



Research Article

PHYTOCHEMICAL SCREENING AND HPTLC FINGERPRINT ANALYSIS OF THE SIDDHA
HERBAL FORMULATION *KADUKKAI CHOORANAM*

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ABSTRACT

The Siddha system of medicine is an indigenous traditional medical system that emphasizes preventive strategies, highlighting the principle that "Prevention is better than cure". In Siddha literature particularly in *Vaithiya Vilakkam Ennum Amirtha Saakaram*, *Kadukkai chooranam* is described as a polyherbal formulation indicated for the management of *Veezhi Akkaram*. **Aim:** The aim of the study was to evaluate the phytochemical profile and HPTLC fingerprint analysis of Siddha polyherbal formulation *Kadukkai Chooranam* (KKC). **Methodology:** The formulation was subjected to standard qualitative phytochemical screening to identify various bioactive constituents. High Performance Thin Layer Chromatography (HPTLC) were performed using silica gel plates with appropriate solvent systems. The developed plates are examined under UV light at 366nm, and the chromatographic fingerprints were recorded. **Results:** The phytochemical screening of *Kadukkai chooranam* (KKC) revealed the presence of alkaloids, flavonoids, steroids, triterpenoids, coumarins, phenols, tannins, saponins, sugars, and betacyanin. HPTLC fingerprint analysis of the formulation revealed seventeen prominent peaks, indicating the presence of diverse phytochemical constituents. The major R_f values of the peaks were observed to range from 0.03 to 0.64. **Conclusion:** Based on the findings, it is concluded that it shows the compendious understanding of presence of phytochemical constituents and HPTLC fingerprint profile of *Kadukkai chooranam* (KKC) and it is competent to assess the quality profile of *Kadukkai chooranam* as a reference standard for the development of a standardized herbal pharmaceutical formulation.

INTRODUCTION

The word Siddha denotes "established truth" and is regarded as the mother system of medicine among the ancient Tamils and Dravidians of South India. It offers a holistic approach to health, emphasizing the balance of body, mind and spirit.

Kadukkai chooranam is a Siddha polyherbal formulation mentioned in *Vaithiya Vilakkam Ennum Amirtha Saakaram*, used for the management of *Veezhi Akkaram*^[1]. The clinical features of *Veezhi akkaram* can be correlated with stomatitis, which is characterized

by inflammation of the tongue, redness of the tongue, burning sensation in the mouth, ulcerative pain, severe headache and chills^[2]. The ingredients present in this formulation demonstrated pharmacological activities including anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, and wound healing activities, which contribute to its therapeutic efficacy.

Standardization of herbal preparations is crucial to ensure their quality, safety, and efficacy. Preliminary phytochemical screening plays an important role in identifying bioactive compounds, while High Performance Thin Layer Chromatography (HPTLC) provides a characteristic fingerprint for quality control. This study describes the phytochemical constituents and the HPTLC profile of *Kadukkai Chooranam*.

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MATERIALS AND METHODS**Collection of raw drug**

The raw drugs of *Kadukkai chooranam* were collected from traditional shops in and around Tirunelveli.

Authentication of raw material

The ingredients of *Kadukkai chooranam* were authenticated by the Department of Gunapadam in Government Siddha Medical College, Palayamkottai.

Ingredients of the drug^[1]

S.No	Name	Quantity
1.	<i>Kadukkai thol</i>	245gm
2.	<i>Lavangam</i>	35gm
3.	<i>Sirunaga poo</i>	70gm
4.	<i>Chukku</i>	140gm
5.	<i>Milagu</i>	175gm
6.	<i>Thippili</i>	210gm
7.	<i>Karunjeeragam</i>	105gm

Purification (Suddhi) of the Trial drug

All the drugs were purified as per classical Siddha texts such as *Sigicha Rathina Deepam^[3]* and *Marunthu sei iyalum Kalaiyum^[4]*

- *Kadukkai* seeds are removed only the outer part was used.
- *Lavangam* was cleaned thoroughly, dried overnight and gently roasted until a golden colouration was obtained.
- *Sirunaga Poo* was cleaned thoroughly, dried overnight and gently roasted until a golden colouration was obtained.
- *Chukku*: One part of dry ginger added with 2 parts of lime stones (*Sunnambu kal*) and heated, washed, dried and outer skin was removed.
- *Milagu* was soaked in fermented butter milk for 3 hours (1 *Samam*) and then dried.
- *Thippili* was soaked in lemon juice for 3 hours and then dried.
- *Karunjeeragam* was soaked in clear limewater (*Sunnambu theliner*) and then dried.

