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Research Article

PHYSICOCHEMICAL, PHYTOCHEMICAL AND HPTLC EVALUATION OF SIDDHA HERBAL FORMULATION *CHITHIRAMOOLA RASAYANAM*

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ABSTRACT

Chithiramoola Rasayanam is a traditional *Siddha* polyherbal formulation commonly used in *Siddha* practice for improving general health and managing chronic illnesses. Even though the medicine has been used for many years in traditional practice, proper scientific standardization is necessary to ensure its quality, purity, and safety. The present study was carried out to evaluate the physicochemical characteristics, phytochemical constituents, and HPTLC fingerprint profile of *Chithiramoola Rasayanam*. The formulation was prepared according to the procedures mentioned in the classical Siddha text *Pulipani Vaidhiyam 500* and the purification methods described in *Sarakugalin Suthi Sei Muraigal*. Physicochemical parameters such as loss on drying, total ash, acid insoluble ash, extractive values, fat content, and pH were analysed using PLIM guidelines. Preliminary phytochemical screening was performed using standard laboratory procedures. HPTLC fingerprint analysis was carried out using methanolic extract of the formulation with Toluene: Ethyl acetate: Acetic acid (8:2:0.5) as the solvent system. The results showed that the formulation possessed acceptable physicochemical properties. Loss on drying was found to be 5.59%, total ash 1.29%, acid insoluble ash 0.82%, water soluble extractive value 46.44%, alcohol soluble extractive value 30.15%, fat content 14.15%, and pH 4.94. Phytochemical analysis revealed the presence of phenols, tannins, and proteins. HPTLC fingerprint profiling performed at 520nm showed several characteristic peaks and distinct bands with Rf values ranging from 0.01 to 0.97, indicating the presence of multiple phytoconstituents in the formulation. The findings of the study provide preliminary standardization data for *Chithiramoola Rasayanam* and may be useful for future quality control studies and further pharmacological research.

INTRODUCTION

Traditional Siddha formulations continue to play an important role in the healthcare practices of South India. Herbal preparations described in Siddha literature are widely used for promoting health and managing chronic diseases. In recent years, there has been increasing scientific interest in validating these traditional medicines through analytical and standardization studies.[6]

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Rasayanam is a semisolid dosage form commonly prepared using herbal ingredients along with adjuvants such as honey, sugar, and ghee. These preparations are traditionally believed to improve vitality, strengthen the body, and support long-term wellbeing.[7] Because of their complex composition, scientific evaluation of *Rasayanam* formulations is necessary to maintain quality standards and ensure batch-to-batch consistency.

Chithiramoola Rasayanam is a classical Siddha herbal preparation mentioned in traditional Siddha texts. The formulation is used in Siddha practice for its therapeutic benefits and rejuvenative properties. Although the medicine has been traditionally utilized for many years, limited analytical documentation is available regarding its standardization profile.

Evaluation of herbal formulations using physicochemical parameters and chromatographic techniques provides supportive evidence for quality control and authentication.[8] Preliminary phytochemical studies help identify the major classes of chemical constituents present in the formulation, while HPTLC fingerprint profiling acts as an important tool for detecting characteristic phytochemical patterns.[9]

The present work was therefore designed to establish analytical parameters for *Chithiramoola Rasayanam* through physicochemical analysis, phytochemical screening, and HPTLC fingerprint profiling. The generated data may contribute towards future research and standardization of *Siddha* formulations.

MATERIALS AND METHODS

Collection and Authentication of Raw Drugs

The raw drugs required for the preparation of *Chithiramoola Rasayanam* were procured from a reliable country drug shop in Chennai, Tamil Nadu. The ingredients were authenticated by experts of the Department of Gunapadam and by a Botanist, Government Siddha Medical College, Chennai.

Purification of Raw Drugs

Purification procedures were carried out according to the methods mentioned in the *Siddha* literature *Sarakugalin Suthi Sei Muraigal*.[2] The purified raw drugs were shade dried and powdered separately.

Preparation of *Chithiramoola Rasayanam*

The formulation was prepared according to the classical *Siddha* text *Pulipani Vaidhiyam 500*.[1] The purified ingredients were dried, powdered, and sieved through *Vasthra Kaayam* to obtain fine powder. The powder was processed by *Pittaviyal* method and mixed with sugar, honey, and ghee to obtain the final *Rasayanam* consistency. The prepared formulation was stored in an airtight container.

