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Research Article

Preliminary Standardization and HPTLC Fingerprinting of Siddha Medicine Soosika Chooranam (SC): Physicochemical & Phytochemical Evaluation

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Abstract

Soosika Chooranam (SC), a traditional polyherbal formulation cited in classical Siddha literature, has been used to treat various ailments, including gastrointestinal disorders, respiratory issues, and gynaecological conditions such as polycystic ovarian disease. With the growing global reliance on herbal medicines, ensuring the quality, safety, and efficacy of such traditional formulations has become imperative. This study aimed to perform preliminary standardisation of *Soosika Chooranam* in accordance with AYUSH-PLIM guidelines by conducting a comprehensive evaluation involving organoleptic, physicochemical, phytochemical, and chromatographic analyses. The organoleptic assessment confirmed SC as a brown, aromatic, fine powder with good flow properties. Physicochemical parameters such as total ash (9.7%), loss on drying (4%), and extractive values were within acceptable limits. Phytochemical screening revealed the presence of glycosides, saponins, flavonoids, phenols, tannins, and terpenoids, indicating potential bioactivity. High-Performance Thin Layer Chromatography (HPTLC) fingerprinting was carried out using both methanol and hydro alcohol extracts, demonstrating consistent and distinctive peaks at 254 nm and 366 nm, thus establishing a reliable chemical profile for SC. Safety assessments, including heavy metal analysis, microbial contamination testing, and aflatoxin testing, confirmed the absence of hazardous substances and pathogens. Overall, the study establishes preliminary quality benchmarks for *Soosika Chooranam*, reinforcing its safety and providing a foundation for further pharmacological and toxicological investigations to support its clinical use and regulatory acceptance.

Keywords: Herbal standardization, HPTLC fingerprinting, Phytochemical screening, *Soosika Chooranam, Siddha medicine*.

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Introduction

The Siddha system of medicine, a traditional healing practice rooted in Tamil heritage, employs an extensive pharmacopoeia sourced from plants, minerals/metals, and animal-based products to develop a wide array of therapeutics, such as herbal formulations and alchemical preparations (1). Among them, in recent years, herbal remedies have been increasingly employed to treat both chronic and acute conditions and support the immune system owing to their diverse phytochemical properties and therapeutic potential (2). However, their growing popularity has also led to increasing reports of adulteration and contamination with heavy metals, microbes, and even undeclared pharmaceuticals, which can severely compromise both their safety and effectiveness (3). Studies have found that around 64% of traditional Indian herbal remedies tested, contained lead, with 41% showing arsenic and 9% cadmium, highlighting widespread heavy metal contamination in these products (4). Hence, standardising Siddha medicines is essential for their commercialisation, ensuring their safety, efficacy, and quality, while meeting AYUSH PLIM guidelines and international drug regulations (5). Modern analytical methods such as physicochemical assays, phytochemical screening, and chromatographic fingerprinting (especially HPTLC) are indispensable for verifying the purity, consistency, and reproducibility of complex polyherbal formulations (6). *Soosika Chooram* (SC) is one of the polyherbal formulations mentioned in *Agathiyar Vaithya Kaviyam 1500, which* indicates for *kiraani, kunmam, vayitriraichal, mel nokkum vaayu* (Gastrointestinal disorders), *Soosika Vayu* (Polycystic Ovarian Disease), *Izhuppu* (Respiratory Disorders) and *Pakka Vaayu* (Hemiplegia). The aim of the study to analyse *Soosika Chooranam* by AYUSH PLIM guidelines through physicochemical, phytochemical analysis and high-performance thin layer chromatograph (HPTLC) to develop a reliable chemical signature for future research and quality control.

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Material and Methods

Procurement and Authentication of Raw Drugs: The raw materials were procured from a reputable indigenous raw drugs store in Chennai. Their authenticity was verified by the Head of the Department from the Postgraduate Department of Pharmacology (GD/ 2024/05/33), Government Siddha Medical College, Arumbakkam, Chennai.

