IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

QUALITY ASSESSMENT OF THE SIDDHA HERBAL FORMULATION KUNDALADHI KULAMBU (K^2) THROUGH SPECTROSCOPIC AND CHROMATOGRAPHIC ANALYSIS

Dr J Monisha[1], Dr M D Saravana Devi[2], Dr N S Janani Sri[3]

[1&3]PG scholar, Department of Gunapadam (Siddha Pharmacology), Government Siddha medical college, Chennai – 106, Tamil Nadu, India.

monisha261097@gmail.com

[2]Guide, Prof., HOD (Retd.), Department of Gunapadam (Siddha Pharmacology), Government Siddha medical college, Chennai – 106, Tamil Nadu, India

ABSTRACT

Kundaladhi Kulambu (K^2) is a traditional Siddha medicine widely indicated for the management of jaundice and liver disorders[1]. Although its clinical use is well established, scientific validation using advanced instrumental techniques is essential to define quality control standards. The present study focused on the standardization of K^2 through High-Performance Thin-Layer Chromatography (HPTLC), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Heavy metal analysis by Atomic Absorption Spectroscopy (AAS). HPTLC profiling established a distinct fingerprint pattern for the formulation, ensuring reproducibility and authentication[2]. FTIR analysis revealed the presence of characteristic functional groups corresponding to phytoconstituents. SEM provided detailed insights into the morphological features and particle size distribution, while AAS confirmed the presence of essential therapeutic trace elements within permissible limits. Collectively, these results provide instrumental validation and comprehensive quality parameters for K^2, supporting its safe application in jaundice and liver-related diseases and facilitating future pharmacological and clinical research.

Key words – Siddha medicine, Kundaladhi Kulambu (K^2), Instrumental analysis, HPTLC, FT-IR, AAS, SEM

INTRODUCTION

Advancements in analytical instrumentation have opened new possibilities for validating traditional medicines with scientific precision. Siddha formulations, owing to their complex herbal-mineral composition, require multidimensional characterization to ensure authenticity, quality, and safety. Techniques such as High-Performance Thin-Layer Chromatography (HPTLC), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Atomic Absorption Spectroscopy (AAS) are increasingly employed in herbal drug research to generate reproducible fingerprints and identify trace elements[4]. Among Siddha formulations, K^2 has long been used for the treatment of jaundice and liver-related disorders. However, its complex composition poses challenges for conventional evaluation. Instrumental standardization offers a comprehensive

understanding of its phytochemical profile, functional groups, microstructural features, and elemental composition. Establishing such quality parameters not only validates traditional claims but also supports regulatory acceptance and integration of Siddha hepatoprotective medicines into evidence-based healthcare systems.

AIM

To standardize and validate *Kundaladhi Kulambu (K^2)* using advanced instrumental techniques for establishing quality control and ensuring its safety in liver disorders.

OBJECTIVES

- To develop the HPTLC fingerprint profile of K^2.
- To identify functional groups using FTIR analysis.
- To study morphology and particle size through SEM.
- To determine elemental composition using AAS.
- To establish instrumental quality parameters for standardization.

MATERIALS AND METHODS

Drug Profile

Drug name : **Kundaladhi Kuzhambu (K^2)**

Dosage : Mullikai Alavu (Kandangathiri kai alavu) ~ 0.5 to 2g (ODS for 6 Days)

Route : Oral Administration

Indications : Kamalai (Jaundice), Pandu Rogam

Ingredients

1. Sanganchedi ver (*Azima tetracantha*) - Vilaangai alavu (70 g)
2. Perungayam (*Ferula asafoetida*) - 2 varagan (8.4 g)
3. Lemon juice (*Citrus limon*) - Kaal araikal padi (40ml)

Collection and Authentication of raw drugs

- All the ingredients were authenticated by Gunapadam (Siddha Pharmacology) experts and Botanist of Government Siddha Medical College (GSMC), Chennai.
- All the ingredients will be purified according to the Siddha literature - *Sarakku suthi sei muraigal.*

Preparation of Kundaladhi Kulambu (K^2)[1]

The roots of *Sanganchedi* (Azima tetracantha) were collected, thoroughly cleaned and finely ground. The resulting material was then combined with *Perungayam* (Ferula asafoetida). To this mixture, fresh *Elumichai pazha charu* (lemon juice) was added and the preparation was placed in a new, unused clay vessel. The vessel containing the formulation was subsequently exposed to direct sunlight results in slight welling up of the mixture within the vessel. This deposited substance was carefully collected and stored in a clean, dry container for subsequent standardizing procedures.[1]

ANALYTICAL INSTRUMENTATION TECHNIQUES - METHODOLOGY

TLC Analysis

Methodology[4]

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro litre 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 are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm

HPTLC Analysis

Methodology[5]

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus, this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of Phyto-therapeutics.