Physicochemical Analysis

Physicochemical parameters were evaluated according to standard PLIM guidelines.[3] The following parameters were analyzed:

- Loss on drying at 105°C
- Total ash value
- Water soluble ash
- Acid insoluble ash
- Water soluble extractive value
- Alcohol soluble extractive value
- Fat content
- pH determination

Preliminary Phytochemical Screening

The formulation was subjected to preliminary phytochemical screening using standard procedures to identify major phytoconstituents such as alkaloids, flavonoids, phenols, tannins, proteins, glycosides, steroids, and saponins.[4]

HPTLC Fingerprint Analysis

Methanolic extract of *Chithiramoola Rasayanam* was prepared and subjected to HPTLC analysis.[5]

Chromatographic Conditions

- Stationary phase: Silica gel 60 F254 TLC plates
- Mobile phase: Toluene : Ethyl acetate : Acetic acid (8:2:0.5)
- Detection: UV 254 nm and UV 366 nm
- Derivatization reagent: Vanillin sulphuric acid

The developed plates were observed under UV light and after derivatization to obtain fingerprint profiles.

RESULTS

Physicochemical Parameters

S.No	Parameter	I	II	Mean
1	Loss on drying	5.40%	5.48%	5.59%
2	Total ash	1.30%	1.29%	1.29%
3	Water soluble ash	Nil	Nil	Nil
4	Acid insoluble ash	0.80%	0.84%	0.82%
5	Water soluble extractives	46.24%	46.63%	46.44%
6	Alcohol soluble extractives	30.22%	30.08%	30.15%
7	Fat content	14.29%	14.01%	14.15%
8	pH	4.94	4.94	4.94

The physicochemical parameters showed that the formulation possessed acceptable quality and stability characteristics.

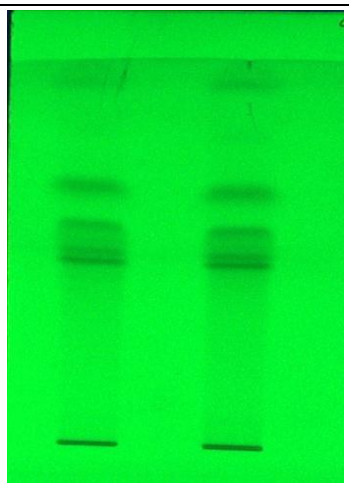
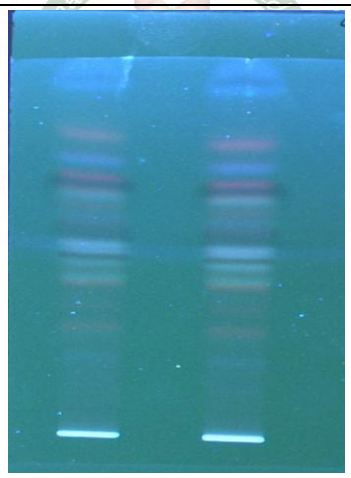
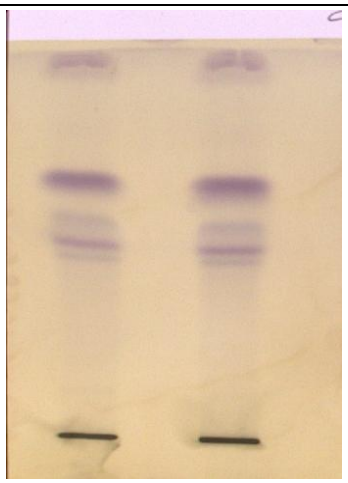
Phytochemical analysis

S.No	Phytochemical test	Result
1.	Phenols	Present
2.	Tannins	Present
3.	Saponins	Absent
4.	Flavonoids	Absent
5.	Protein	Present
6.	Cardiac glycosides	Absent
7.	Glycosides	Absent
8.	Alkaloid	Absent
9.	Quinones	Absent
10.	Steroids	Absent
11.	Anthraquinones	Absent
12.	Coumarins	Absent
13.	Acids	Absent
14.	Reducing sugar	Absent

The phytochemical analysis revealed the presence of phenols, tannins, and proteins, which may contribute to the therapeutic activity of the formulation.

