approaches to health through herbal, mineral, and animal-based formulations. Among its many classical preparations, Saathiladhi Chooranam, documented in siddha traditional manuscript 'Aathmaaratchamirdham ennum vaithiya saarasangiragam' [1] holds a prominent place for its therapeutic application in the management of stomach ulcers and other gastrointestinal disorders. Peptic ulcer disease remains a common clinical concern, often resulting from an imbalance between gastric acid secretion and mucosal defense mechanisms, and traditional formulations such as Saathiladhi Chooranam are widely utilized for their perceived gastroprotective, anti-inflammatory, and healing properties. Hence, scientific validation and quality standardization are essential to ensure safety, efficacy, and reproducibility, particularly for formulations intended for internal use.

The process of standardization involves establishing consistent physicochemical characteristics, identifying active phytoconstituents, confirming purity through chromatographic profiling, and assessing safety through heavy metal, microbial, and aflatoxin analysis. High-Performance Thin Layer Chromatography (HPTLC) is a particularly valuable tool in herbal standardization, providing a unique chemical fingerprint that can be used for authentication and quality control. Furthermore, adherence to AYUSH [4] and WHO guidelines [5] for testing ensures that herbal products meet international safety and quality benchmarks, strengthening their acceptance in both traditional and modern healthcare frameworks.

The present study was undertaken to comprehensively standardize Saathiladhi Chooranam by evaluating its physicochemical parameters, HPTLC profile, phytochemical composition, microbial quality, heavy

metal content, and aflatoxin levels. This integrated approach aims to provide a reliable reference profile for regulatory purposes and to support the safe, evidence-based use of Saathiladhi Chooranam in the management of stomach ulcers within the Siddha system of medicine.

Materials and Methods

Saathiladhi chooranam consists of 11 ingredients which were purchased from an indigenous raw drug shop. The raw drugs were authenticated in the Post graduate department of Siddha Pharmacology, Government Siddha Medical College, Chennai. Raw drugs were purified as mentioned in Siddha texts MARUNDHU SEI IYALUM KALAYUM [2] and SIKITCHA RATNA DEEPAM ENNUM VAIDHIYA NOOL [3]. The purified drugs were taken in equal quantities. Then the ingredients were crushed and powdered well by an iron mortar. And sieved in a fine cloth (vasthirakaayam) to obtain fine powder.

Table 1: Ingredients of Saathiladhi chooranam

S.NO	DRUG	SCIENTIFIC NAME	PARTS USED
1	Chithiramoolam	*Plumbago indica*	Root
2	Perungayam	*Ferula asafoetida*	Resin extract
3	Thippili	*Piper longum*	Fruit
4	Omam	*Trachyspermum ammi*	Fruit
5	Milagu	*Piper nigrum*	Fruit
6	Malli	*Coriandrum sativum*	Fruit
7	Seeragam	*Cuminum cyminum*	Fruit
8	Ponmusuttai ver	*Cissampelos pareira*	Root
9	Sadamanjil	*Nardostachys jatamansi*	Root
10	Thippili moolam	*Piper longum*	Root
11	Indhuppu	*Sodium chloride*	

Standardization Parameters

Saathiladhi Chooranam was subjected to standardization parameters including physicochemical analysis, HPTLC fingerprinting, heavy metal estimation, preliminary phytochemical analysis, microbial load determination, and aflatoxin estimation.

Physicochemical Investigations

Physicochemical analysis was performed to determine pH, total ash, acid-insoluble ash, moisture content, water-soluble extractive value, and alcohol-soluble extractive value according to AYUSH protocols.

HPTLC Fingerprinting

TLC methodology

Weigh 4 g of the sample and add 40 mL of distilled methanol. Keep the mixture overnight with occasional shaking. Then, boil it for 10 minutes in a water bath, allow it to cool, and filter. Concentrate the filtrate and make up the volume to 10 mL in a volumetric flask.

