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Research Article

Standardization and Quality Control of Siddha Herbo-Mineral Formulation Siva Nama Rasam (SNR)

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ABSTRACT

Background: The fundamental causes of ailments could be more effectively and efficiently treated with siddha medications. Standardising herbal formulations is a key step towards assuring pharmaceutical reliability and efficacy. Siva Nama Rasam is a traditional siddha Herbo mineral preparation. This formulation was stated in Anuboga Vaithiya Navaneedham, suggested for Iraippirumal (Bronchial asthma), Kurainoi (Leprosy), Sanni (Delirium), Moolanoi (Haemorrhoids), Sayam (Tuberculosis), and other forms of valippu noi (Epilepsy). **Objectives:** The purpose of this study is to create a standard for Siva Nama Rasam (SNR) in accordance with PLIM (Pharmacopeia Laboratory of Indian Medicine) guidelines. **Materials and Methods:** Four of the six chemicals that make up the preparation are inorganic, while the other two are substances derived from plants. The recognizable proof of different substance constituents is identified by HPTLC test. Along with this organoleptic character, physico-chemical parameters, solubility profile, confirmatory specification, particle size determination and heavy metal analysis were additionally done for the prepared medicine. **Conclusion:** The results obtained can be considered viable for this preparation.

Keywords: Herbo-Mineral Medicine, HPTLC, Physiochemical analysis, SNR, PLIM Guidelines.

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INTRODUCTION

Siva Nama Rasam is classified under the category of karuppu by the Siddha Pharmacopoeia Committee. The name of it comes from the final product's black coloration.[1] Siddha's scope is relatively extensive in comparison to that of other ancient medical systems. In the Siddha system, thousands of raw medications are utilized. The purification of raw materials was a top priority for Siddha physicians prior to medication preparation. More than 80% of Siddha medicines are made from herbs. In any case, home grown cures are incapable in a few difficult circumstances. In that circumstance, Siddhars mentioned herbo-metal and herbo-mineral formulations. These medicines are produced through the application of standard procedures for preparation and purification.

The finished product is extremely small, measuring in nano microns. Accordingly, it is immediately integrated into our framework and never causes toxic effects.[2] This formulation is indicated for Kaakkai vali (Epilepsy), Magotharam (Ascites), Kunmam (Peptic ulcer). Currently, there is not enough data to support the quality and analysis aspects of this formulation. This will be conducted by precise standardization of Siva Nama Rasam exercising PLIM- Guideline centred methods for analysis like Organoleptic characters, Physicochemical study, Particle size, Heavy metal analysis and HPTLC. This paper deals gives the detailed study of Siva Nama Rasam to establish standards. This drug is known to possess pharmacological activities such as Analgesic, Anti- Inflammatory and Anti-Epileptic actions.

MATERIALS AND METHODS

Choice of the drug

Siva Nama Rasam is a herbo-mineral formulation used for treating "rheumatism, haemorrhoids, and convulsions. It is documented in the Siddha text Anuboga Vaithiya Navaneedham–Part *7*, authored by Hakim P. Mohammed Abdullah Sayabu" on pages 76-78.[3]

Procurement of raw materials

The raw drug was procured from a country medical shop and verified by a "botanist from the Department of Gunapadam at the National Institute of Siddha, Chennai" under certification number NISMB6662024, GUN/AUT/09/24.

Ingredients for Siva Nama Rasam

The ingredients are depicted in Table 1.

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Purification of Raw Drugs

Purification of Gandhagam (Sulphur)

Quantity sufficient of ghee was taken. When ghee is melted, sulfur is added, and the mixture is poured into a vessel containing milk, where it solidifies. The solidified material is then washed with warm water and allowed to air dry. This process is repeated 30 times.[4]

Purification of Rasam (Hydrargyrum)

Mercury (Rasam) underwent purification in a traditional grinder (Kalvam) using brick powder as the initial medium. Following this, it was processed with Turmeric Choornam, grinding each powder for a duration of 1 hr. The purified mercury was then collected in an earthen vessel. Subsequently, it was subjected to controlled heating along with a specific quantity of Acalypha indica herb juice. Finally, the mercury was meticulously washed to ensure thorough purification.[4]

