

STANDARDIZATION AND PHYTOCHEMICAL PROFILING OF KIRUBHAKADATCHADHIRAVAGAM USING PHYSICOCHEMICAL PARAMETERS, GC-MS AND HPTLC FINGERPRINTING

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ABSTRACT

Traditional Siddha formulations require systematic scientific validation to ensure their quality, safety, and reproducibility. The present study focuses on the standardization of *Kirubha Kadatcha Dhiravagam* (KKD), a classical Siddha preparation, using physicochemical evaluation, preliminary phytochemical screening, gas chromatography-mass spectrometry (GC-MS), and high-performance thin layer chromatography (HPTLC) fingerprinting. Physicochemical parameters such as pH, specific gravity, and total solids were analyzed to assess the formulation's quality and stability, while preliminary phytochemical screening was carried out to identify major classes of bioactive constituents. Advanced analytical techniques, including GC-MS, were employed to detect and characterize chemical constituents, and HPTLC fingerprinting was performed to establish a chromatographic profile for the formulation. This study aims to develop a comprehensive

standardization protocol for KKD that can serve as a reference for quality control, ensure batch-to-batch consistency, and support further pharmacological and clinical investigations of Siddha formulations.

KEYWORDS: Siddha medicine, Kirubha Kadatcha Dhiravagam, standardization, HPTLC, GC–MS, phytochemical analysis.

1. INTRODUCTION

Siddha medicine is a traditional system widely used for managing various diseases through herbal formulations. However, lack of scientific standardization limits its global acceptance. Establishing physicochemical and phytochemical standards ensures quality, safety, and consistency.

Advanced analytical techniques such as GC–MS and HPTLC play a crucial role in identifying bioactive compounds and generating fingerprint profiles. HPTLC provides reproducible chromatographic patterns for quality control, while GC–MS helps in identifying volatile and semi-volatile constituents.

Kirubha Kadatcha Dhiravagam (KKD) is traditionally used for gastrointestinal disorders as described in Siddha literature.^[1] The present study aims to standardize KKD using modern analytical techniques. Siddha formulations are increasingly gaining attention due to their holistic therapeutic approach however, lack of standardized protocols remains a major limitation for their global acceptance. Scientific validation through physicochemical and chromatographic techniques is essential to ensure identity, purity, and consistency of herbal formulations.^[2,3] Advanced analytical tools such as GC–MS and HPTLC play a crucial role in identifying bioactive compounds and generating reproducible fingerprints for quality control.^[2] Therefore, standardization of Kirubha Kadatcha Dhiravagam is necessary to establish reliable quality parameters and support its therapeutic application.

2. MATERIALS AND METHODS

2.1 Drug Preparation

Kirubha Kadatcha Dhiravagam (KKD) was prepared as per the traditional Siddha *Dhiravagam* (distillation) method. The raw drugs used in the formulation included Poduthalai (*Phyla nodiflora*), Vallarai (*Centella asiatica*), Veppam ilai (leaf of *Azadirachta indica*), Veppam pattai (bark of *Azadirachta indica*), and Veppam poo (flower of *Azadirachta indica*).

All the raw materials were cleaned thoroughly to remove extraneous matter and used in fresh form. The ingredients were placed in a traditional *Dhiravaga Valai* apparatus. The setup was subjected to controlled heating, while cold water was continuously poured over the lid to

facilitate condensation. Through this process of cooling distillation, the vapours generated from the boiling mixture were condensed and collected as *Dhiravagam*. The distillate obtained was clear and was collected carefully and stored in a clean, dry, airtight container for further analysis.

2.2 Physicochemical Analysis

Parameters such as pH, specific gravity, and total solids were determined using standard procedures.

2.3 Phytochemical Screening

Preliminary qualitative tests were conducted to detect major phytoconstituents including alkaloids, carbohydrates, phenols, flavonoids, and tannins.

2.4 GC–MS Analysis

GC–MS analysis was performed to identify bioactive compounds. The obtained spectra were compared with standard libraries.

2.5 HPTLC Analysis

HPTLC was carried out using silica gel plates and CAMAG system. The mobile phase consisted of chloroform:n-butanol:methanol:water:acetic acid (4:1:1:0.5:0.5). Plates were visualized under UV at 366 nm and chromatographic fingerprints were recorded.

Analytical procedures were carried out using standard protocols to ensure reproducibility and accuracy. The instruments were calibrated prior to analysis, and all experiments were performed under controlled laboratory conditions to minimize variability.