Preparation

After purification, each ingredient was separately powdered into a fine powder and then mixed thoroughly. The prepared *Chooranam* was stored in an airtight container.

Dosage: Children aged 6 -12 years - ½ - 1 gram (twice a day after food)

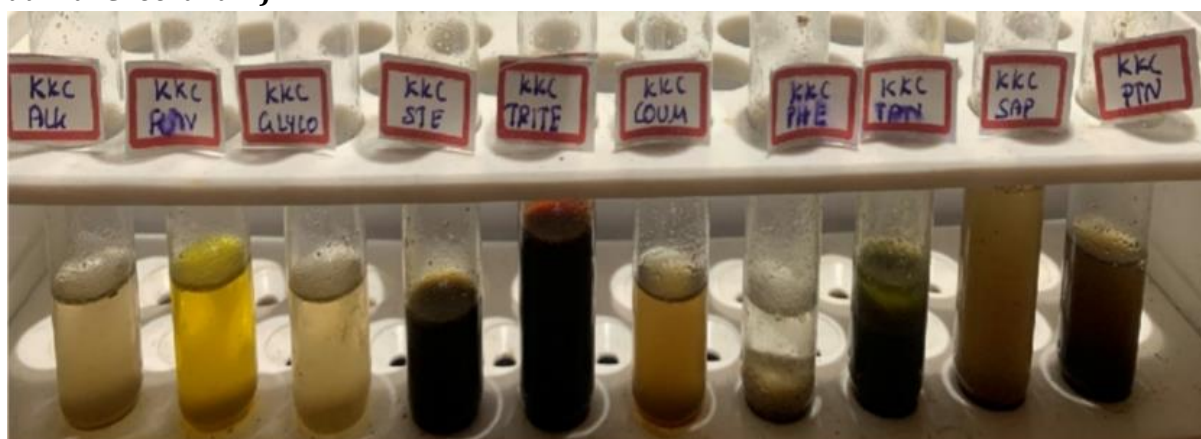
Route of administration: Oral

Phytochemical Analytical Report^[6,7]

S.No	Experiment	observation	Inference
1	Test for Alkaloids Mayer's test Two milliliters of Mayer's reagent was added to the test sample.	A dull white precipitate revealed the presence of alkaloids.	Presence
2	Test for Flavonoids Alkaline reagent test Two or three drops of sodium hydroxide were added to two milliliters of extract.	Initially a deep yellow color appeared but it gradually turned colorless upon adding a few drops of dilute hydrochloric acid, indicating the presence of flavonoids.	Presence
3	Test for Glycosides Borntrager's test Two milliliters of filtered hydrolysate, three milliliters of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it.	The presence of glycosides is indicated by the formation of a pink colour.	Absence
4	Test for Steroids Two milliliters of chloroform was added with few drops of concentrated sulphuric acid. The mixture was shaken well.	The upper layer turned red, and the sulphuric acid layer showed a yellow colour with green fluorescence, indicating the presence of steroids.	Presence
5	Test for Triterpenoids Liebermann-Burchard test: A few drops of acetic anhydride were added to the chloroform solution, and the mixture was shaken well.	One milliliter of concentrated sulphuric acid was added from the sides of the test tube. The presence of triterpenoids is indicated by the appearance of a red ring.	Presence

6.	Test for coumarins One milliliter of 10% sodium hydroxide was added to the test sample.	The presence of coumarins is indicated by the formation of yellow colour.	Presence
7	Test for Phenols Lead acetate test Three milliliters of 10% lead acetate solution was added to the test sample.	The presence of phenolic compounds is indicated by the formation of a bulky white precipitate.	Presence
8	Test for Tannins Ferric chloride was added to the test sample.	The formation of a dark blue or greenish-black color indicated the presence of tannins.	Presence
9	Proteins Biuret Test To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide.	The formation of violet purple colour indicated the presence of proteins.	Absence
10	Saponins Five milliliters of water was added to the test sample and the tube was shaken vigorously.	The presence of saponins was indicated by the formation of a large amount of foam or lather.	Absence
11	Test for carbohydrates Benedict's test About 0.5ml of Benedict's reagent was added to the test sample which was then heated on a boiling water bath for 2 minutes.	The formation of a red precipitate indicated the presence of sugar.	Presence
12	Anthocyanin One milliliter of 2N sodium hydroxide was added to the test sample and the mixture was heated for five minutes at 100°Celsius.	The formation of bluish-green colour indicated the presence of anthocyanin.	Absence
13	Betacyanin To the test sample mix the solvent with ethanol.	The formation of red violet indicated the presence of betacyanin.	Presence

