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SHORT COMMUNICATION

Standardization and HPTLC Profile of *Siddha* Polyherbal Formulation *Thontha Sura Kiyaazham* (TSK) and *Thontha Sura Kiyaazham Chooranam* (TSKC)

R. Uthaya Ganga[1*], K. Suresh[1], E. M. Manikgantan[2], S. Kalpana[3] and Senthilkumar B. Rajamohan[4]

[1]Department of Kuzhanthai Maruthuvam, National Institute of Siddha, Affiliated to the Tamil Nadu Dr. M. G. R. Medical University, Chennai - 600045, Tamil Nadu, India; uthayaganga@gmail.com

[2]Department of Siddha, The Tamil Nadu Dr. M. G. R. Medical University, Chennai - 600032, Tamil Nadu, India

[3]Department of Epidemiology, The Tamil Nadu Dr. M. G. R. Medical University, Chennai - 600032, Tamil Nadu, India

[4]Department of Udal Thathuvam (Physiology), National Institute of Siddha, Affiliated to The Tamil Nadu Dr. M. G. R. Medical University, Chennai - 600032, Tamil Nadu, India

Abstarct

Background: Standardizing process is a comprehensive evaluation of physico-chemical and pharmacological profiles of a drug which enables the establishment of benchmark parameters that provide confidence in safety and efficacy of traditional *Siddha* medicines. *Siddha* system is one of the medical sciences of India. **Aim:** The objective of the study was to do systematic standardisation of *Thontha Sura kiyaazham* Chooranam (TSKC - Coarse powder for decoction) and *Thontha Sura kiyaazham* (TSK - Decoction) to create documentary evidence. **Methods:** The phytochemical analysis, physicochemical analysis and HPTLC finger print analytical techniques based on the PLIM guidelines had been evaluated. **Results:** The study results portrayed a wide range of Phytochemicals both in TSKC and TSK, including phenols, tannins, flavonoids, and triterpenoids, which were known for their therapeutic properties. In physicochemical analysis TSKC and TSK showed pH of 2.95 (strong acidic) and 5.98 (weak acidic) respectively. HPTLC chromatogram was observed at UV 254 nm, 366 nm and 520 nm before and after derivatization and their respective Rf values were analyzed. **Conclusion:** It is the need of the hour to standardize the traditional herbal formulation. So, this study was conducted to create scientific based profiles for research drug. It highlighted the physicochemical and phytochemical differences between TSKC and TSK. These variations were likely to influence therapeutic efficacy and bioavailability. Standardizing polyherbal formulations with advanced analytical methods ensures consistent quality, safety and efficacy to promote their integration in healthcare systems and will make pavement for the global acceptance of *Siddha* system of medicine.

Major Findings: In physicochemical analysis, TSK (decoction form) revealed week acidic pH and TSKC (Coarse powderfor decoction) revealed strong acidic pH. The absorption of weakly acidic drugs in the stomach is generally more favourable than that of strong acidic drugs. Hence the liquid form of TSK facilitates easy absorption in gastro intestinal tract.

Keywords: Fever, Herbal Medicine, HPTLC, *Siddha*, *Thontha Sura Kiyaazham* (TSK)

1. Introduction

Indian society has long relied on traditional healthcare methods to treat and prevent both acute and chronic illnesses[1]. In India, traditional therapies-particularly *Siddha* medical system have a well-established role in the treatment of a wide range of illnesses. These treatments have been shown to be safe, effective, and well-liked by people of all ages[2].

*Author for correspondence

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Numerous traditional medicinal formulations are documented in *Siddha* literature, addressing various ailments and improving human well-being[3,4]. *Siddha* medicine encompasses two broad categories: Internal and external medicines. Among the internal medicines, *Kiyaazham Chooranam* (Decoction Powder form) is one of the forms. The *Kiyaazham* (Decoction) is prepared in precise concentrated proportions. *Kiyaazham* is also known as *Kudineer* in *Siddha* literature. It is an excellent medication especially for children, facilitating easy absorption and promoting health[5].