Purification of Manosilai (Red orpiment)

Manosilai is grinded well with lemon juice for 3 hr on Kalvam without interruption and then dried.[4]

Purification of Venkaaram (Borax)

A measured quantity of Venkaaram was subjected to frying in an earthen pot.[4]

Purification of Karunabi (*Aconitum spicatum*)

For purification, they are soaked in cow's urine for three days and exposed to sunlight daily. After exposure to sunlight cow's urine is replaced by fresh water. After third day, they are dried and preserved.[5]

Purification of Milagu (*Piper nigrum*)

For purification, the black pepper seeds were soaked in buttermilk and left undisturbed for one hour and 30 min. After which the pepper was strained and thoroughly dried. the pepper was then roasted and preserved.[5]

Preparation of Siva Nama Rasam

Rasam and Gandhagam were finely ground together until the mixture turned completely black. The remaining three ingredients were individually weighed, ground into a fine powder, and sifted through a cloth before being added to the mixture. The combined mixture was then continuously ground for 2 hr, followed by the addition of pepper powder, and further ground for an additional 12 hr. The final product was stored in an airtight container.[3]

Physico-chemical analysis by PLIM Guidelines

The medicine Siva Nama Rasam (SNR) was taken for physicochemical characteristics and evaluated for the active ingredients and properties via HPTLC in accordance with AYUSH-PLIM guidelines.[6,7] Physico-chemical studies like pH, loss on drying at 105°C, total ash, water-soluble ash, acid insoluble ash, water and alcohol soluble extract were done at "Siddha Central Research Institute, Anna Govt. Hospital Campus, Arumbakkam, Chennai". The results are given in (Table 2).

Physicochemical Evaluation

Loss on Drying Analysis of Siva Nama Rasam

A precisely weighed SNR of 2 g was placed in an evaporating dish coated with tar. The raw medication was baked at 105°C for 5 hr, or until it reached a steady weight, and then it was weighed. Using the shade-dried material as a guide, the sample's percentage moisture content was determined.

Estimation of Total Ash in SNR

A 2 g sample of SNR was accurately weighed and placed in a silica dish, then incinerated in a furnace at 400°C until it turned white, signifying the complete elimination of carbon. The total ash content was subsequently calculated in relation to the weight of the air-dried sample.

Estimation of Water-soluble Ash in SNR

A 25 mL portion of water was added to the sample obtained from the total ash test and boiled for 5 min. The remaining residue was then transferred to a crucible, rinsed with hot water, and heated at 450°C for 15 min. The water-soluble ash content was determined by deducting the weight of the insoluble residue from the total ash weight.

Estimation of Acid Insoluble Ash in SNR

The ash obtained from the total ash test was boiled in 25 mL of HCl for 6 min and then filtered using ashless filter paper. The insoluble residue collected on the filter paper was rinsed with hot water and heated in a muffle furnace until a constant weight was achieved. The percentage of acid-insoluble ash was then determined based on the weight of the air-dried ash.

Estimation of Water-Soluble Extractive in SNR

5 g of SNR were mixed with 100 mL of chloroform water in a sealed flask, shaken for 6 hr, and left to stand for 18 hr. The solution was then quickly filtered to prevent solvent loss and dried at 105°C until a constant weight was obtained. A 25 mL portion of the filtrate was transferred to a tarred dish and weighed. The water-soluble extract percentage was determined based on the weight of the air-dried sample.

Estimation of Alcohol Soluble Extractive in SNR

SNR was macerated with 100 mL of alcohol in a sealed flask for 24 hr, shaken frequently for 6 hr and left to stand for 18 hr. To prevent solvent loss, the solution was quickly filtered, then dried at 105°C until a constant weight was reached. After complete evaporation,

the residue was weighed in a tarred dish. The alcohol-soluble extract percentage was calculated based on the air-dried sample.

Estimation pH value in SNR

About 5 g of SNR was dissolved in 25 mL of distilled water, filtered, and left to stand for 30 min. The pH of the solution was then measured.