3. RESULTS

3.1 Physicochemical Parameters

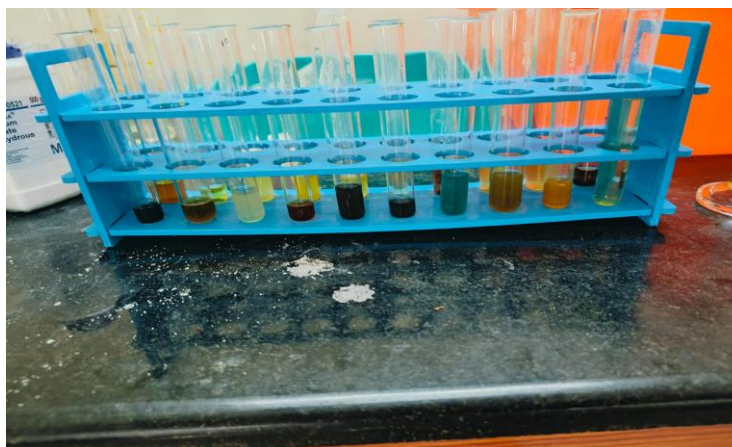
Table 1: Physicochemical Parameters of KKD.

PARAMETER	RESULT
Appearance	Colourless liquid
P ^H	4.05
Specific gravity	1.02
Total solids	Negligible

3.2 Phytochemical Screening

Table 2: Phytochemical Constituents.

PHYTOCONSITUTENT	RESULT
Alkaloids	Present
Carbohydrates	Present
Phenolic compounds	Present
Flavinoids	Absent
Tanins	Absent



The physicochemical parameters indicate that the formulation is stable and suitable for storage. The presence of alkaloids and phenolic compounds suggests potential pharmacological activity, particularly antioxidant and antimicrobial effects.

3.3 GC–MS Analysis

Table 3: Identified Compounds.

Compound	Activity
Piperine	Bio availability enhancer
Phenyl ethyl alcohol	Anti microbial
Amide derivative	Anti inflammatory

The compounds identified through GC–MS analysis (Table 3, page 4) demonstrate the presence of biologically active constituents such as piperine and phenyl ethyl alcohol, which are known for their bioavailability-enhancing and antimicrobial properties.

3.4 HPTLC Fingerprinting

Table 4: HPTLC Peak Values.

Peak	R _f value
1	0.02
2	0.09
3	0.13

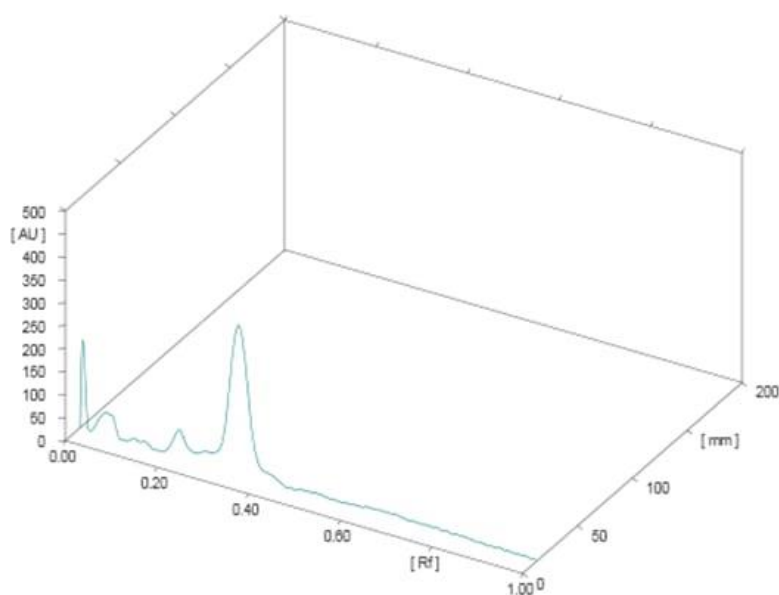
4	0.18
5	0.29

The HPTLC chromatogram of KKD is shown in Figure 1. The 3D chromatogram (Figure 2) and densitometric scan (Figure 3) revealed five prominent peaks with R_f values ranging from 0.02 to 0.29.

The HPTLC chromatogram (Figures 1–3, pages 5–7) shows distinct peaks with specific R_f values, indicating the presence of multiple phytoconstituents. These fingerprint profiles can serve as a reference standard for ensuring batch-to-batch consistency of the formulation.



Figure 1: HPTLC Chromatogram of KKD at 366 nm.