Apply 10 µL of the sample solution onto an E. Merck aluminum plate pre-coated with silica gel 60 F254 using a CAMAG automatic sample applicator. Develop the plate in a solvent system composed of Toluene: Ethyl acetate: Formic acid (7.5:3.5:0.01) up to a distance of 80 mm. Dry the plate and observe it under UV light at 254 nm and 366 nm using the CAMAG TLC Visualizer. Capture photographs and scan the developed plate.

Dip the plate in vanillin–sulphuric acid reagent and heat it in a hot air oven at 105 °C until colored spots appear. Place the plate in the CAMAG TLC Scanner. Scan all the tracks, integrate the data. Record the fingerprint of each track.

TLC plate was developed using Toluene: Ethyl acetate: Formic acid (7.5: 3.5: 0.01) as mobile phase. After development allow the plate to dry in air, record the finger print and densitometric chromatogram of the two batch samples of the single compound scanned at 254 and 366 nm.

Heavy Metal Analysis

Heavy metals (lead, cadmium, arsenic, mercury) were estimated as per AYUSH protocol.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis was carried out on methanol and hydroalcohol extracts using standard qualitative tests.

Microbial Analysis

Microbial Load Determination

The microbial quality, including the isolation and identification of pathogenic bacteria from commercial and homemade herbal medicines, was tested according to the regulations of the Unani Pharmacopoeia (2016) and WHO standards (2007). The tests were used to quantify the number of bacteria and fungi isolated that are able to grow aerobically in 1 g of sample. The sample were homogenized by mixing vigorously with water. One gram of sample was transferred to 9 mL of peptone broth. Then, serial dilutions were made to achieve an appropriate concentration. All microbial analyses were carried out in triplicate. Briefly, serial dilutions were made, and viability was assessed using the pour plate method on Casein soyabean digest agar and Sabouraud dextrose agar for bacterial counts and fungal identification, respectively. All dehydrated media were prepared according to the manufacturer's instructions and seeded and incubated at 37 °C for 24 to 48 hours for bacterial screening and at 25 °C for 48 to 72 hours for fungal screening. At the end of

the incubation period, the number of colony-forming units per gram (CFU/g) was calculated by multiplying the average number of colonies by the dilution factor. The obtained CFU/g of sample was compared with WHO standards. Samples that presented bacterial growth greater than 105 CFU in 1 g of herbal medicine were considered unsatisfactory or inadequate according to WHO guidelines for aerobic bacteria.

Identification of Bacteria

For bacterial isolation and identification, the samples were diluted in water, according to the solubility, and homogenized by vigorously mixing. The 1-mL aliquots were transferred to 9 mL of peptone broth and cultured at the recommended time and temperature. All microbial analyses were carried out in triplicate. For investigating *Escherichia coli*, *Salmonella spp*., *Pseudomonas aeruginosa* and *Staphylococcus aureus* EMB agar, MacConkey agar, Deoxycholate citrate agar, Cetrimide agar and Mannitol salt agar culture media were used respectively. At the end of the incubation period, pathogenic bacterial isolates were preliminarily characterized by colony morphology, Gram staining, and biochemical tests (oxidase, gas and catalase production).

Aflatoxin test using afla-test fluorometer

Aflatoxins are a group of naturally occurring toxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, two common mold species. AflaTest is a quantitative method for the detection of aflatoxin in B1, B2, G1, G2, M1, and M2. One gram of Saathiladhi chooranam and 0.4 g of sodium chloride was mixed with methanol: 2% Tween 20 or phosphate buffer (60:40 v/v). Vortex the mixture of extract on high speed 3 minutes. Filter the extract through fluted filter paper. Add 10 ml of filtered extract in measuring cylinder, in that 20 mL purified water was added and vortex on high for 1 minute. Then filter the diluted extract through a pre-wet glass microfiber filter (1.5µm). Pass 10 mL of diluted extract through AflaTest WB column. Apply pressure to get 1-2 drops per second. Wash the column with 10 mL 2% Tween 20. Wash column with 10 mL purified water twice. Elute AflaTest WB columns by passing 1 mL HPLC-grade methanol (100%) through column, apply pressure to get 1 drop per second. Collect eluate in sterile VICAM cuvette. In that add 1.0 mL of AflaTest Developer and mix well, then immediately place in fluorometer (VICAM fluorometer-series 4EX). Fluorometer will read concentration after 60 seconds.