Microscopic Evaluation of Particle Size in SNR

The particle size of the sample was analyzed using optical microscopy. The sample was diluted approximately 1:100 with sterile distilled water, and a drop of the diluted solution was mounted onto a microscope slide. The slide was then positioned appropriately on the microscope stage. Using a calibrated micrometer scale, light microscopic images were captured to measure particle size. At least 30 individual observations were recorded to calculate the average particle size of the sample, ensuring accuracy and reliability in the results.

Solubility Analysis of SNR

A pinch of sample was placed in a dry test tube, mixed with "chloroform, ethanol, water, ethyl acetate, and DMSO" then shaken for a minute. The results were observed individually.

Instrumental Analysis of Siva Nama Rasam

The drug was analyzed to obtain its fingerprint profile using High-Performance Thin Layer Chromatography (HPTLC).

SNR Assessment in High-Performance Thin Layer Chromatography (HPTLC)

The advanced HPTLC method, derived from TLC, ensures accurate and efficient separation using pre-coated plates and an autosampler. It serves as a cost-effective tool for evaluating botanical materials, offering high sensitivity, selectivity, and speed for routine quality control. CAMAG Twin Trough chambers were used for chromatogram development, with elution based on adsorption properties. After drying, the plates were examined under UV light, scanned at 366 nm, and analyzed using CAMAG software. The chromatographic fingerprint identified phytoconstituents, recording their R_f values.[8]

Heavy Metal Analysis of SNR Using Atomic Absorption Spectrometry (AAS)

Sigma standards for "arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg)" were used in this study. Atomic Absorption Spectrometry (AAS), specifically the AA 240 Series model, was chosen for its accuracy in detecting metals in environmental samples. This method measured the total concentrations of these heavy metals in the test sample. For sample preparation, 1 mol/L HCl was used for mercury and arsenic digestion, while 1 mol/L HNO_3 was used for lead and cadmium. Standard solutions of 100 ppm for As and Hg were prepared in HCl, and Cd and Pb in HNO_3.[9]

RESULTS

Organoleptic characters

The organoleptic characters of SNR were done and noted in Table 2.

Physico-chemical parameters

The physicochemical analysis of SNR was done and noted in Table 3.

Results of Particle Size Analysis in SNR

Microscopic analysis of particle size showed an average size of 16.27±4.22 µm, as presented in Figure 1.

Solubility Profile of SNR

The solubility profile analysis of SNR was done and noted in Table 4.

Analytical Techniques for Siva Nama Rasam (SNR) Using Instrumentation

HPTLC Photo documentation

The HPTLC fingerprinting analysis of Siva Nama Rasam (SNR) involved the generation of 3D chromatograms at wavelengths of 254, 366, and 520 nm. The fingerprints were scanned at these wavelengths, highlighting the corresponding peaks. The chromatograms along with peak details are presented in Figures 2- 5.

Table 1: Ingredients of Siva Nama Rasam.

Sl. No.	Ingredients	Botanical name/Chemical name	Quantity
1.	Purified Gandhagam	Sulphur	50 g
2.	Purified Rasam	Hydrargyrum	50 g
3.	Purified Manosilai	Red orpiment	50 g
4.	Purified Vengaram	Borax	50 g
5.	Purified Karunabi	*Aconitum spicatum*	50 g
6.	Purified Milagu	*Piper nigrum*	50 g