Table 1: Ingredients and their quantities of *Soosika Choornam* (SC)

S.I.No	Plant	Botanical name	Family	Part	Quantity
1	Musmusukai	*Mukia maderaspatana* (L.) Roem.	Cucurbitaceae	Whole plant	380gms
2	Ponnankanni	*Alternanthera sessilis* (L.) R.Br. ex-DC.	Amaranthaceae	Whole plant	380gms
3	Poduthalai	*Phyla nodiflora* (L.) Greene	Verbenaceae	Whole plant	380gms
4	Paruthi Vithai	*Gossypium hirsutum* L.	Malvaceae	Seed	380gms
5	Siru Payaru	*Vigna radiata* (L.) R.Wilczek	Fabaceae	Seed	380gms
6	Seeragam	*Cuminum cyminum* L.	Apiaceae	Seed	380gms
7	Vellaatu neer	Goat's urine			100ml

Procedure

All the aforementioned raw drugs (Table 1), each weighing 380 g, except *Seeragam*, were mildly roasted, cooled, and finely powdered. An equal quantity of *Seeragam* was soaked in fresh goat's urine for three days with occasional stirring, then drained, sun-dried, powdered, and sieved. The processed *Seeragam* was then mixed with the previously prepared powder, blended thoroughly, and stored in a clean, airtight container (7).

Standardisation according to PLIM Guideline

A single laboratory-prepared batch of *Soosika Chooranam* was analysed as part of a preliminary standardization study. Following the AYUSH-PLIM guidelines (8), the formulation was evaluated for its organoleptic characteristics, physicochemical parameters, phytochemical constituents, chromatographic profile, and safety parameters, including heavy metal content, microbial load, and aflatoxin contamination, to establish baseline quality control data.

Organoleptic Characters

The organoleptic characters of the sample drug were evaluated. 1gm of the SC was taken and the state, appearance, nature, odour, and other morphological characters were viewed by the naked eye under natural light and results were noted. (Table 2)

Physicochemical Analysis of *Soosika Chooranam* (SC)

Physicochemical studies like total ash, acid-insoluble ash, water, and alcohol soluble extract, loss on drying at 105°C were done at Research and Development Wing for Indian Systems of Medicine ISM, AAGHIM west campus, Arumbakkam, Chennai. Results were noted in (Table 3).

Preliminary Phytochemical Screening of *Soosika Chooranam* (SC)

The preliminary phytochemical screening of the aqueous extract of *Soosika Chooranam* (SC) was carried out using standard procedures. The analysis included the detection of various phytoconstituents such as alkaloids, carbohydrates, reducing sugars, glycosides, saponins, proteins and amino acids, phenols and tannins, flavonoids, phytosterols, terpenoids, lignin, quinones, gum, and mucilage. These tests were conducted at the Research and Development Wing for Indian Systems of Medicine (ISM), AAGHIM West Campus, Arumbakkam, Chennai. The results of the screening indicate the presence of several bioactive compounds in the extract of SC. The detailed findings are summarized in Table 4.

Instrumental Analysis of *Soosika Chooranam* (SC)

The test drug SC was analysed to generate the fingerprint using High-Performance Thin Layer Chromatography (HPTLC) in Regional Research Institute of Unani Medicine – Chennai.

Sample Preparation for TLC

Two sets of samples (SC, 1 g each) were dissolved separately in 10 mL of methanol and hydroalcoholic solvent (Ethanol: Water, 50:50 v/v). The solutions were sonicated for 15 minutes and filtered. The resulting filtrates were used for TLC analysis.

HPTLC Procedure

An 8 µl aliquot of each sample was applied as bands using the ATS4 sample applicator. The TLC plates were developed in a mobile phase of toluene: ethyl acetate: formic acid (7.6: 2.4: 0.1, v/v) and then dried. Plates were photo-documented using Camag's TLC Visualiser under UV light at 254 nm and 366 nm, followed by scanning with Camag's Scanner 4 (D_2 lamp in absorption mode, Hg lamp in fluorescence mode) to obtain fingerprint profiles. Subsequently, plates were dipped in 5% vanillin–sulphuric acid reagent and heated at 105 °C until coloured spots developed. Final images were photo-documented. (Figure No.1)