HPTLC Profile

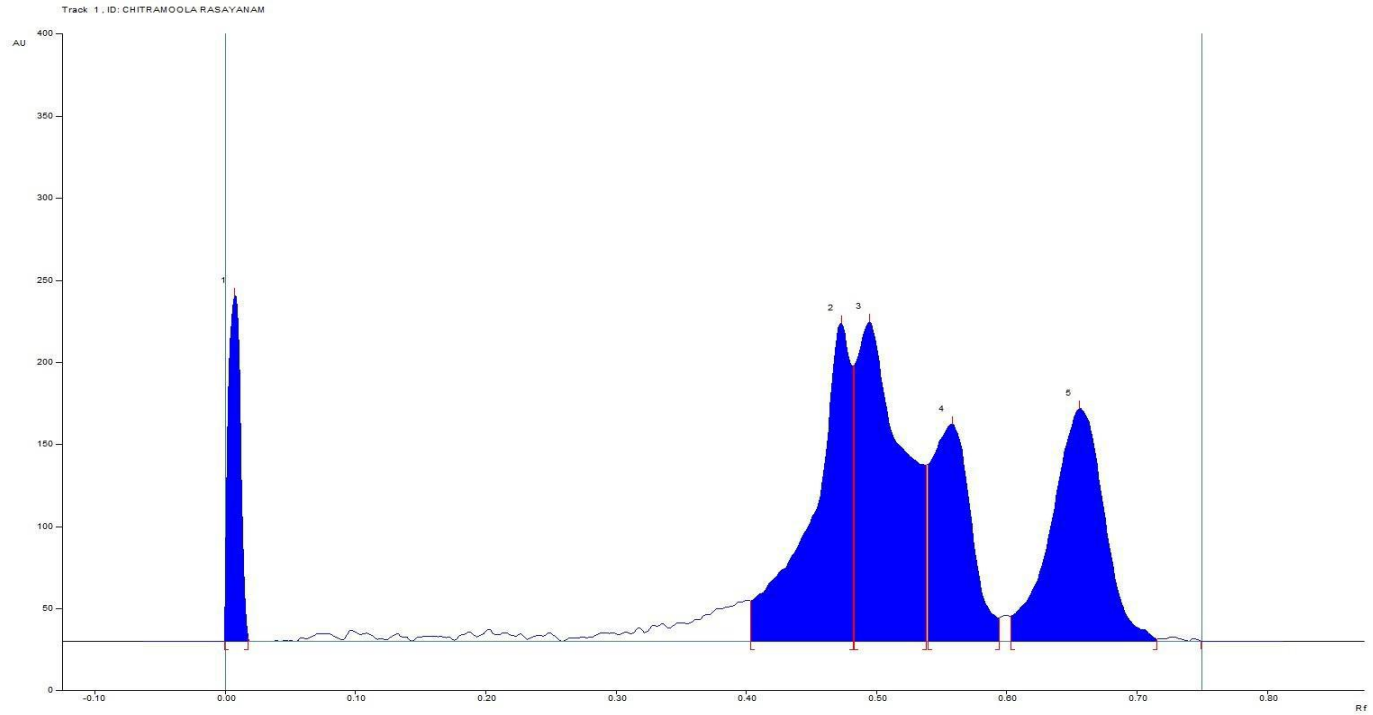
TLC Photo documentation

UV 254 nm		UV 366 nm		Derivatized plate	
Rf value	**Color**	**Rf value**	**Color**	**Rf value**	**Color**
0.46	Green	0.20	Blue	0.44	Purple
0.49	Green	0.28	Red	0.48	Purple
0.55	Green	0.40	Green	0.54	Purple
0.65	Green	0.45	Green	0.64	Purple
0.93	Green	0.49	Blue	0.94	Purple
		0.62	Green		
		0.66	Black		
		0.71	Blue		
		0.77	Red		
		0.91	Blue		

The HPTLC chromatogram exhibited multiple bands at different Rf values under UV 254nm, UV 366nm, and after derivatization, indicating the presence of various phytoconstituents in the formulation.