The success of the treatment depends on the purity and quality of the used raw materials and the quality of finished products[6]. If the drug that does not meet specified standards, it will not meet the requirements of prescription. Standardisation is essential for quality assurance, fulfil the regulatory compliances and global acceptance[7,8]. Hence the study had been framed in accordance with AYUSH laws, to standardize *Thontha Sura Kiyaazha* Chooranam (TSKC) and *Thontha Sura Kiyaazham* (TSK) by phytochemical, and physicochemical and HPTLC evaluation[1].

PLIM guidelines help in various parameters testing for different categories of simple and compound formulations. It ensures the safety and quality parameters in medicines[9]. The following parameters are the analytical specifications of *Kiyaazham* (*Kudineer)* chooranam (Coarse powder for decoction). The aim of this study was to standardize the poly herbal formulation *Thontha Sura Kiyaazham Chooranam and Thontha Sura Kiyaazham* for scientific validation and clinical use of the society. The objective of this study was to assess the physico-chemical properties, phytochemical composition, and perform HPTLC analysis.

2. Materials and Methods

2.1 Ingredients of *Thontha Sura Kiyaazham Chooranam* (TSKC)[10]

2.2 Identification, Authentication and Purification of Raw Drugs

The raw materials depicted in Table 1 were identified and authenticated by the experts of *Gunapadam* (*Siddha* pharmacology) and Medicinal Botany Department of National Institute of *Siddha*, Tambaram Sanatorium, Chennai. Drug authentication certificate number is NISMB6242023. All the raw drugs specified above were purified as per *Siddha* literature[11].

2.2 Phytochemical Analysis

All the phytochemical parameters were carried out as per the standard test procedures[12,13].

2.3 Instrumental Analysis

2.3.1 Sample Preparation for TLC of Thontha Sura Kiyaazham Chooranam

1 gm of sample was taken and dissolved in 10 ml of chloroform.Then sonicated for 15 minutes and was filtered. The filtrate was utilized for HPTLC analysis[14].

2.3.2 Sample Preparation for TLC of Thontha Sura Kiyaazham

Kiyaazham was first prepared by taking 30 grams of sample in 1000 ml beaker and added 650 ml of distilled water then reduced to 160 ml on water bath. Then the *Kiyaazham* was filtered. 10 ml of *Kiyaazham* was evaporated to dryness. To this dried *Kiyaazham* extract, added 10 ml of ethanol and sonicated for 20 min. Then filtered and collected in asample vial[14].

2.3.3 High Performance Thin Layer Chromatography (HPTLC)

Test sample was used to Thin Layer Chromatography (TLC) as per conventional one-dimensional ascending method applying silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate

Table 1. Ingredients of *thontha sura kiyaazham chooranam*

S. No.	Tamil Name	Botanical Name	Family	Parts Used
1	*Nilavembu*	*Andrographis paniculate* (Burm. f.) Nees	*Acanthaceae*	Whole plant
2	*Parpadaagam*	*Mollugo cerviana* Linn	*Molluginaceae*	Whole plant
3	*Koraikizhangu*	*Cyperus rotundus* Linn	*Cyperaceae*	Tuber
4	*Seendilkodi*	*Tinospora cordifolia* (Willd.) Hook. f. and Thoms	*Menispermaceae*	Stem
5	*Kadakarohini*	*Picrorhiza kurroa* Royle ex Benth.	*Scrophulariaceae*	Root

markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro litter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system after the run plates were dried and were observed by using visible light short-wave UV light 254 nm and light long-wave UV light 366 nm[15]. A volume of 15 µl of the extract was applied onto a silica gel-coated TLC plate (60F254) using the Camag ATS4 sample applicator. The plate was developed in a CAMAG twin trough chamber (20 × 10 cm), which had been pre-saturated with the mobile phase. For *Thontha Sura Kiyaazham Chooranam*, the mobile phase consisted of toluene, ethyl acetate, and acetic acid in the ratio 8.5:1.5:0.6 (v/v/v). For *Thontha Sura Kiyaazham*, the mobile phase was a mixture of chloroform, ethyl acetate, methanol, and acetic acid in the proportion 3:6:2:0.5 (v/v/v/v). The chromatogram was developed up to a distance of 85 mm from the baseline. After development, the plate was documented photographically using the Camag TLC visualizer under UV light at wavelengths of 254 nm and 366 nm. Subsequently, the plate was scanned with the Camag Scanner 4 at 254 nm (D2 lamp, absorption mode) and 366 nm (Hg lamp, fluorescence mode) to obtain fingerprint profiles of the extracts. The plate was then dipped in a 5% sulfuric acid solution in alcohol and heated at 105° C until coloured spots appeared. Finally, the plate was photographed under white light using the Camag TLC visualizer[16,17].