Test for Heavy Metals

To determine the concentration of heavy metals such as mercury (Hg), arsenic (As), lead (Pb), and cadmium (Cd) in the test formulation *Soosika Chooranam* (SC), Atomic Absorption Spectroscopy (AAS) was employed using Model: AA 240 Series. SC sample was digested with 1 mol/L HCl for the determination of arsenic and mercury (8). Results were tabulated in (Table 10)

Microbial Contamination

Test for Specific Pathogen

The microbial quality of *Soosika Chooranam* was evaluated in accordance with WHO guidelines (2007) to assess the presence of pathogenic microorganisms. One gram of the sample was homogenized in 9 mL of peptone broth, followed by serial dilutions. Microbial enumeration was performed in triplicate using the pour plate method. Bacterial colonies were cultured on Casein Soyabean Digest Agar and incubated at 37°C for 24–48 hours, while fungal growth was assessed on Sabouraud Dextrose Agar incubated at 25°C for 48–72 hours. After incubation, the total number of colony-forming units per gram (CFU/g) was calculated and evaluated

against WHO limits, with bacterial counts exceeding 10^5 CFU/g considered non-compliant (9). (Table 11)

Identification of Bacteria

For bacterial isolation and identification, the samples were diluted in sterile water or Tween solution based on their solubility and homogenized thoroughly. One millilitre of the diluted sample was transferred into 9 mL of peptone broth and incubated under appropriate conditions. All tests were performed in triplicate. Selective media were used for the detection of specific pathogens: EMB agar and MacConkey agar for *Escherichia coli*, Deoxycholate Citrate Agar for *Salmonella spp.*, Cetrimide agar for *Pseudomonas aeruginosa*, and Mannitol Salt Agar for *Staphylococcus aureus*. After incubation, suspected colonies were identified based on colony morphology, Gram staining, and biochemical tests such as oxidase, catalase, and gas production (9).

Aflatoxin Analysis for SC

Aflatoxins were analyzed using the AflaTest WB method (VICAM Series 4EX fluorometer), which quantitatively detects aflatoxins B_1, B_2, G_1, G_2, M_1, and M_2. One gram of *Soosika Chooranam* (SC) was extracted with methanol:2% Tween 20 or phosphate buffer (60:40 v/v) containing 0.4 g sodium chloride and vortexed for 3 minutes. The extract was filtered through fluted filter paper, and 10 mL of the filtrate was diluted with 20 mL purified water and vortexed again. This was then filtered through a pre-wetted glass microfiber filter (1.5 µm). Ten milliliters of the final extract were passed through an AflaTest WB immunoaffinity column at 1–2 drops/sec. The column was washed with 10 mL of 2% Tween 20 and twice with 10 mL purified water. Aflatoxins were eluted with 1 mL of 100% methanol at 1 drop/sec. The eluate was mixed with 1 mL AflaTest Developer and immediately read in a VICAM fluorometer, with results recorded after 60 seconds (9,10). Results were tabulated (Table12).

Results

Organoleptic characters of *Soosika Chooranam* were evaluated and showed in table 2. It was done to assess the quality of the polyherbal sample.

Table 2: Organoleptic characters of *Soosika Chooranam* (SC)

S. No	Parameter	Result
1	State	Solid
2	Appearance	Brown colour
3	Nature	Fine powder
4	Odour	Aromatic
5	Flow property	Free flowing

The physicochemical analysis of *Soosika Chooranam* (SC) was done and noted in table 3.

Table: 3 Physicochemical parameters of *Soosika Chooranam* (SC)

S. No	Name of the Test	Value (%)
1	Loss on drying at 105°C (%	4.00
2	Total Ash (% w/w)	9.70
3	Acid Insoluble Ash (% w/w)	1.40
4	Water soluble extractive (%	13.60
5	Alcohol soluble extractive (%	4.40
6	pH (5% solution)	6.20
7	Rancidity	Nil

Phyto-chemical Screening

The Preliminary phytochemical studies of Aqueous extract of *Soosika Choornam* (SC) were done using standard procedures. The results were presented in table 4. The present study reveals that the bioactive compounds were present in the aqueous extracts of SC.