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

Scanning - Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

FTIR analysis (functional groups identification)

Methodology[6]

Sample processed using Bruker Alpha-E by ATR module (attenuated total reflectance). Sample positioned on the Crystal platform with perfect alignment of keeping anvil in up position. To ensure that the sample makes good contact angle with the crystal prior to start of the IR radiation exposure. Spectra measurement was achieved with desired wavelength and the corresponding observational peaks/ waves were recorded with wavenumber were subjected to further interpretation. Software used for the analysis is OPUS version 7 for functional group analysis. Signal detection processed through DTGS detector. Baseline correction adjusted as per the requirement.

SEM analysis (morphology, particle size)

Methodology[7]

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross sections. The test sample powder was sputter coated with gold and viewed under SEM (FEI Quanta 200 FEG, Berlin, Germany) to determine the morphology at ×100,000 magnification and the particle size at ×200,000 magnification.

AAS analysis (elemental composition, safety limits)

Methodology[8]

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Standard: Hg, As, Pb and Cd – Sigma

Sample Digestion - Test sample was digested with 1mol/L HCl for determination of arsenic and mercury.

Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO_3.

Standard reparation

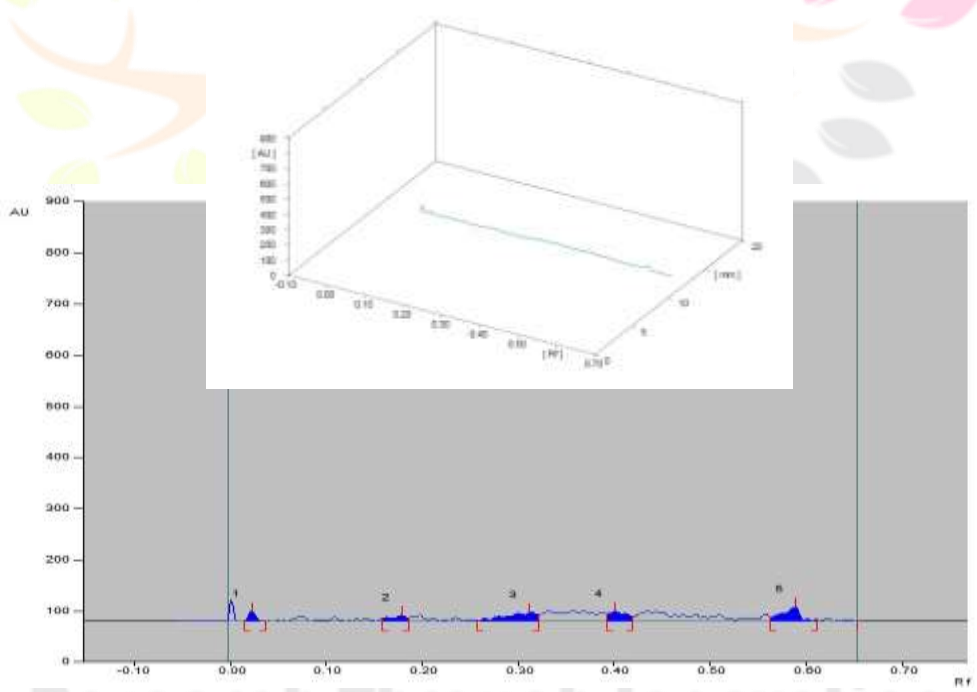
As & Hg- 100 ppm sample in 1mol/L HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO_3

RESULTS

TLC Visualization of K² at 366 nm

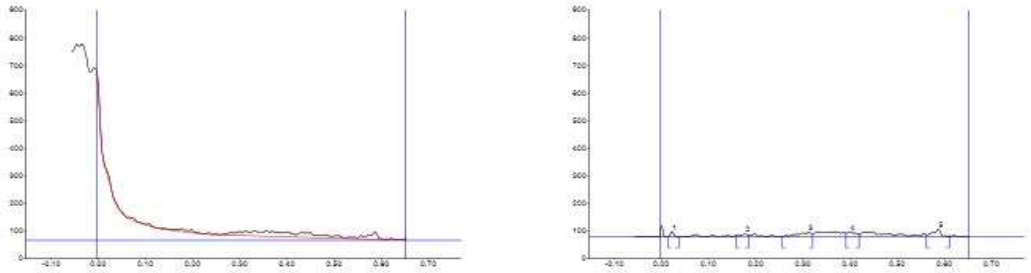
3D- Chromatogram



Report - Five distinct peaks appeared at Rf values ranging approximately from 0.00 to 0.60, indicating the presence of at least five separable constituents in the K2 sample. The varied peak heights reflect differences in concentration or detector response among compounds. The good resolution and absence of overlapping suggest that the selected solvent system and development conditions are suitable for fingerprinting this polyherbal formulation.