Results

Physicochemical parameters

S.No.	Test Parameter(s)	Result(s)
1	Ash (%w/w) Total ash	 14.07 %
2	Acid-insoluble ash	1.73 %
3	pH	5.69
4	Moisture Content	9.85 %
5	Water Extractive Value	26.02%
6	Alcohol Extractive Value	11.48 %

Preliminary Phytochemical Analysis

S.NO	Preliminary Test	Methanol extract	Hydro alcohol extract
1	Alkaloids Wagner's test Picric acid test	 - -	 - -
2	Carbohydrates Fehling's test	 +	 +
3	Glycosides Conc. H2SO4 test Aqueous NaOH test	 + +	 + +
4	Proteins & amino acids Biuret test Ninhydrin test	 - -	 - -
5	Flavonoids Lead acetate test	 -	 -
6	Phenolic compounds Ferric chloride test Lead acetate test	 + +	 + +
7	Tannins Braymer's test	 +	 +
8	Phytosterols Salkowski's test	 -	 -
9	Cholesterol	-	-
10	Terpenoids	-	-
11	Quinones	-	-
12	Anthocyanin	-	-
13	Carboxylic acid	+	+
14	Gums & mucilage	+	-
15	Fixed oil & fat	-	-

Carbohydrates

Fig 1 Fehling's test

Glycosides

Fig 2Conc. H2SO4 test

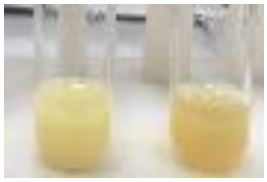


Fig 3Aqueous NaOH test

Phenolic compounds

Fig 4: Ferric chloride test

Fig 5: Lead acetate test

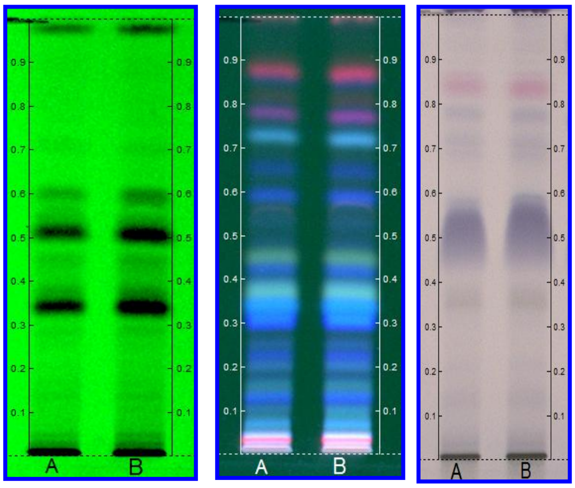
Tannins

Fig 6: Braymer's test

Fig 7: Test for carboxylic acid

Fig 8: Gums & mucilage

HPTLC finger print profile of Saathiladhi Chooranam Alcohol extract



UV – 254 nm **UV – 366 nm** **V – S Reagent**

Fig 9: Solvent System: Toluene: Ethyl acetate: Formic acid (7.5: 3.5: 0.01) 8 µl

Rf values

Sample Name /Track No.	UV-254 nm		UV-366 nm		Derivatised with vanillin sulfuric Acid	
	Rf	Colour	Rf	Colour	Rf	Colour
Track – B	0.04 0.13 0.16 0.25 0.28 0.34 0.44 0.51 0.60 0.71 0.82 0.90 0.98	Green Green Green Green Green Dark Green Green Dark Green Green Green Green Green Dark Green	0.02 0.06 0.15 0.20 0.24 0.34 0.41 0.44 0.52 0.57 0.60 0.72 0.77 0.87	Light pink Light red Dark blue Light blue Light green Light yellow Blue Dark yellow Light grey Light red Blue Blue Pink Dark red	0.03 0.12 0.21 0.35 0.53 0.68 0.70 0.77 0.83 0.89 0.92	Light grey Light blue Light grey Light green Dark blue Light grey Light blue Blue Pink Light grey Light grey