Table 4: Phytochemical extract of *Soosika Chooranam* (SC)

S. No	Phyto-chemicals	Test Name	H_2O Extract	Observation
1	Alkaloids	Mayer's test	-ve	No cream precipitate formed
2	Carbohydrates	Barfoed's test	-ve	No red precipitate formed
3	Reducing sugar	Fehling's test	-ve	No brick-red precipitate formed
4	Glycosides	Borntrager's test	+ve	Pink colour formed
5	Saponins	Foam test	+ve	Persistent foam formed
6	Proteins and Amino Acids	Ninhydrin test	-ve	No purple colour observed
7	Flavonoids	Alkaline reagent test	+ve	Yellow colour formed
8	Phenolic Compounds	Ferric chloride test	+ve	A bluish-black colour formed
9	Tannins	Gelatin test	+ve	Grey colour formed
10	Phytosterols	Salkowski's test	-ve	No colour change observed
11	Terpenoids	H_2SO_4 Test	+ve	Reddish-brown ring formed
12	Lignin	Furfuraldehyde test	-ve	No red colour observed
13	Quinone	Conc. HCL test	-ve	No colour change observed
14	Gums and Mucilage	Alcohol test	-ve	No precipitate observed

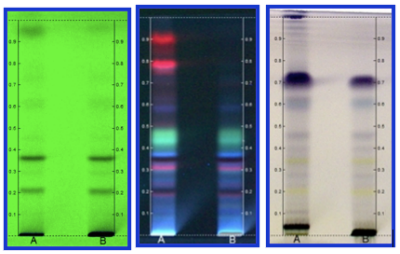
Instrumental Analysis

HPTLC fingerprinting was employed for the purpose of creating quality standards for polyherbal compositions. For sample SC fingerprint was mentioned below.

Table 5: TLC Rf and Colour of Spots

λ 254nm		λ 366nm		Derivatized plate under White Light			
				Methanol Extract		Hydroalcohol extract	
Rf	Colour	Rf	Colour	Rf	Colour	Rf	Colour
0.21	Green	0.01	Blue	0.04	Dark brown	0.03	Dark brown
0.30	Green	0.08	Blue	0.11	Violet	0.20	Yellow
0.36	Dark green	0.18	pink	0.20	Yellow	0.34	Yellow
0.46	Green	0.26	blue	0.34	Yellow	0.39	Violet
0.62	Green	0.32	pink	0.45	Violet	0.45	Violet
0.74	Green	0.39	blue	0.60	Blue	0.60	Blue
0.79	Light green	0.45	Fluorescent green	0.72	Violet	0.71	Violet
0.96	Green	0.60	Violet				
		0.65	Dark Violet				
		0.82	Fluorescent pink				
		0.94	Pink				

Figure 1: TLC Chromatogram of *Soosika chooranam* (SC)



UV – 254 nm	UV – 366 nm	V - S Reagent

Solvent System: Toluene: Ethyl acetate: Formic acid (7.6: 2.4: 0.1)
Track A: Methanol extract (8 µl); Track B: Hydro alcohol extract (8 µl)

HPTLC @ 254nm

Fig No: 2 At 254nm in Methanol extract (Absorption mode)