HPTLC fingerprinting

HPTLC finger printing of K^2



Peak

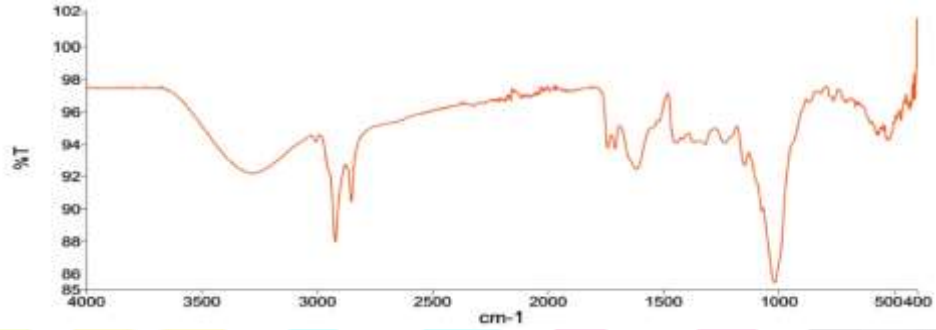
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	0.5	0.02	19.3	20.48	0.04	0.1	98.9	7.85
2	0.16	5.4	0.18	10.9	11.57	0.19	5.8	133.5	10.59
3	0.26	1.3	0.31	17.6	18.69	0.32	12.4	389.6	30.92
4	0.39	13.9	0.40	19.1	20.31	0.42	8.9	270.6	21.48
5	0.56	7.2	0.59	27.2	28.94	0.61	3.9	367.4	29.16

REPORT

HPTLC finger printing analysis of the sample reveals the presence of five prominent peaks corresponds to the presence of versatile phytocomponents present within it. The major Rf value of the peaks ranges from 0.01 to 0.56

FTIR spectra interpretation

FT-IR SPECTRUM OF SAMPLE K^2



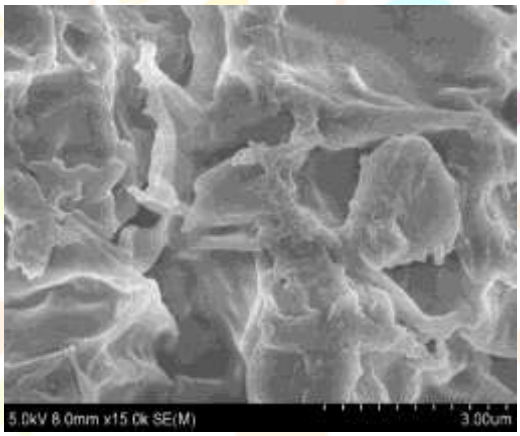
FT-IR Peak Table

Report:

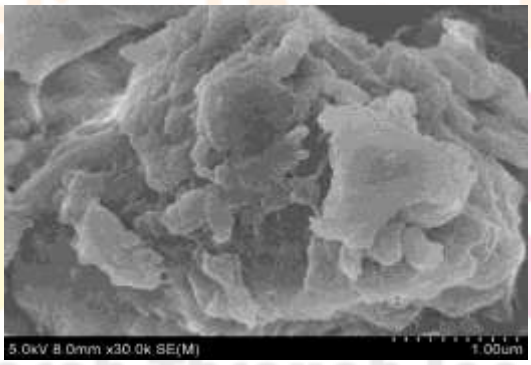
The FTIR analysis of the polyherbal sample KK reveals the presence of key functional groups indicative of bioactive compounds. The broad O-H stretch (~3400 cm^{-1}) suggests phenols or flavonoids, while the C=O stretching (~1650 cm^{-1}) indicates carbonyl-containing compounds like alkaloids or esters. The presence of C-O-C (~1000-1100 cm^{-1}) and C-H (~2900 cm^{-1}) vibrations suggests polysaccharides, glycosides, or terpenoids. These findings confirm a complex phytochemical profile, supporting the sample's potential pharmacological applications. Further studies such as HPLC or GC-MS may validate specific bioactive constituents.