3. Results

3.1 Preliminary Phytochemical Analysis

The phytochemical analysis for TSKC and TSK were tested for various compounds like phenols, tannin, flavonoids, triterpenoids. Proteins, glycosides, reducing sugar, anthraquinones, alkaloids, saponins, steroids *etc* and as shown in Table 2.

3.2 Physico-chemical Analysis

The physicochemical analysis of TSKC (*Thontha Sura Kudineer Chooranam*) and TKC (*Thontha Sura Kudineer*) revealed consistent results. For TSKC, the mean values observed were: Loss on drying at 105° C- 10.58%, total ash- 10.11 %, water-soluble ash- 0.83 %, acid-insoluble ash- 3.85 %, water-soluble extractive- 15.86 %, alcohol-soluble extractive- 11.72 %, and pH (10% solution)- 2.95. For TKC, the specific gravity was 1.015, total solids were 4.15 %, and the pH was 5.98. These parameters help establish the quality and consistency of the formulation (Table 3 and 4).

Table 2. Phytochemical analysis of TSKC and TSK

S. No.	Phytochemical Tests	*Thontha Sura Kiyaazham Chooranam* (TSKC)	*Thontha Sura Kiyaazham* (TSK)
1	Test for phenol	Present	Present
2	Test for tannin	Present	Present
3	Test for flavonoids	Present	Present
4	Test for triterpenoids	Present	Present
5	Test for proteins	Absent	Absent
6	Test for glycosides	Present	Present
7	Test for reducing sugar	Present	Present
8	Test for anthraquinones	Absent	Absent
9	Test for quinones	Present	Present
10	Test for alkaloids	Present	Present
11	Test for saponins	Present	Present
12	Test for cardiac glycoside	Present	Present
13	Test for steroids	Present	Present
14	Test for coumarin	Present	Present
15	Test for acids	Present	Present

Table 3. Physicochemical parameters of *thontha sura kiyaazha chooranam* (TSKC)

S. No.	Name of the Experiment	I	II	Mean Value
1	Loss on drying (at 105° C)	10.05	11.12	10.58
2	Total ash	10.30	9.92	10.11
3	Water soluble ash	0.65	1.00	0.83
4	Acid insoluble ash	3.95	3.74	3.85
5	Water soluble extractive	16.04	15.67	15.86
6	Alcohol soluble extractive	12.37	11.06	11.72
7	pH value (10%)	2.95	2.95	2.95

Table 4. Physical parameters of *thontha sura kiyaazham* (TSK)

S. No.	Name of the Experiment	I	II	Result
1	Specific gravity	1.016	1.014	1.015
2	Total solids	4.16	4.14	4.15 %
3	pH	5.98	5.98	5.98

3.3 HPTLC Finger Print Profile

HPTLC analysis of TSKC and TSK were performed and chromatogram showed peak corresponding to various compounds (Figures 1 and 2). R_f values were mentioned in Table 5.

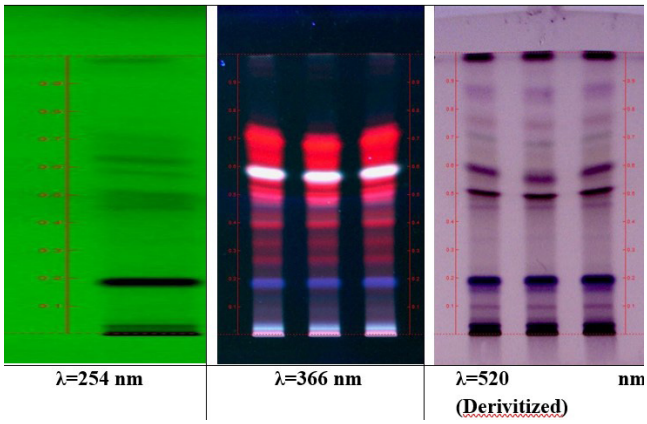


Figure 1. TLC photo documentation of *Thontha Sura Kiyaazham Chooranam* (TSKC).