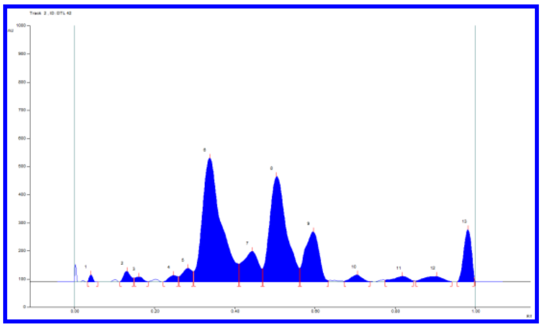


Fig 10: HPTLC finger print at 254 nm (Absorbance mode)

Table 2: Rf values at 254 nm (Absorbance mode)

Track 2, ID: DTL 42

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	1.1 AU	0.04 Rf	25.2 AU	1.68 %	0.06 Rf	0.0 AU	186.1 AU	0.40 %
2	0.11 Rf	0.0 AU	0.13 Rf	37.5 AU	2.50 %	0.15 Rf	13.2 AU	549.2 AU	1.19 %
3	0.15 Rf	13.4 AU	0.16 Rf	17.6 AU	1.17 %	0.18 Rf	0.6 AU	287.2 AU	0.62 %
4	0.22 Rf	0.2 AU	0.25 Rf	22.7 AU	1.51 %	0.26 Rf	19.2 AU	407.6 AU	0.89 %
5	0.26 Rf	19.3 AU	0.28 Rf	48.1 AU	3.21 %	0.30 Rf	37.0 AU	994.5 AU	2.16 %
6	0.30 Rf	37.3 AU	0.34 Rf	439.8 AU	29.30 %	0.41 Rf	63.7 AU	17016.0 AU	36.99 %
7	0.41 Rf	64.1 AU	0.44 Rf	108.0 AU	7.20 %	0.47 Rf	46.1 AU	3435.4 AU	7.47 %
8	0.47 Rf	47.1 AU	0.51 Rf	373.9 AU	24.91 %	0.56 Rf	48.0 AU	13038.7 AU	28.34 %
9	0.56 Rf	48.2 AU	0.60 Rf	177.8 AU	11.84 %	0.63 Rf	5.1 AU	5148.4 AU	11.19 %
10	0.67 Rf	2.1 AU	0.71 Rf	24.8 AU	1.65 %	0.74 Rf	0.8 AU	579.4 AU	1.26 %
11	0.78 Rf	6.3 AU	0.82 Rf	19.6 AU	1.30 %	0.85 Rf	3.9 AU	633.0 AU	1.38 %
12	0.85 Rf	3.6 AU	0.90 Rf	19.5 AU	1.30 %	0.94 Rf	0.5 AU	774.4 AU	1.68 %
13	0.96 Rf	0.2 AU	0.98 Rf	186.4 AU	12.42 %	1.00 Rf	15.6 AU	2956.1 AU	6.43 %

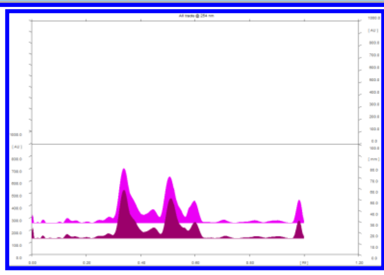


Fig 11: Densitometric chromatogram at 254 nm in Alcohol (Absorbance mode)