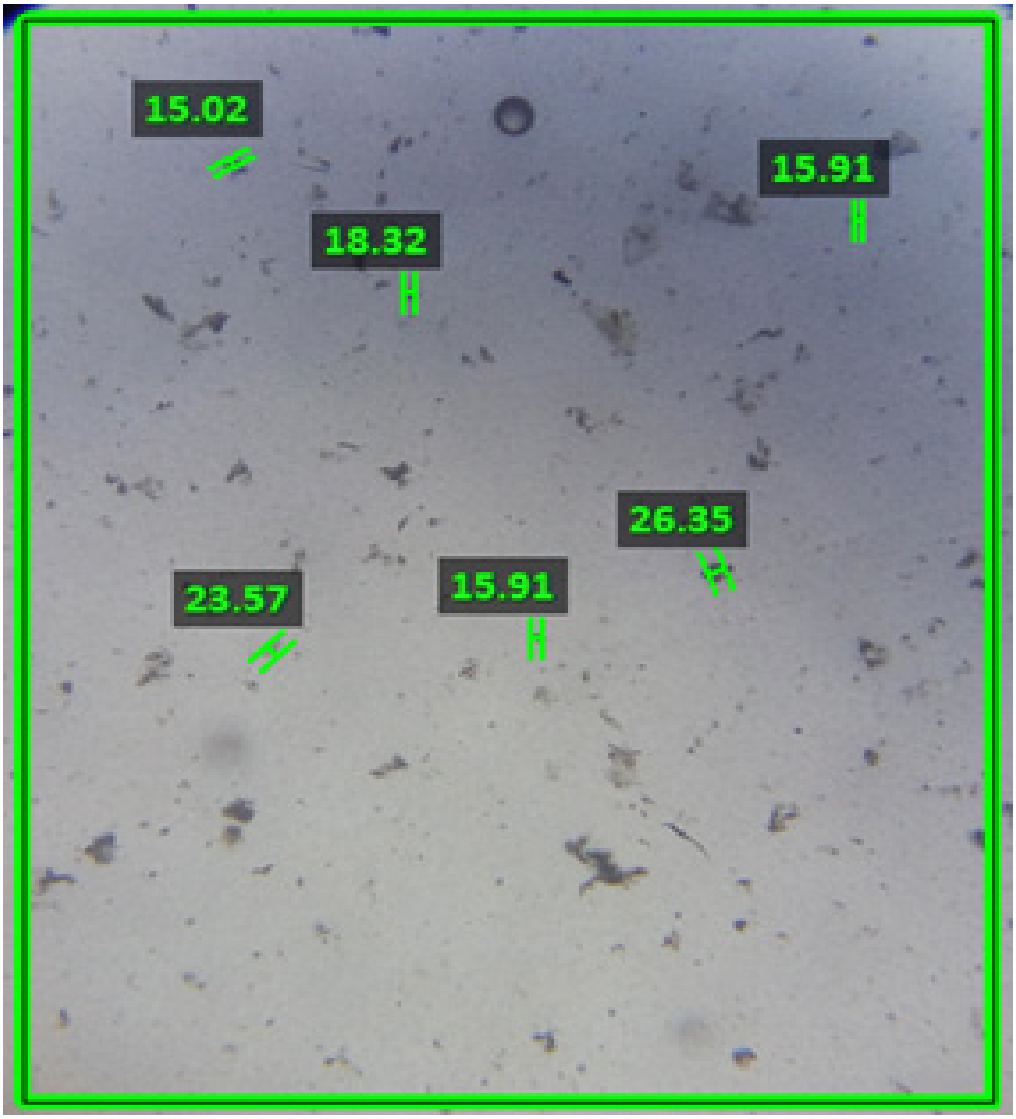


Figure 1: Microscopic Observation of Particle Size for the sample SNR.

SNR Analysis in Heavy Metal Assessment Using Atomic Absorption Spectrometry (AAS)

The findings of this study confirm that the sample contains no detectable traces of heavy metals such as mercury, arsenic, and cadmium. However, lead was present at a concentration of 0.060 ppm, as shown in Table 5.