3.4 Thin Layer Chromatography (TLC)

Thin layer chromatography is a technique used for qualitative analysis phytoconstituents in plant mixtures.

4. Discussion

Herbal materials chemical composition is influenced by various factors, even within the same species. Geographical location, harvesting time, post-harvest processing techniques, and storage conditions all contribute to the unique chemical fingerprint of each batch[17]. In this study, we observed physicochemical similarities between TSK and TSKC, which may have implications for their therapeutic potential. The formulation exhibits a mean moisture content of 10.58%, which is below the PLIM guideline threshold of 14%, indicating reduced risk of microbial contamination and enhanced shelf life[18]. Our findings revealed a higher moisture content in TSKC compared to TSK, which is consistent with the observations made by Panossian and colleagues[18]. This higher moisture content could impact the overall stability and require careful consideration during storage and packaging to prevent degradation.

The total ash content, representing inorganic residues, is 10.11%, suggesting acceptable purity levels. The total ash content, an indicator of inorganic substances, was significantly higher in TSKC[19]. This finding is notable because inorganic elements, such as minerals, can influence the pharmacokinetics and pharmacodynamics of herbal medicines[19,20]. Higher

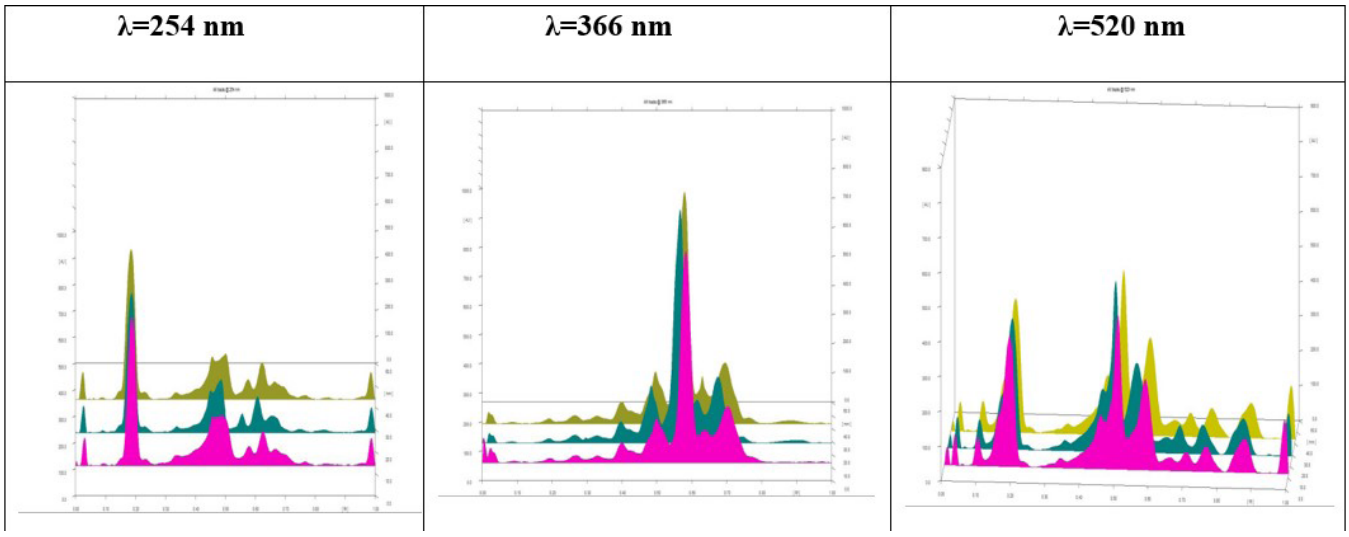


Figure 2. 3D Chromatograms of *Thontha Sura Kiyaazham Chooranam* (TSKC).