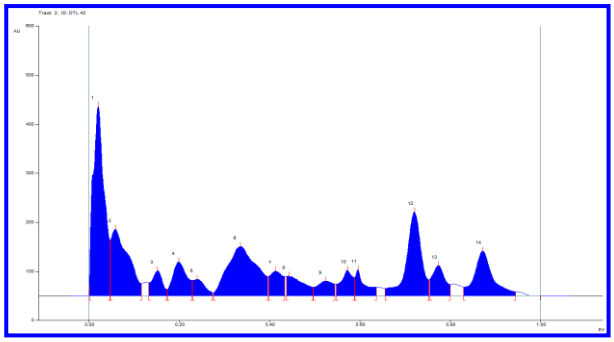


Fig 12: HPTLC finger print at 366 nm (Fluorescence mode)

Table 3: Rf values at 366 nm (Fluorescence mode)

Track 2, ID: DTL 42

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	20.6 AU	0.02 Rf	386.7 AU	29.17 %	0.05 Rf	12.2 AU	7976.2 AU	22.24 %
2	0.05 Rf	112.9 AU	0.06 Rf	135.7 AU	10.24 %	0.12 Rf	25.0 AU	4308.2 AU	12.01 %
3	0.13 Rf	26.6 AU	0.15 Rf	51.2 AU	3.87 %	0.17 Rf	12.6 AU	1010.7 AU	2.82 %
4	0.17 Rf	13.3 AU	0.20 Rf	68.9 AU	5.20 %	0.23 Rf	31.4 AU	1795.1 AU	5.01 %
5	0.23 Rf	31.4 AU	0.24 Rf	33.2 AU	2.51 %	0.28 Rf	6.0 AU	744.3 AU	2.08 %
6	0.28 Rf	6.3 AU	0.34 Rf	100.5 AU	7.58 %	0.40 Rf	39.3 AU	5422.1 AU	15.12 %
7	0.40 Rf	39.5 AU	0.41 Rf	50.2 AU	3.79 %	0.43 Rf	39.4 AU	1205.3 AU	3.36 %
8	0.44 Rf	39.2 AU	0.44 Rf	39.8 AU	3.01 %	0.50 Rf	17.2 AU	1256.2 AU	3.50 %
9	0.50 Rf	17.2 AU	0.52 Rf	29.9 AU	2.26 %	0.55 Rf	24.0 AU	880.0 AU	2.45 %
10	0.55 Rf	23.9 AU	0.57 Rf	52.3 AU	3.95 %	0.59 Rf	37.5 AU	1119.3 AU	3.12 %
11	0.59 Rf	38.3 AU	0.60 Rf	53.2 AU	4.01 %	0.64 Rf	17.4 AU	941.2 AU	2.62 %
12	0.66 Rf	15.0 AU	0.72 Rf	171.0 AU	12.90 %	0.75 Rf	32.2 AU	4658.3 AU	12.99 %
13	0.76 Rf	32.9 AU	0.77 Rf	61.6 AU	4.65 %	0.80 Rf	23.2 AU	1463.3 AU	4.08 %
14	0.83 Rf	17.6 AU	0.87 Rf	91.1 AU	6.88 %	0.95 Rf	8.1 AU	3080.9 AU	8.59 %

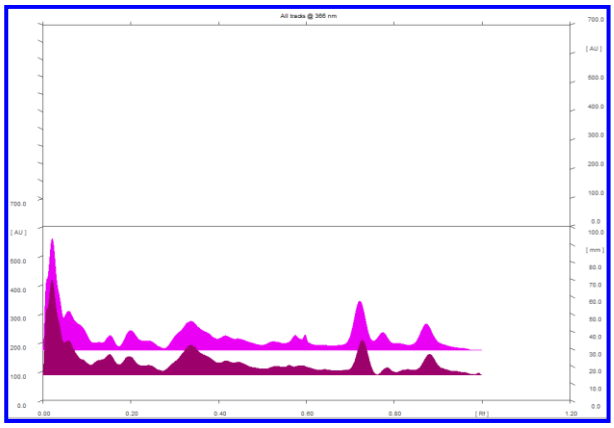


Fig 13: Densitometric chromatogram at 366 nm in Alcohol (Fluorescence mode)