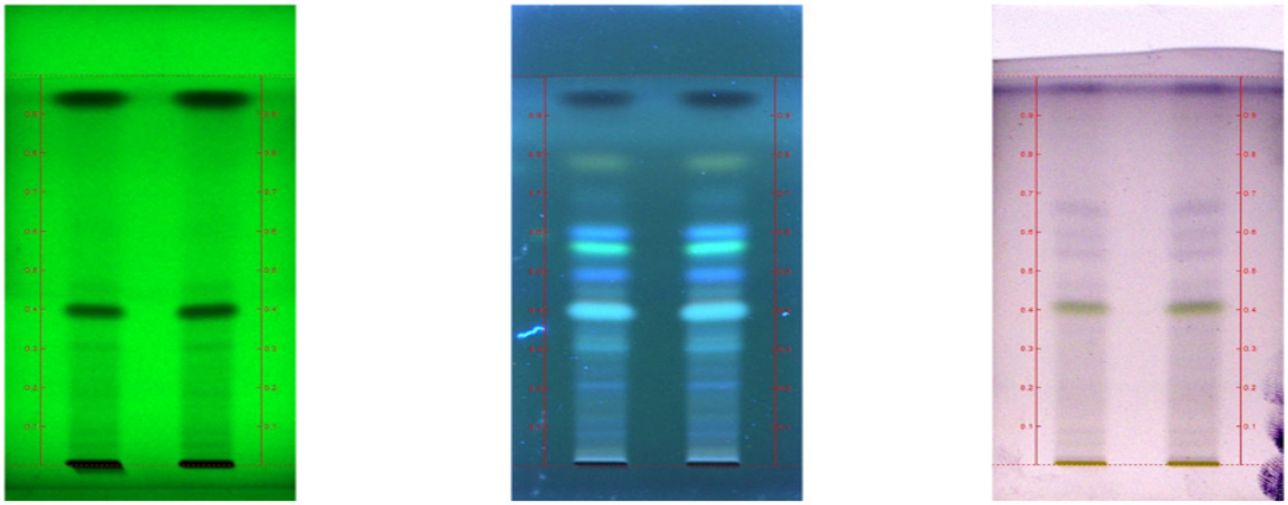
Table 2: Results of organoleptic characters of SNR.

Sl. No.	Parameters	Results
1.	Colour	Black
2.	Odour	odourless
3.	Texture	Fine powder
4.	Touch	Soft
5.	Flow Property	Smooth and Free flowing

DISCUSSION

The drug Siva Nama Rasam was made using several purifying techniques described in the literature, all in accordance with accepted Siddha sources. Furthermore, physicochemical standards were devised to evaluate the ultimate product's quality. The dark blackish appearance, unique odour, powder consistency, and free-flowing nature of the final formulation, along with its organoleptic characteristics, all attest to the validity of the raw ingredients and formulation. Loss on drying is a measurement often used in various fields such as chemistry, pharmaceuticals, and food science to determine the amount of moisture in a sample. The loss of mass can be caused by the release of water and other volatiles.[10] The product's quality and longevity are indicated by the 9.38% loss on drying value of SNR. The total ash value of SNR is 15.37%. The ash value provides information about the mineral content of the plant material.

It is used in various applications, including quality control, standardization, and assessing the purity of herbal products, foodstuffs, and other plant-based materials. It primarily



(a) (b) (c)

Figure 2: HPTLC Results Demonstrating the Fingerprint of Siva Nama Rasam (a) Under Short UV (254 nm), (b) Under Long UV (366 nm), (c) Under White light after Derivatization (520 nm).

Table 3: **Results of Physicochemical Analysis of SNR.**

Name of the Experiment	Experiment 1	Experiment 2	Mean Value
Loss on drying (at 105°C)	8.94	9.83	9.38 %
Total Ash	15.26	15.49	15.37 %
Water soluble ash	0.88	0.69	0.78 %
Acid insoluble ash	0.44	0.35	0.39 %
Water soluble extract	29.68	28.90	29.26 %
Alcohol soluble extract	12.27	12.29	12.28 %
pH	8.6	8.6	8.6

ensures the quality of powdered medicines. Calculating ash values helps verify the absence of mineral impurities, including adulteration, spent medicinal substances, and earth, sand, or floor sweepings.[11] The Acid Insoluble Ash of SNR is 0.39%, and the water-soluble ash is 0.78%. The Acid Insoluble Ash indicates the reduction of inorganic adulterants.[12] The drug SNR has a pH of 8.6, indicating its alkaline nature. A pH greater than 7 is basic. Therefore, oral consumption is unlikely to cause strong alkali or acid-like irritation to the gastrointestinal tract or any physical discomfort.[13] The extractive values of SNR that are soluble in alcohol and water are 12.28% and 29.26%, respectively. The higher water-soluble extractive value indicates that water is an effective solvent for extracting the formulation's components. The particle size of SNR was found to be 16.27±4.22 µm. Furthermore, the sample has particles ranging in size from 9 µm to 30 µm. Therefore, the drug SNR is easily absorbed and suitable for oral administration.[14] SNR is well soluble in major solvents like Water, Ethanol and DMSO, which proves its efficiency of solubility and increases bioavailability in the stomach indirectly.[15]