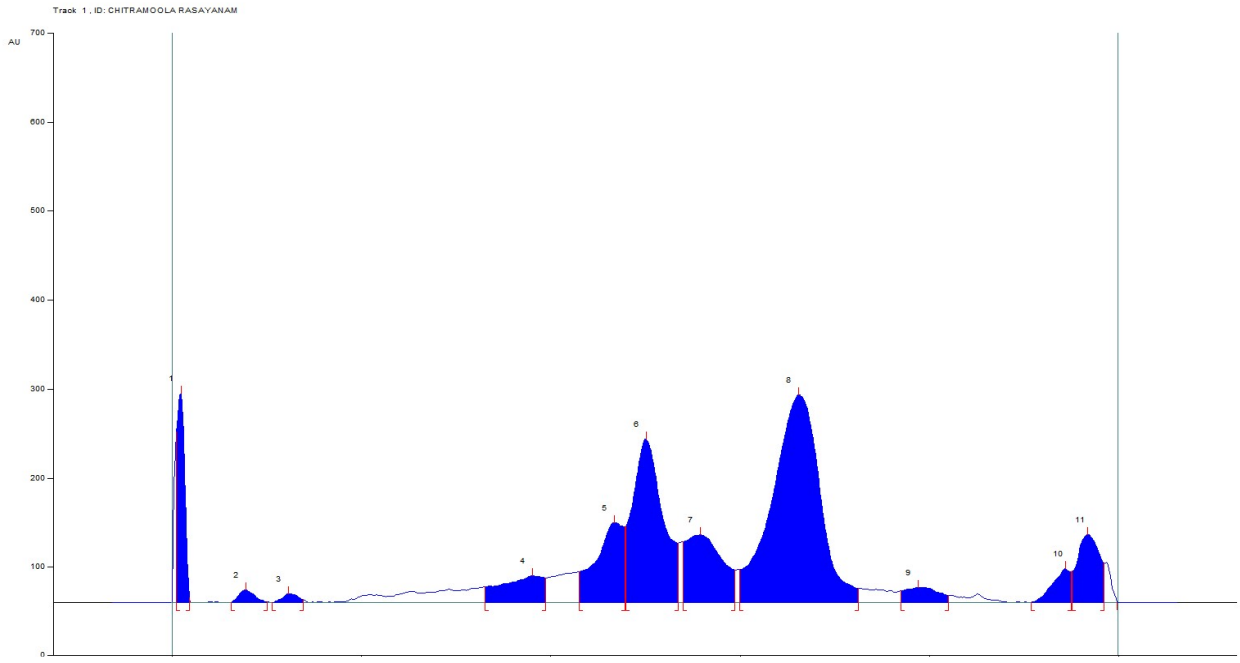
HPTLC Finger print profiling at UV 254 nm



Track 1, ID: CHITRAMOOLA RASAYANAM

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	210.6 AU	24.12 %	0.02 Rf	2.7 AU	1799.9 AU	7.95 %
2	0.40 Rf	24.6 AU	0.47 Rf	193.5 AU	22.17 %	0.48 Rf	67.1 AU	5370.7 AU	23.73 %
3	0.48 Rf	168.0 AU	0.50 Rf	194.6 AU	22.30 %	0.54 Rf	07.4 AU	6447.3 AU	28.49 %
4	0.54 Rf	107.6 AU	0.56 Rf	132.2 AU	15.15 %	0.60 Rf	14.3 AU	3697.7 AU	16.34 %
5	0.60 Rf	15.4 AU	0.66 Rf	141.9 AU	16.26 %	0.72 Rf	1.3 AU	5317.3 AU	23.49 %