Qualitative Phytochemical Investigation (KKC-Kadukkai Chooranam)



HPTLC Analysis

HPTLC is an ultra-modern, sophisticated, and automated separation technique derived from conventional thin layer chromatography (TLC). The use of pre-coated HPTLC plates along with an automated sampler ensures precise, sensitive, and efficient separation of components, both qualitatively and quantitatively. It serves as a cost-effective tool for the quality evaluation of botanical materials. The HPTLC method offers a high degree of selectivity, sensitivity, and rapid analysis with minimal sample preparation, making it suitable for routine quality

control. Furthermore, it provides a chromatographic fingerprint of phytoconstituents, which aids in confirming the identity and purity of phytotherapeutic formulations.

Chromatogram development

Chromatogram development was performed using CAMAG Twin Trough chambers. Sample elution was performed based on the adsorption capability of the components to be analyzed. After elution process was completed, the plates were removed from the chamber and dried.

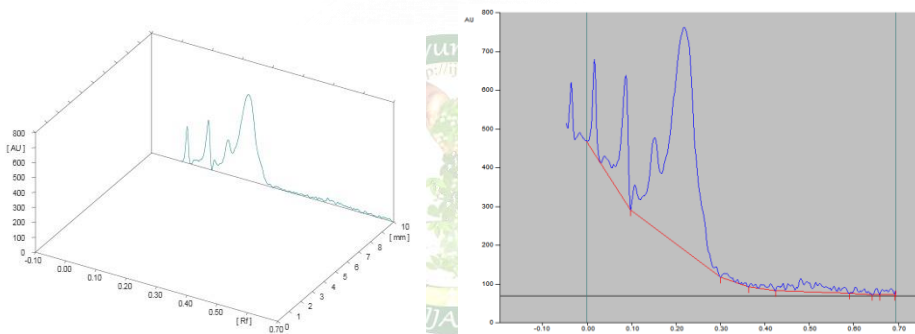
Scanning

The plates were examined under ultraviolet light at 366nm. The resulting data were acquired and integrated using CAMAG software. A chromatographic fingerprint profile was developed to identify the phytoconstituents present in each sample, and their respective Rf values were recorded and tabulated.

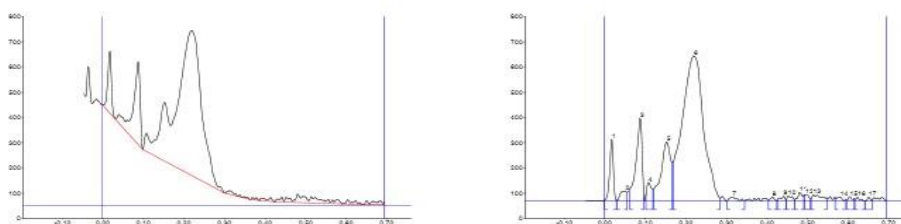
HPTLC plate of KKC observed at 366 nm



3D - Chromatogram of KKC



HPTLC finger printing of KKC



Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	2.8	0.02	244.8	14.34	0.03	2.5	1583.2	4.63
2	0.03	2.6	0.05	39.4	2.31	0.06	33.4	472.6	1.38
3	0.06	48.6	0.09	327.8	19.20	0.10	13.4	3498.5	10.23
4	0.10	26.0	0.11	72.5	4.25	0.12	44.1	657.4	1.92
5	0.12	45.3	0.15	233.3	13.67	0.17	152.7	4143.6	12.11
6	0.17	154.9	0.22	575.0	33.69	0.29	10.6	21904.8	64.02
7	0.30	1.0	0.32	14.7	0.86	0.35	0.7	195.0	0.57
8	0.41	5.1	0.41	16.0	0.94	0.43	0.0	102.0	0.30
9	0.43	5.6	0.44	18.8	1.10	0.45	3.0	150.1	0.44
10	0.45	7.1	0.45	19.3	1.13	0.47	3.7	172.3	0.50
11	0.47	5.9	0.48	34.1	2.00	0.49	23.1	293.1	0.86
12	0.49	24.4	0.50	25.8	1.51	0.51	7.7	192.3	0.56
13	0.51	9.9	0.52	24.8	1.46	0.55	7.9	413.5	1.21
14	0.57	12.8	0.58	15.4	0.90	0.59	3.3	153.3	0.45
15	0.60	2.3	0.61	16.4	0.96	0.62	8.6	112.5	0.33
16	0.62	5.5	0.63	14.2	0.83	0.64	0.0	95.2	0.28
17	0.64	0.3	0.65	14.4	0.84	0.66	5.3	73.3	0.21