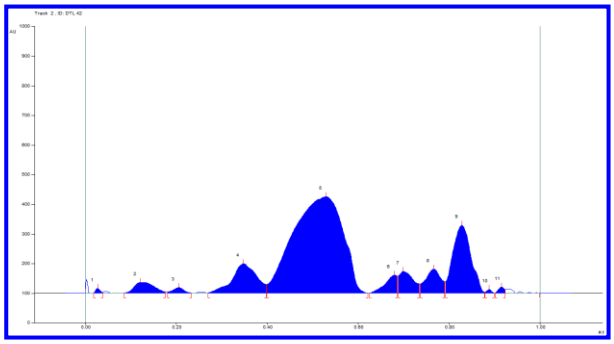


Fig 14: HPTLC finger print at 545nm (Absorbance mode)

Table 4: R_f values at 520 nm (Absorbance mode)

Track 2, ID: DTL 42

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.7 AU	0.03 Rf	16.0 AU	1.65 %	0.04 Rf	3.6 AU	134.0 AU	0.27 %
2	0.09 Rf	0.1 AU	0.12 Rf	36.1 AU	3.72 %	0.18 Rf	4.3 AU	1329.5 AU	2.68 %
3	0.18 Rf	4.2 AU	0.21 Rf	18.7 AU	1.93 %	0.23 Rf	0.2 AU	362.6 AU	0.73 %
4	0.27 Rf	0.3 AU	0.35 Rf	98.8 AU	10.18 %	0.40 Rf	29.6 AU	4421.4 AU	8.93 %
5	0.40 Rf	29.7 AU	0.53 Rf	325.3 AU	33.51 %	0.62 Rf	0.1 AU	29834.6 AU	60.23 %
6	0.63 Rf	0.0 AU	0.68 Rf	60.4 AU	6.22 %	0.69 Rf	57.4 AU	1335.4 AU	2.70 %
7	0.69 Rf	57.5 AU	0.70 Rf	73.1 AU	7.53 %	0.74 Rf	31.1 AU	1932.1 AU	3.90 %
8	0.74 Rf	31.2 AU	0.77 Rf	81.1 AU	8.35 %	0.79 Rf	38.8 AU	2250.2 AU	4.54 %
9	0.79 Rf	39.3 AU	0.83 Rf	228.6 AU	23.55 %	0.88 Rf	3.4 AU	7612.0 AU	15.37 %
10	0.88 Rf	3.5 AU	0.89 Rf	12.3 AU	1.26 %	0.90 Rf	0.5 AU	104.1 AU	0.21 %
11	0.90 Rf	0.3 AU	0.92 Rf	20.3 AU	2.09 %	0.93 Rf	12.2 AU	218.4 AU	0.44 %

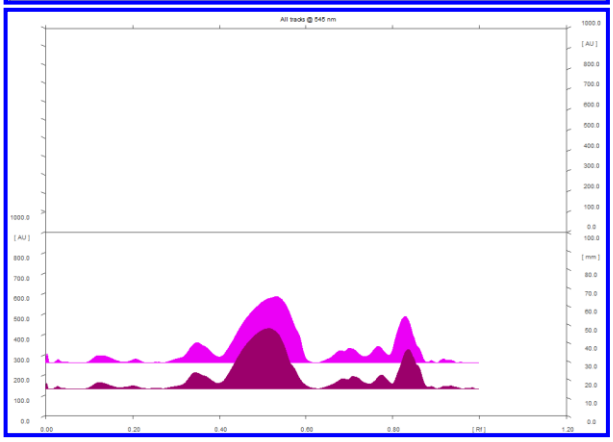


Fig 15: Densitometric chromatogram at 520 nm in Alcohol (Absorbance mode)

Heavy Metal Analysis

S.No.	Test Parameter(s)	Result(s)	WHO Permissible limit (ppm)	Test Method
1.	Heavy Metals a. Lead b. Cadmium c. Arsenic d. Mercury	0.0316 0.0036 0.1112 0.0220	10 01 03 0.3	UPI/Ayush Protocol