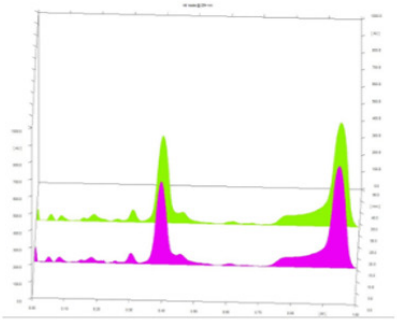
The HPTLC fingerprinting analysis of SNR at 254 nm revealed the presence of eleven distinct phytocomponents, with R_f values ranging from 0.03 to 0.82. Among them, the peak at an R_f value of 0.82 exhibited the highest area percentage of 41.44%, indicating the predominant compound. At 366 nm, fifteen phytocomponents were detected, with R_f values between 0.00 and 0.95. The most abundant compound at this wavelength corresponded to an R_f value of 0.00, contributing to the highest area percentage of 15.03%. Similarly, at 520 nm, fourteen phytocomponents were identified, with R_f values spanning from 0.01 to 0.91. The peak at an R_f value of 0.91 recorded the highest area percentage of 21.88%, highlighting its significant presence. The investigation results confirm that SNR is free from heavy metals such as arsenic

Table 4: **Results of Solubility profile of SNR.**

Sl. No.	Solvent Used	Solubility / Dispensability
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

Table 5: Results of Heavy Metal Analysis in SNR (BDL-Below Detection Limit).

Heavy metals	Wavelength (nm)	Results	Maximum limit
Lead	217.0	0.060	10 ppm
Arsenic	193.7	BDL	3 ppm
Cadmium	228.8	BDL	0.3 ppm
Mercury	253.7	BDL	1 ppm



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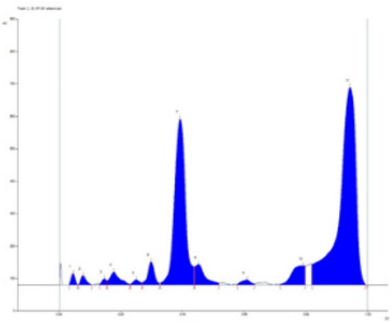
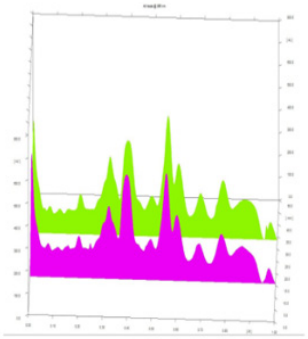


(b)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.4 AU	0.05 Rf	35.3 AU	2.40 %	0.06 Rf	0.1 AU	388.7 AU	0.74 %
2	0.06 Rf	0.1 AU	0.08 Rf	28.5 AU	1.94 %	0.11 Rf	0.2 AU	432.3 AU	0.83 %
3	0.13 Rf	2.1 AU	0.15 Rf	19.0 AU	1.29 %	0.16 Rf	13.0 AU	231.8 AU	0.44 %
4	0.16 Rf	13.1 AU	0.18 Rf	40.2 AU	2.74 %	0.23 Rf	2.0 AU	1096.2 AU	2.09 %
5	0.23 Rf	2.1 AU	0.25 Rf	16.1 AU	1.10 %	0.27 Rf	7.1 AU	293.4 AU	0.56 %
6	0.27 Rf	7.4 AU	0.30 Rf	71.5 AU	4.87 %	0.33 Rf	6.3 AU	1355.6 AU	2.59 %
7	0.33 Rf	7.0 AU	0.39 Rf	511.6 AU	34.80 %	0.44 Rf	56.2 AU	15840.9 AU	30.27 %
8	0.44 Rf	56.3 AU	0.45 Rf	63.6 AU	4.33 %	0.52 Rf	6.1 AU	1801.8 AU	3.44 %
9	0.58 Rf	2.6 AU	0.61 Rf	16.0 AU	1.09 %	0.63 Rf	3.3 AU	409.5 AU	0.78 %
10	0.72 Rf	2.4 AU	0.79 Rf	59.0 AU	4.02 %	0.80 Rf	58.3 AU	2178.7 AU	4.16 %
11	0.82 Rf	63.9 AU	0.94 Rf	609.2 AU	41.44 %	0.99 Rf	0.9 AU	28308.4 AU	54.09 %

(c)

Figure 3: HPTLC Finger Printing Analysis of SNR (a) 3D chromatogram of 254 nm, (b) HPTLC fingerprints scanning of 254 nm, (c) peak 254 nm.