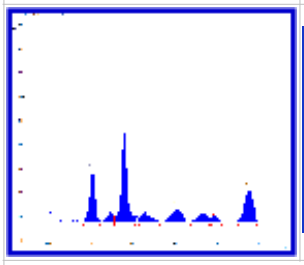


Table: 6 Peak table @254nm in Methanol extract (Absorption mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	121.2 AU	0.01 Rf	124.2 AU	14.34 %	0.03 Rf	0.0 AU	619.4 AU	3.21 %
2	0.16 Rf	0.2 AU	0.21 Rf	201.7 AU	23.30 %	0.25 Rf	0.0 AU	4531.7 AU	23.47 %
3	0.26 Rf	0.1 AU	0.30 Rf	41.6 AU	4.80 %	0.32 Rf	15.6 AU	836.1 AU	4.33 %
4	0.32 Rf	15.7 AU	0.36 Rf	349.4 AU	40.35 %	0.42 Rf	11.2 AU	7684.5 AU	39.69 %
5	0.44 Rf	3.2 AU	0.47 Rf	25.8 AU	2.98 %	0.53 Rf	0.7 AU	808.9 AU	4.19 %
6	0.54 Rf	1.0 AU	0.63 Rf	52.2 AU	6.03 %	0.68 Rf	0.5 AU	2604.9 AU	13.49 %
7	0.70 Rf	2.8 AU	0.74 Rf	32.9 AU	3.79 %	0.80 Rf	4.4 AU	1256.6 AU	6.51 %
8	0.93 Rf	0.0 AU	0.97 Rf	38.2 AU	4.41 %	1.00 Rf	2.7 AU	988.4 AU	5.12 %

Fig No: 3 At 254nm in Hydroalcohol extract (Absorption mode)

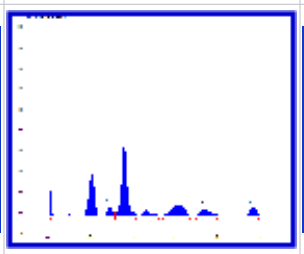
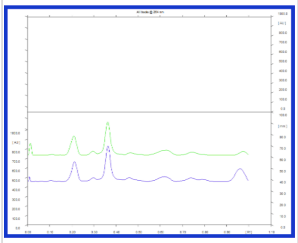


Table: 7 Peak table @254nm in Hydroalcohol extract (Absorption mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	121.2 AU	0.01 Rf	124.2 AU	14.34 %	0.03 Rf	0.0 AU	619.4 AU	3.21 %
2	0.16 Rf	0.2 AU	0.21 Rf	201.7 AU	23.30 %	0.25 Rf	0.0 AU	4531.7 AU	23.47 %
3	0.26 Rf	0.1 AU	0.30 Rf	41.6 AU	4.80 %	0.32 Rf	15.6 AU	836.1 AU	4.33 %
4	0.32 Rf	15.7 AU	0.36 Rf	349.4 AU	40.35 %	0.42 Rf	11.2 AU	7684.5 AU	39.69 %
5	0.44 Rf	3.2 AU	0.47 Rf	25.8 AU	2.98 %	0.53 Rf	0.7 AU	808.9 AU	4.19 %
6	0.54 Rf	1.0 AU	0.63 Rf	52.2 AU	6.03 %	0.68 Rf	0.5 AU	2604.9 AU	13.49 %
7	0.70 Rf	2.8 AU	0.74 Rf	32.9 AU	3.79 %	0.80 Rf	4.4 AU	1256.6 AU	6.51 %
8	0.93 Rf	0.0 AU	0.97 Rf	38.2 AU	4.41 %	1.00 Rf	2.7 AU	988.4 AU	5.12 %

Fig No: 4 Densitometric chromatogram at 254 nm



HPTLC @ 366nm

Fig No.5 At 366nm in Methanol extract (Fluroscence mode)

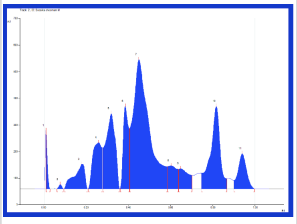


Table: 8 Peak table @ 366nm in Methanol extract (Fluroscence mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	219.0 AU	0.01 Rf	219.0 AU	10.03 %	0.03 Rf	0.2 AU	1104.6 AU	1.35 %
2	0.06 Rf	0.4 AU	0.08 Rf	16.1 AU	0.74 %	0.09 Rf	0.8 AU	190.9 AU	0.23 %
3	0.10 Rf	0.8 AU	0.18 Rf	92.5 AU	4.24 %	0.21 Rf	0.5 AU	3941.7 AU	4.82 %
4	0.21 Rf	0.8 AU	0.26 Rf	173.7 AU	7.95 %	0.28 Rf	53.6 AU	6242.0 AU	7.63 %
5	0.28 Rf	153.7 AU	0.32 Rf	282.6 AU	12.93 %	0.36 Rf	2.2 AU	10630.6 AU	13.00 %
6	0.36 Rf	2.5 AU	0.39 Rf	310.3 AU	14.20 %	0.41 Rf	26.8 AU	6459.9 AU	7.90 %
7	0.41 Rf	227.7 AU	0.45 Rf	485.5 AU	22.22 %	0.59 Rf	83.0 AU	30935.0 AU	37.83 %
8	0.59 Rf	83.2 AU	0.60 Rf	86.8 AU	3.97 %	0.64 Rf	74.0 AU	3104.3 AU	3.80 %
9	0.64 Rf	74.1 AU	0.65 Rf	75.7 AU	3.46 %	0.70 Rf	51.2 AU	2952.5 AU	3.61 %
10	0.75 Rf	59.1 AU	0.82 Rf	309.9 AU	14.18 %	0.87 Rf	39.2 AU	11800.8 AU	14.43 %
11	0.90 Rf	32.1 AU	0.94 Rf	132.5 AU	6.07 %	1.00 Rf	0.4 AU	4401.4 AU	5.38 %