Microbial Load

S. No.	Parameters	Results	Remarks
1	Total Bacterial Count (TBC)	$2.3x10^4$cfu/g	Within permissible limits
2	Total Fungal Count (TFC)	Less than 16 cfu/g	
3	Enterobacteriaceae	Absent	
4	*Escherichia coli*	Absent	

5	Salmonella Spp	Absent	Within permissible limits
6	*Staphylococcus aureus*	Absent	
7	*Pseudomonas aeruginosa*	Absent	

Discussion

The standardization of Saathiladhi Chooranam was undertaken to establish its physicochemical parameters, phytochemical profile, chromatographic fingerprint, heavy metal content, and microbiological quality in accordance with AYUSH, and WHO guidelines.

Physicochemical Characteristics

The total ash value (14.07 % w/w) was within the permissible range, indicating the presence of inorganic matter largely attributed to the plant's natural mineral composition and minimal extraneous matter. The acid-insoluble ash content (1.73 % w/w) reflected negligible silica contamination, suggesting appropriate processing and raw material purity. The moisture content (9.85 %) was below the threshold for microbial growth, thus supporting shelf-life stability. Water extractive value (26.02 %) exceeded the alcohol extractive value (11.48 %), indicating that the majority of the phytoconstituents are water-soluble, which aligns with the traditional aqueous preparation methods in Siddha practice. The pH value of 5.69 suggested a mildly acidic nature, which is favorable for stability and palatability.

Phytochemical Screening

Preliminary phytochemical analysis revealed the presence of carbohydrates, glycosides, phenolic compounds, tannins, carboxylic acids, and gums/mucilage in both methanol and hydroalcoholic extracts. These constituents are known for their potential therapeutic roles, including antioxidant, anti-inflammatory, and gastroprotective effects, which may support its traditional indication for digestive ailments such as peptic ulcers. The absence of alkaloids, flavonoids, steroids, and toxic secondary metabolites further supports its safety profile.

HPTLC Fingerprinting

The HPTLC profile, developed in the solvent system Toluene: Ethyl acetate: Formic acid (7.5:3.5:0.01), displayed distinct Rf values under UV 254 nm, UV 366 nm, and after derivatization with vanillin–sulphuric acid. Multiple spots with varied coloration confirmed the polyherbal nature and complex phytoconstituent profile of Saathiladhi Chooranam. These fingerprints serve as a reproducible reference for authentication and quality control in future batches.

Heavy Metal Analysis

Lead (0.0316 ppm), cadmium (0.0036 ppm), arsenic (0.1112 ppm), and mercury (0.0220 ppm) levels were far below WHO permissible limits. This indicates that the formulation is free from significant heavy metal contamination, reflecting good sourcing and processing practices.

Microbial Quality and Aflatoxin Levels

The total bacterial count (2.3×10^4 CFU/g) and fungal count (<16 CFU/g) were within WHO safety limits. E. coli, Salmonella spp., and Pseudomonas aeruginosa were absent. The total aflatoxin content was 12 ppb, well within the WHO limit (≤15 ppb), indicating minimal fungal toxin contamination.

Overall Quality Assessment

The results collectively confirm that Saathiladhi Chooranam meets the essential quality and safety parameters for Siddha drug formulations. The established physicochemical constants, phytochemical markers, and HPTLC profiles can be used for routine quality control. Heavy metal and microbial load analyses support the safety of the formulation for internal use.

Conclusion

These findings not only validate the traditional use of Saathiladhi Chooranam but also provide a scientific framework for its pharmacopoeial standardization. Future studies focusing on quantitative phytochemical analysis, stability profiling, and bioactivity correlation could further strengthen its therapeutic claim and regulatory acceptance.

Sponsorship and Conflict of Interest

Nil

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Author contribution

Dr. U.Chithra designed and supervised the project. Dr R. Soundarya collected, analysed the data and prepared the manuscript. Dr. Sudhamathi Pushparaj reviewed the final version. All authors approved the final manuscript.

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