Table 5. HPTLC fingerprint of TSKC and TSK

λ=254 nm		λ=366nm		Derivitized	
HPTLC Fingerprint of TSKC					
Rf	**Colour**	**Rf**	**Colour**	**Rf**	**Colour**
0.03	Green	0.02	Fluorescent blue	0.04	Blue
0.19	Green	0.06	Pink	0.10	Violet
0.34	Green	0.10	Pink	0.16	Purple
0.46	Green	0.18	Blue	0.20	Purple
0.49	Green	0.27	Red	0.35	Grey
0.56	Green	0.33	Red	0.45	Violet
0.61	Green	0.39	Red	0.49	Violet
0.67	Green	0.42	Red	0.55	Purple
0.99	Green	0.46	Blue	0.63	Pale yellow
		0.48	Pink	0.68	Light green
		0.50	Red	0.74	Orange
		0.56	Fluorescent white	0.83	Purple
		0.61	Red	0.86	Purple
		0.68	Red	0.98	Violet
HPTLC Fingerprint of TSK					
0.05	Green	0.04	Blue	0.05	Brown
0.10	Green	0.10	Blue	0.11	Brown
0.13	Green	0.14	Blue	0.23	Brown
0.17	Green	0.24	Blue	0.30	Light brown
0.23	Green	0.31	Blue	0.34	Yellow
0.30	Green	0.39	Blue	0.38	Blue
0.46	Green	0.59	Blue	0.43	Pink
0.60	Green	0.67	Blue	0.47	Brown
0.83	Dark green	0.80	Blue	0.60	Yellow
0.92	Green	0.92	Blue	0.84	Blue

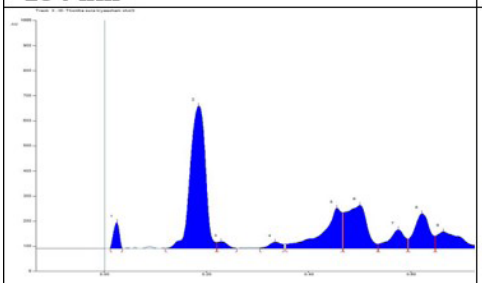
ash content may contribute to the overall efficacy, but it also emphasizes the importance of understanding the mineral composition to ensure safety and avoid potential side effects associated with certain minerals[21]. A water-soluble ash value of 0.83% implies limited water-soluble inorganic constituents, while an acid-insoluble ash value of 3.85% indicates minimal siliceous matter, both reflecting good quality (Tables 3 and 4).

The pH of a formulation can also affects its therapeutic efficacy and bioavailability. The formulation's acidic pH of 2.95 supports optimal absorption in the stomach. TSK exhibited a week acidic pH compared to TSKC, which may enhance its absorption in the gastrointestinal tract. Oladimeji and colleagues highlighted the role of acidic environments and bioenhancers in improving drug absorption[21]. The lower pH of TSK could, therefore, contribute to its therapeutic benefits, particularly in conditions where an acidic environment is optimal for absorption.

The extraction process and the specific parameters used can significantly impact the final phytochemical composition of herbal formulations. The water and alcohol extractive values are 15.86 % and 11.72 %, respectively, highlighting the presence of bioactive compounds like alkaloids and flavonoids, which contribute to the drug's bioavailability (Tables 3 and 4). TSKC's solubility in solvents such as DMSO, ethanol, and water suggests effective dissolution in gastric fluids, enhancing absorption. Additionally, the specific gravity of 1.015 and total solids content of 4.15 % confirm the consistency and concentration of the preparation. Both TSKC and TSK contained a wide range of phytochemicals, including phenols, tannins, flavonoids, and triterpenoids, which are known for their therapeutic properties[22]. However, subtle differences in the extraction process could lead to variations in the concentration and presence of specific compounds, ultimately influencing their biological activity.

HPTLC analysis provided valuable insights into the chemical fingerprints of TSKC and TSK. The distinct Rf values and spot patterns indicated noticeable differences in the concentration and distribution of phytoconstituents between the two formulations[22]. The HPTLC chromatogram was visualized under daylight as well as UV light at 254 nm and 366 nm, both before and after derivatization. The highest peak area percentages of TSK at 254 nm, 366 nm and 520 nm were 38.50%, 46.2%, 23.15% at Rf values 0.12, 0.53, 0.12 respectively. The lowest peak area percentages of TSK at 254 nm, 366 nm and 520nm were 0.97%, 0.78%, and 0.50% at Rf values 0.74, 0.16 and 0.01 respectively. The highest peak area percentages of TSKC at 254 nm, 366 nm and 520 nm were 39.30%, 36.24% and 38.38% at Rf values 0.75, 0.89, 0.0.67 respectively and the lowest peak area of TSKC were 1.07, 0.93 and 0.06 at R_f of 0.03, 0.09, 0.5 respectively (Table 5). In previous published papers on HPTLC the colour bands blue, bluish violet colour under visible light confirmed the presence of terpenoids.