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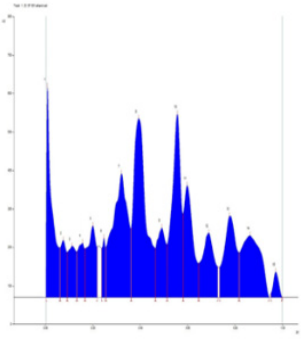
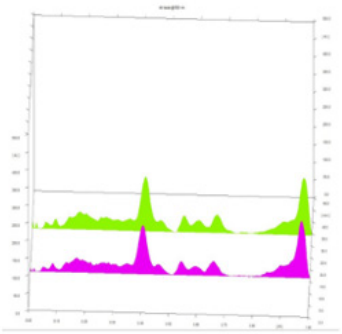


(b)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	548.0 AU	15.03 %	0.06 Rf	28.6 AU	10722.2 AU	8.39 %
2	0.06 Rf	128.8 AU	0.08 Rf	147.9 AU	4.05 %	0.09 Rf	17.3 AU	3101.4 AU	2.43 %
3	0.09 Rf	117.4 AU	0.11 Rf	133.1 AU	3.65 %	0.13 Rf	19.1 AU	3766.7 AU	2.95 %
4	0.13 Rf	119.4 AU	0.16 Rf	141.2 AU	3.87 %	0.17 Rf	26.7 AU	3421.3 AU	2.68 %
5	0.17 Rf	126.9 AU	0.20 Rf	185.4 AU	5.08 %	0.22 Rf	31.1 AU	5815.6 AU	4.55 %
6	0.24 Rf	128.0 AU	0.25 Rf	153.3 AU	4.20 %	0.25 Rf	29.3 AU	1925.2 AU	1.51 %
7	0.26 Rf	130.6 AU	0.32 Rf	321.5 AU	8.82 %	0.36 Rf	79.1 AU	17818.2 AU	13.95 %
8	0.36 Rf	181.3 AU	0.39 Rf	464.4 AU	12.73 %	0.46 Rf	26.9 AU	21195.9 AU	16.59 %
9	0.47 Rf	127.9 AU	0.49 Rf	180.5 AU	4.95 %	0.51 Rf	37.8 AU	5806.6 AU	4.55 %
10	0.51 Rf	138.8 AU	0.56 Rf	476.0 AU	13.05 %	0.58 Rf	16.7 AU	15321.5 AU	11.99 %
11	0.58 Rf	219.1 AU	0.60 Rf	290.7 AU	7.97 %	0.65 Rf	88.8 AU	9519.2 AU	7.45 %
12	0.65 Rf	89.1 AU	0.69 Rf	166.9 AU	4.58 %	0.73 Rf	79.2 AU	7396.4 AU	5.79 %
13	0.73 Rf	78.7 AU	0.78 Rf	211.5 AU	5.80 %	0.82 Rf	16.4 AU	9336.0 AU	7.31 %
14	0.82 Rf	116.5 AU	0.86 Rf	160.4 AU	4.40 %	0.94 Rf	1.2 AU	11307.7 AU	8.85 %
15	0.95 Rf	9.9 AU	0.97 Rf	66.1 AU	1.81 %	1.00 Rf	1.2 AU	1286.3 AU	1.01 %

(c)

Figure 4: HPTLC Finger Printing Analysis of SNR 3D chromatogram of 366 nm, (b) HPTLC fingerprints scanning of 366 nm, (c) peak 366 nm.