RESULTS AND DISCUSSION

Phytochemical screening of *Kadukkai Chooranam* revealed the presence of:

- Alkaloids
- Flavonoids
- Steroids
- Triterpenoids
- Coumarins
- Phenols
- Tannins
- Saponins
- Carbohydrates
- Betacyanin

Alkaloids exhibit notable antimicrobial and anti-inflammatory properties, which help reduce infection and inflammation in affected tissues. Likewise, saponins demonstrate immunomodulatory and antimicrobial activities and also play a role in boosting the body's defense mechanisms and facilitating tissue healing.^[8,9]

Flavonoids found in the preparation are widely acknowledged for their strong antioxidant, anti-inflammatory, and antimicrobial activities. Recent studies indicate that flavonoids exhibit antibacterial effects through multiple mechanisms, such as disrupting microbial cell membranes, inhibiting nucleic acid synthesis, and preventing the formation of biofilms. These effects are particularly important in cases of oral infections, where they play a major role in controlling microbial growth and oxidative stress.^[8,10,13]

Steroids and triterpenoids identified in the preparation are widely documented for their anti-inflammatory and analgesic effects. These compounds play an important role in alleviating pain, swelling, and irritation associated with ulcerative oral conditions.^[9]

Tannins, another key group of compounds identified in the preparation, exhibit strong astringent and antimicrobial properties. They aid in mucosal healing by forming a protective layer over inflamed tissues and decreasing microbial adhesion, which is especially advantageous in conditions such as stomatitis.^[11]

Coumarins demonstrate anti-inflammatory and antimicrobial activities. In the context of oral disorders, they contribute to the reduction of inflammation and microbial load within the oral cavity. Their antimicrobial action helps control pathogenic microorganisms responsible for infections, while their anti-inflammatory properties aid in relieving pain, swelling, and irritation associated with inflammatory condition.^[9,13]

Phenolic compounds typically have powerful antioxidant and antibacterial properties, which help

safeguard tissues against oxidative damage and support the healing process.^[8]

Carbohydrates play a supportive role in tissue repair and healing. Certain polysaccharides exhibit immunomodulatory and wound-healing activities and help maintain cellular energy supply, promote epithelial cell proliferation and regeneration, and enhance mucosal integrity by supporting extracellular matrix formation. These effects are beneficial in oral ulcerative conditions, where rapid tissue repair is essential.^[9,14]

Betacyanin are known for their strong antioxidant and anti-inflammatory properties. They play an important role in neutralizing free radicals and reducing oxidative stress, which is a major contributor to the development of inflammatory oral conditions such as stomatitis. By minimizing oxidative damage, betacyanin support the protection of the oral mucosa and aid in the rapid recovery of ulcerative lesions.^[15]

The HPTLC chromatogram of *Kadukkai Chooranam* showed a clearly defined fingerprint pattern with seventeen distinct peaks. The Rf values ranged from 0.03 to 0.64, indicating the presence of phytoconstituents with varying polarities. Compounds with lower Rf values are more polar, such as tannins and phenolic compounds, whereas those with higher Rf values are less polar, such as terpenoids. The presence of multiple peaks confirms the polyherbal nature and chemical diversity of the formulation. This HPTLC fingerprint profile can serve as a standard reference for identification and quality control.^[16]

The findings of the present study provide detailed insights into the phytochemical constituents and the HPTLC fingerprint profile of *Kadukkai Chooranam* (KKC). The presence of multiple phytoconstituents supports its traditional use in Siddha medicine and highlights its potential synergistic therapeutic effects, particularly in the management of inflammatory oral conditions such as *Veezhi Akkaram* (stomatitis). The established HPTLC fingerprint profile serves as a valuable tool for ensuring product quality and detecting possible adulteration. However, further studies, including quantitative phytochemical analysis and comprehensive pharmacological evaluation, are necessary to substantiate its clinical efficacy and safety.

CONCLUSION

Based on the findings, it is concluded that it shows the compendious understanding of presence of phytochemical constituents and HPTLC fingerprint profile of *Kadukkai chooranam* (KKC) and it is competent to assess the quality profile of *Kadukkai chooranam* as a reference standard for the development of a standardized herbal pharmaceutical formulation. The presence of multiple bioactive

constituents supports the traditional use of *Kadukkai chooranam* in Siddha medicine and demonstrates its potential synergistic therapeutic effects, particularly in the management of inflammatory oral conditions such as *Veezhi Akkaram* (stomatitis). However, further studies involving preclinical and clinical studies are necessary to affirm the efficacy of *Kadukkai chooranam* for stomatitis.

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