HPTLC finger print profile of Chloroform extract scanning 254 nm



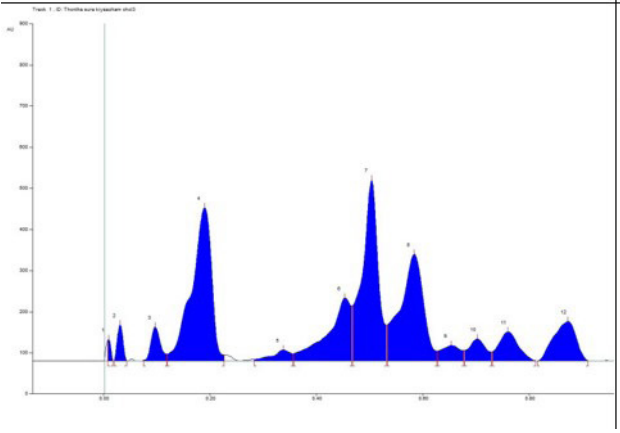
Rf Values of TSKC at **254 nm**

Track 3, ID: Thontha sura kiyaazham chcl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.0 AU	0.03 Rf	103.4 AU	7.10 %	0.04 Rf	0.4 AU	1011.1 AU	2.65 %
2	0.12 Rf	0.6 AU	0.19 Rf	568.5 AU	39.03 %	0.22 Rf	24.2 AU	14666.7 AU	38.50 %
3	0.22 Rf	24.5 AU	0.23 Rf	27.2 AU	1.87 %	0.26 Rf	0.1 AU	461.0 AU	1.21 %
4	0.31 Rf	2.9 AU	0.33 Rf	25.6 AU	1.76 %	0.35 Rf	16.8 AU	544.2 AU	1.43 %
5	0.36 Rf	17.1 AU	0.46 Rf	160.9 AU	11.04 %	0.47 Rf	43.0 AU	5245.3 AU	13.77 %
6	0.47 Rf	143.3 AU	0.50 Rf	172.4 AU	11.83 %	0.54 Rf	17.9 AU	6044.1 AU	15.87 %
7	0.54 Rf	18.2 AU	0.58 Rf	74.9 AU	5.14 %	0.59 Rf	38.2 AU	1960.1 AU	5.15 %
8	0.60 Rf	38.3 AU	0.62 Rf	139.4 AU	9.57 %	0.65 Rf	50.0 AU	3543.2 AU	9.30 %
9	0.65 Rf	50.2 AU	0.66 Rf	66.9 AU	4.59 %	0.74 Rf	7.0 AU	2706.1 AU	7.10 %
10	0.74 Rf	7.0 AU	0.77 Rf	15.3 AU	1.05 %	0.79 Rf	2.0 AU	368.4 AU	0.97 %
11	0.92 Rf	0.3 AU	0.99 Rf	102.1 AU	7.01 %	1.00 Rf	3.6 AU	1543.2 AU	4.05 %

Figure 3. HPTLC finger print profile of chloroform extract scanning 254, 366 and 520 nm (TSKC).