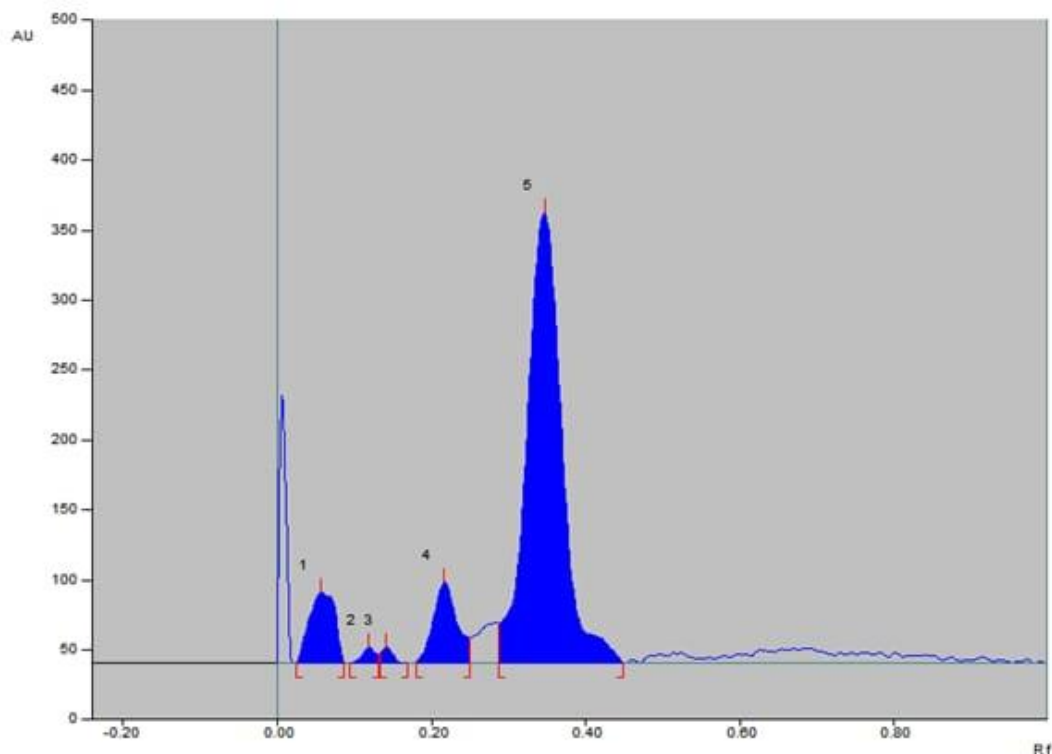


Figure 2: 3D HPTLC Chromatogram of KKD.

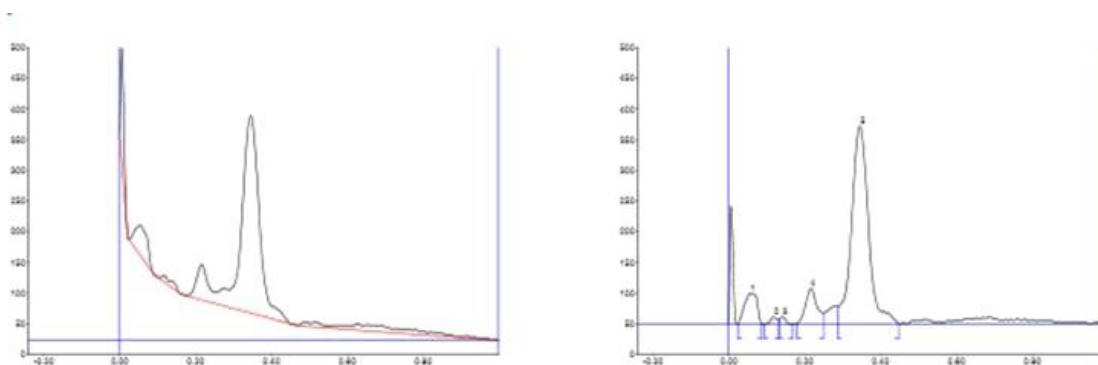


Figure 3: HPTLC Densitogram of KKD.

4. DISCUSSION

The physicochemical parameters confirmed the stability and quality of the formulation. The presence of alkaloids and phenolic compounds suggests antioxidant and antimicrobial potential. GC–MS analysis identified bioactive compounds such as piperine, which enhances drug bioavailability and exhibits pharmacological effects.^[4]

HPTLC fingerprinting provides a reliable method for quality control and ensures batch-to-batch consistency. The observed peaks confirm the presence of multiple phytoconstituents contributing to therapeutic efficacy.

The integration of traditional Siddha knowledge with modern analytical techniques validates the formulation scientifically. The presence of phenolic compounds and alkaloids supports the traditional use of the formulation in managing gastrointestinal disorders due to their known antioxidant and therapeutic properties. The GC–MS and HPTLC findings further validate the formulation by confirming the presence of multiple active constituents responsible for its pharmacological activity.^[4]

5. CONCLUSION

The study successfully establishes standardization parameters for Kirubha Kadatcha Dhiravagam using physicochemical, phytochemical, GC–MS, and HPTLC analysis. The results confirm the presence of bioactive compounds and provide a reproducible fingerprint profile, supporting its traditional use and future pharmacological studies.

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