Fig No: 6 At 366nm in Hydroalcohol extract (Fluroscence mode)

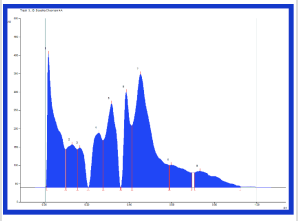
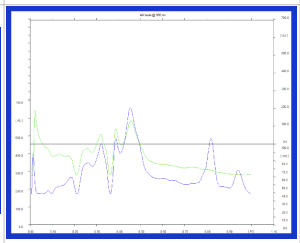


Table: 9 Peak table @ 366nm in Hydroalcohol extract (Fluroscence mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	7.3 AU	0.02 Rf	363.6 AU	22.22 %	0.10 Rf	02.5 AU	12081.8 AU	17.87 %
2	0.10 Rf	102.7 AU	0.13 Rf	118.2 AU	7.10 %	0.16 Rf	03.7 AU	4404.1 AU	6.51 %
3	0.16 Rf	103.8 AU	0.17 Rf	107.5 AU	6.57 %	0.21 Rf	0.8 AU	2760.2 AU	4.08 %
4	0.21 Rf	0.2 AU	0.26 Rf	140.4 AU	9.86 %	0.28 Rf	27.7 AU	5387.3 AU	7.97 %
5	0.28 Rf	127.9 AU	0.32 Rf	228.0 AU	13.93 %	0.36 Rf	1.0 AU	8901.5 AU	13.16 %
6	0.36 Rf	0.4 AU	0.39 Rf	260.0 AU	15.89 %	0.41 Rf	67.9 AU	6186.1 AU	9.15 %
7	0.42 Rf	168.4 AU	0.45 Rf	308.3 AU	18.84 %	0.59 Rf	60.5 AU	20051.1 AU	29.65 %
8	0.59 Rf	60.5 AU	0.60 Rf	60.9 AU	3.72 %	0.70 Rf	39.9 AU	3969.6 AU	5.87 %
9	0.71 Rf	40.1 AU	0.73 Rf	43.9 AU	2.68 %	0.93 Rf	2.6 AU	3878.6 AU	5.74 %

Fig No: 7 Densitometric chromatogram at 366 nm



Heavy metal Analysis of *Soosika chooranam* (SC)

The heavy metal analysis showed that there were no traces of heavy metal residues such as lead, arsenic and cadmium, mercury was within WHO permissible limits to ensure the safety of the drug SC (Table 10).

Table: 10 Heavy metal Analysis of SC

S.No.	Parameters	Results	WHO Permissible limit (ppm)
1	Lead	Nil	10
2	Cadmium	0.0068 mg/L	0.3
3	Arsenic	Nil	3
4	Mercury	0.023367 µg/L	1

Microbial Contamination of *Soosika chooranam* (SC)

The drug SC was free from the viable microorganisms and the total bacterial and fungal count were within permissible limits which indicates that the drug SC is of good quality and safer drug (Table 11).

Aflatoxin for Soosika chooranam (SC)

The results shows that there were no spots were being identified in the test sample SC loaded on TLC plates when compared to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2. The results are tabulated in Table12.