HPTLC finger print profile of Chloroform extract scanning 366 nm



Rf Values of TSKC at **520 nm**

Track 1, ID: Thontha sura kiyaazham chcl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	50.8 AU	0.01 Rf	52.6 AU	2.79 %	0.02 Rf	0.1 AU	262.1 AU	0.50 %
2	0.02 Rf	0.3 AU	0.03 Rf	87.4 AU	4.63 %	0.04 Rf	1.6 AU	810.4 AU	1.54 %
3	0.07 Rf	1.5 AU	0.10 Rf	82.4 AU	4.37 %	0.12 Rf	16.5 AU	1266.0 AU	2.41 %
4	0.12 Rf	16.8 AU	0.19 Rf	372.8 AU	19.77 %	0.23 Rf	14.8 AU	12183.2 AU	23.15 %
5	0.28 Rf	3.6 AU	0.34 Rf	26.8 AU	1.42 %	0.36 Rf	17.5 AU	800.0 AU	1.52 %
6	0.36 Rf	17.8 AU	0.45 Rf	153.3 AU	8.13 %	0.47 Rf	33.1 AU	5780.3 AU	10.98 %
7	0.47 Rf	133.9 AU	0.50 Rf	440.0 AU	23.33 %	0.53 Rf	87.0 AU	11416.6 AU	21.69 %
8	0.53 Rf	87.5 AU	0.58 Rf	260.0 AU	13.79 %	0.63 Rf	24.2 AU	9830.5 AU	18.68 %
9	0.63 Rf	24.3 AU	0.65 Rf	37.4 AU	1.98 %	0.68 Rf	24.9 AU	1172.4 AU	2.23 %
10	0.68 Rf	25.2 AU	0.70 Rf	53.5 AU	2.84 %	0.73 Rf	22.0 AU	1459.8 AU	2.77 %
11	0.73 Rf	22.2 AU	0.76 Rf	70.8 AU	3.76 %	0.81 Rf	0.3 AU	2257.3 AU	4.29 %
12	0.82 Rf	0.1 AU	0.87 Rf	95.7 AU	5.08 %	0.91 Rf	0.3 AU	3527.4 AU	6.70 %
13	0.97 Rf	1.5 AU	0.99 Rf	153.1 AU	8.12 %	0.99 Rf	08.5 AU	1860.7 AU	3.54 %

Figure 4. HPTLC finger print profile of chloroform extract scanning 254, 366 and 520 nm (TSK).

Yellow or yellowish blue coloured fluorescence zone confirmed the presence of flavonoids. Orange or brown coloured band for alkaloids, violet blue coloured spots for saponins, red and yellow-orange colour bands detected phenolic compounds[15,16]. In future studies, Principal Component Analysis (PCA) integrated with Factor Analysis (FA), along with Hierarchical Cluster Analysis (HCA) will be employed as multivariate exploratory techniques to analyze data obtained from the digitization of individual colour scale fingerprints. These methods are crucial for identifying the principal components corresponding to the colour bands of TSKC and TSK[15]. The given samples *Thontha Sura Kiyaazham Chooranam* and *Thontha Sura Kiyaazham* have been standardized as per standard testing protocols. Outcome of physico-chemical parameters, HPTLC documentation incorporates photo images, densitometric scan and tables and figures of corresponding R_f values (Table 5, Figures 1, 2, 3 and 4).

TSKC exhibited a higher number of spots, suggesting a greater diversity of phytochemicals, while TSK showed fewer but more intense spots, indicative of a higher concentration of specific compounds[22]. These variations could result from differences in manufacturing processes, such as grinding, mixing, and heating, which can affect the stability and concentration of phytochemicals[23].

5. Conclusion

In conclusion, this study highlights physicochemical and phytochemical differences between TSK and

TSKC, despite their shared phytochemical profiles. These variations are likely to influence therapeutic efficacy and bioavailability. The week acidic pH of TSK may enhance its efficacy and better tolerated in sensitive individuals. The variations in HPTLC profiles suggest that TSK may have enhanced bioavailability of certain phytochemicals, potentially leading to different therapeutic outcomes.

These findings underscore the critical importance of TSKC and TSK thorough characterization and standardization of herbal formulations to ensure consistent quality, efficacy, and safety. Future research should focus on clinical trials to evaluate the therapeutic effects of these formulations in various conditions, considering their physicochemical properties and phytochemical compositions. Additionally, further studies should aim to elucidate the underlying mechanisms of their therapeutic efficacy, providing a deeper understanding of how these formulations can be optimized for maximum clinical benefit.

6. Recommendations

Similar standardization methods for various *Kiyaazham chooranam* (Coarse powder for decoction) and *Kiyaazham* (decoction) will be conducted to make strong evidence for absorption and bioavailability of traditional *Siddha* medicines.

7. Acknowledgements

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8. Authors Contribution

Uthaya ganga: Concept, methods and writing; Suresh: Supervision and data collection; Manikgantan: Formal analysis; Kalpana: Data management and interpretation; Senthilkumar: Review and editing. All authors have read and approved the final version of the manuscript.

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