HPTLC Finger print profiling at 520 nm



Track 1, ID: CHITRAMOOLA RASAYANAM

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	191.9 AU	0.01 Rf	235.6 AU	23.41 %	0.02 Rf	5.3 AU	1822.6 AU	6.54 %
2	0.06 Rf	0.1 AU	0.08 Rf	14.2 AU	1.41 %	0.10 Rf	0.6 AU	220.0 AU	0.79 %
3	0.11 Rf	0.1 AU	0.12 Rf	10.2 AU	1.01 %	0.14 Rf	3.4 AU	151.3 AU	0.54 %
4	0.33 Rf	17.7 AU	0.38 Rf	30.2 AU	3.00 %	0.40 Rf	27.9 AU	1241.0 AU	4.45 %
5	0.43 Rf	34.6 AU	0.47 Rf	90.2 AU	8.97 %	0.48 Rf	85.5 AU	2493.1 AU	8.94 %
6	0.48 Rf	86.6 AU	0.50 Rf	183.9 AU	18.28 %	0.54 Rf	67.1 AU	5418.6 AU	19.44 %
7	0.54 Rf	68.5 AU	0.56 Rf	76.2 AU	7.57 %	0.60 Rf	36.6 AU	2774.3 AU	9.95 %
8	0.60 Rf	37.4 AU	0.66 Rf	234.1 AU	23.26 %	0.73 Rf	16.1 AU	10850.1 AU	38.93 %
9	0.77 Rf	13.3 AU	0.79 Rf	16.9 AU	1.68 %	0.82 Rf	7.6 AU	569.5 AU	2.04 %
10	0.91 Rf	0.2 AU	0.94 Rf	38.1 AU	3.78 %	0.95 Rf	34.7 AU	664.2 AU	2.38 %
11	0.95 Rf	35.3 AU	0.97 Rf	76.6 AU	7.62 %	0.99 Rf	44.3 AU	1669.2 AU	5.99 %

HPTLC densitometric scanning performed at 520nm revealed 11 characteristic peaks with Rf values ranging from 0.01 to 0.97. Among them, the major peak was observed at Rf 0.66 with an area percentage of 38.93%, followed by peaks at Rf 0.50 and 0.56. The chromatographic profile indicates the presence of multiple phytochemical constituents in *Chithiramoola Rasayanam.*

S.No	Parameter	Result
1	Loss on drying	5.59%
2	Total ash	1.29%
3	Acid insoluble ash	0.82%
4	Water soluble extractive	46.44%
5	Alcohol soluble extractive	30.15%
6	Fat content	14.15%
7	pH	4.94

DISCUSSION

Standardization of traditional herbal formulations plays an important role in ensuring the quality and safety of medicines used in clinical practice.[10] In the present study, *Chithiramoola Rasayanam* was analysed using physicochemical evaluation, preliminary phytochemical screening, and HPTLC fingerprint profiling.

The loss on drying value of the formulation was found to be 5.59%, which indicates the presence of minimal moisture content. Lower moisture content is generally considered beneficial because it reduces the possibility of microbial growth and improves the stability of the formulation during storage. The total ash and acid insoluble ash values were low, suggesting that the formulation contains very little inorganic impurities or extraneous matter.

The water-soluble extractive value was found to be higher than the alcohol soluble extractive value. This may indicate the presence of more polar constituents in the formulation. The phytochemical screening also confirmed the presence of phenols and tannins, which are known to possess antioxidant and therapeutic activities.[11] Proteins were also detected in the formulation.

The fat content observed in the formulation may be due to the addition of ghee during preparation. In *Siddha* formulations, ghee is traditionally believed to improve the therapeutic action and enhance the absorption of active constituents. The pH of the formulation was mildly acidic, which may help in maintaining stability.

HPTLC fingerprint analysis showed several distinct bands under UV 254nm, UV 366nm, and after derivatization. The HPTLC densitogram obtained at 520nm demonstrated multiple peaks with varying area percentages, suggesting the presence of several bioactive compounds within the formulation. The major peak observed at Rf 0.66 may indicate the predominance of certain phytoconstituents. Such fingerprint profiles can serve as useful reference standards for identification and quality control of the formulation. [12]

Overall, the study generated useful analytical data for *Chithiramoola Rasayanam* and supports its identity and quality as a traditional *Siddha* formulation.

CONCLUSION

The analytical evaluation carried out in the present study provided useful information regarding the quality characteristics of *Chithiramoola Rasayanam*. The physicochemical parameters obtained were within acceptable limits and may serve as preliminary standards for identification and quality assessment of the formulation.

Phytochemical screening indicated the presence of certain bioactive constituents, especially phenols and tannins, which may contribute to the medicinal value of the drug. The HPTLC chromatographic profile demonstrated multiple characteristic bands, confirming the presence of diverse phytochemical compounds.

The study findings can be considered as supportive scientific documentation for *Chithiramoola Rasayanam* and may help in future standardization and quality control studies of Siddha herbal formulations. Further pharmacological and clinical investigations are required to explore the therapeutic potential of the medicine in detail.

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