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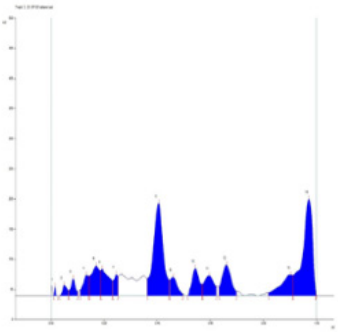

(b)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.02 Rf	14.9 AU	2.04 %	0.03 Rf	0.0 AU	62.7 AU	0.31 %
2	0.03 Rf	0.1 AU	0.05 Rf	17.7 AU	2.42 %	0.07 Rf	8.9 AU	259.6 AU	1.28 %
3	0.07 Rf	9.0 AU	0.08 Rf	28.0 AU	3.82 %	0.10 Rf	6.7 AU	411.6 AU	2.04 %
4	0.11 Rf	9.7 AU	0.13 Rf	33.7 AU	4.61 %	0.14 Rf	32.4 AU	636.6 AU	3.15 %
5	0.15 Rf	32.6 AU	0.17 Rf	49.8 AU	6.80 %	0.19 Rf	41.6 AU	1386.6 AU	6.86 %
6	0.19 Rf	41.9 AU	0.19 Rf	43.9 AU	6.00 %	0.23 Rf	24.6 AU	1165.3 AU	5.76 %
7	0.23 Rf	24.7 AU	0.25 Rf	35.2 AU	4.81 %	0.25 Rf	32.6 AU	463.2 AU	2.29 %
8	0.36 Rf	28.3 AU	0.41 Rf	153.6 AU	20.98 %	0.45 Rf	25.9 AU	4952.7 AU	24.49 %
9	0.45 Rf	26.0 AU	0.46 Rf	30.8 AU	4.21 %	0.50 Rf	3.2 AU	722.6 AU	3.57 %
10	0.51 Rf	0.0 AU	0.54 Rf	45.4 AU	6.21 %	0.57 Rf	18.6 AU	1081.2 AU	5.35 %
11	0.57 Rf	18.7 AU	0.60 Rf	33.2 AU	4.53 %	0.62 Rf	14.7 AU	1027.1 AU	5.08 %
12	0.63 Rf	14.6 AU	0.66 Rf	50.9 AU	6.96 %	0.70 Rf	7.3 AU	1513.0 AU	7.48 %
13	0.82 Rf	4.9 AU	0.90 Rf	34.8 AU	4.75 %	0.91 Rf	34.0 AU	1362.4 AU	6.74 %
14	0.91 Rf	33.8 AU	0.97 Rf	160.1 AU	21.88 %	1.00 Rf	5.5 AU	5180.0 AU	25.61 %

(c)

Figure 5: HPTLC Finger Printing Analysis of SNR 3D (post derivatized) chromatogram of 520 nm, (b) HPTLC fingerprints scanning of 520 nm, (c) peak 520 nm.

and cadmium, ensuring the safety of the drug. Moreover, the levels of mercury and lead are below permissible limits, further ensuring safety.

CONCLUSION

In conclusion, this study shows that Siva Nama Rasam (SNR) follows traditional Siddha practices and meets quality standards. The findings confirm its safety and effectiveness, supporting its use in traditional medicine. This research also provides useful information for future studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

SNR: Siva Nama Rasam; **PLIM:** Pharmacopeia Laboratory of Indian Medicine; **HPTLC:** High-Performance Thin-Layer Chromatography; **TLC:** Thin-Layer Chromatography; **AAS:** Atomic Absorption Spectrometry; **As:** Arsenic; **Pb:** Lead; **Cd:** Cadmium; **Hg:** Mercury; **HCl:** Hydrochloric acid; **HNO_3:** Nitric acid; **DMSO:** Dimethyl Sulfoxide; **AYUSH:** Ayurveda, Yoga naturopathy, Unani, Siddha and Homeopathy; **BQL:** Below Quantification Limit.

ETHICAL APPROVAL

As the present study is an *in vitro* investigation, it does not involve the use of human or animal subjects and therefore, ethical clearance is not applicable.

AUTHOR CONTRIBUTION

Conceptualization: KAP; Medicine Preparation: KAP and SR; Data collection and compilation: KAP and SR; Manuscript Writing: KAP, SR and SS; Proofreading and editing: KAP, SR and SS.

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