Table: 11 Microbial contaminations of *Soosika chooranam* (SC)

S. No.	Parameters	Results	Remarks
1	Total Bacterial Count (TBC)	1x10³cfu/g	Within permissible limits
2	Total Fungal Count (TFC)	Less than 10 cfu/g	
3	Enterobacteriaceae	Absent	
4	*Escherichia coli*	Absent	
5	Salmonella Spp	Absent	
6	*Staphylococcus aureus*	Absent	
7	*Pseudomonas aeruginosa*	Absent	

Table: 12 Aflatoxin Analysis for *Soosika chooranam* (SC)

S.No.	Parameters	Method / Reference	Results
1	Total Aflatoxin B1+B2+G1+G2	Vicam Aflatest Fluorometer Instruction Manual	1ppb
(Note: Detection Limit - 1ppb)			

Discussion

Herbal medicines are gaining strong acceptance worldwide, with billions relying on them for primary healthcare and wellness. Their perceived safety, affordability, and cultural familiarity bolster demand even as growing concerns about quality, standardization, and safety come to light (11). The organoleptic assessment of SC revealed a solid, brown, aromatic, fine-powder form that lacks free-flowing properties, characteristics comparable to those reported in similar classical Siddha formulations (12). These sensory observations provide baseline quality indicators that help ensure consistency between batches. Physicochemical analysis showed moderate moisture content (loss on drying ~4%), total ash (~9.7%), acid-insoluble ash (~1.4%), extractive values in water (13.6%) and alcohol (4.4%), and a slightly acidic pH (6.2). These values fall within typical ranges for well-standardized polyherbal powders, confirming the formulation's integrity (13). The nil rancidity result further supports good storage stability. Preliminary phytochemicals indicated the presence of glycosides, saponins, flavonoids, phenolics, tannins, and terpenoids bioactive groups commonly linked to therapeutic effects in Siddha drugs. The fingerprint profile of methanol extract under 254nm revealed that the major peak is R_f 0.36 with an area 35.13% of followed by the peaks at R_f 0.21(18.86%) and 6 more minor peaks (Figure No.2); under 366nm, the major peak appeared at R_f 0.45 with an area of 37.83%, second major peak at R_f 0.82 with an area of 14.43%, along with 9 more peaks (Figure No.5). The fingerprint profile of successive hydroalcohol extract under 254 nm revealed that the major peak is R_f 0.36 with an area of 39.69% followed by the peaks at R_f 0.21 (23.47%) and 6 more minor peaks (Figure No.3); under 366nm, the major peak appeared at R_f 0.02 with an area of 17.87%, second major peak at R_f 0.45 (29.65%), along with 7 more peaks (Figure No. 6). Densitometric chromatogram at 254nm and 366nm are represented in the Figures No. 4 and 7. Heavy metals (lead, arsenic, cadmium, mercury) were undetectable or within WHO and AYUSH permissible limits (14). Likewise, microbial counts (total bacterial ~10^3 cfu/g; fungal <10 cfu/g), absence of pathogenic species, and negative aflatoxin results (<1 ppb) underscore the preparation's microbiological purity, matching benchmarks from other well-standardized herbals (15). Overall, these findings affirm that *Soosika Chooranam* adheres to essential quality, safety, and efficacy standards expected of polyherbal Siddha powders. This investigation represents a preliminary standardization based on a single batch preparation. Future studies involving multiple batches will be undertaken to establish batch-to-batch consistency and reproducibility.

Conclusion

By integrating organoleptic observation, physicochemical and phytochemical profiling, HPTLC fingerprinting, and rigorous safety testing, this study confirms that *Soosika Chooranam* adheres to quality standards set by PLIM/AYUSH. The clearly defined moisture, ash, extractive, and pH values, coupled with the presence of key bioactive compounds and a consistent chromatographic profile, provide a strong basis for identity confirmation and batch uniformity. Furthermore, the absence of heavy metals, microbial contaminants, and aflatoxins validates its safety for therapeutic use. These essential preliminary findings serve as a platform for progressing into detailed toxicological evaluation and pharmacological validation, ultimately supporting its inclusion in standardized, evidence-based herbal medicine frameworks.

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