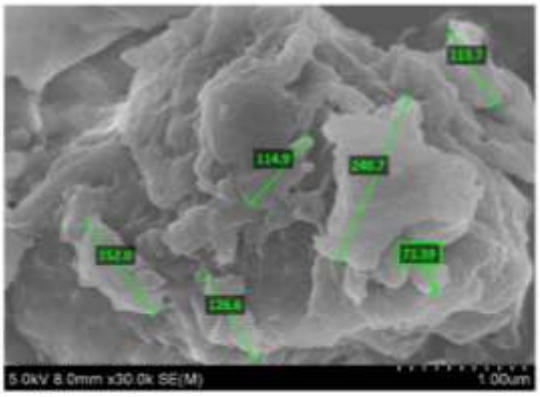
SEM images and findings

SEM image of K^2 - Cluster View



SEM image of K^2- Categorised View





Report:

It was observed from the SEM analysis of sample K^2 that the average particle size of the sample ranges from 71 µm to 152 nm.

AAS elemental profile

Test Report

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	4.01	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

*BDL- Below Detection Limit

Report - AAS Results of the present investigation have clearly shows that the sample has no traces of heavy metal such as Mercury, Arsenic and Cadmium were as the sample evident the presence of Lead at 7.87 and 4.01 ppm levels as listed in the table.

DISCUSSION

The present study employed various modern analytical techniques to characterize and standardize the Siddha formulation *Kundaladhi Kulambu* (K^2). Each instrumental method provided complementary data supporting the formulation's chemical complexity, structural characteristics, and safety profile.

Thin Layer Chromatography (TLC) analysis of K^2 revealed multiple distinct spots at varying Rf values, confirming the polyherbal nature of the formulation. The clear separation of spots indicated the presence of diverse phytochemical constituents with different polarities, serving as a preliminary fingerprint for identification and quality assessment.

High-Performance Thin Layer Chromatography (HPTLC) fingerprinting demonstrated five well-resolved peaks with Rf values ranging from 0.01 to 0.56, suggesting the presence of several bioactive compounds. The appearance of multiple peaks supports the chemical diversity and synergistic nature of the ingredients, which may contribute to the overall therapeutic efficacy of K^2 inmanaging hepatic disorders.

Fourier Transform Infrared (FTIR) spectral analysis revealed the presence of significant functional groups such as O–H (~3400 cm^{-1}), C=O (~1650 cm^{-1}), C–H (~2900 cm^{-1}), and C–O–C (~1000–1100 cm^{-1}). These peaks are characteristic of phytoconstituents like phenols, flavonoids, alkaloids, glycosides, and terpenoids, indicating the formulation's complex phytochemical matrix. Such compounds are known for their antioxidant and hepatoprotective properties, aligning with the traditional use of K^2

Scanning Electron Microscopy (SEM) analysis revealed that the average particle size of the sample ranged from 71 µm to 152 nm, indicating a heterogeneous mixture with fine micro- and nanosized particles. The small particle size enhances surface area and improves solubility and bioavailability, which may contribute to the better absorption and therapeutic efficiency of the formulation.

Atomic Absorption Spectroscopy (AAS) analysis confirmed the absence of toxic heavy metal contamination. Lead was detected at 4.01 ppm, which is well below the permissible limit (10 ppm), while arsenic, cadmium, and mercury were below detectable levels (BDL). This ensures that the formulation is safe for internal administration and adheres to regulatory safety standards.

Overall, the combined analytical findings substantiate that *Kundaladhi Kulambu* (K^2) is a chemically diverse, structurally stable, and toxicologically safe Siddha formulation. The integrated use of TLC, HPTLC, FTIR, SEM and AAS establishes a comprehensive fingerprint profile that ensures its authenticity, consistency and quality for clinical application.

CONCLUSION

The comprehensive analytical evaluation of *Kundaladhi Kulambu* (K^2) using modern instrumental techniques has successfully established its chemical complexity, safety, and quality profile. The combined results from TLC and HPTLC confirm the polyherbal composition and diverse phytoconstituents responsible for the formulation's therapeutic potential. FTIR analysis provided valuable insight into the presence of functional groups corresponding to bioactive compounds with known hepatoprotective and antioxidant properties. SEM observations revealed a heterogeneous micro- and nanoscale particle distribution, which may enhance solubility and bioavailability, thereby supporting pharmacological efficacy. Furthermore, AAS results verified the absence of harmful heavy metals within permissible limits, reinforcing the safety of K^2 for internal use. Collectively, these findings validate *Kundaladhi Kulambu* as a standardized, structurally stable and toxicologically safe Siddha formulation, supporting its continued clinical use and encouraging further pharmacological and clinical investigations to elucidate its mechanisms of action in hepatic disorders.

Acknowledgment:

The authors are highly thankful to The Tamil Nadu Dr. MGR Medical University, Chennai, Noble Research Solutions, Chennai, The Principal and the faculties of Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Chennai. A special thanks to my guide who is always a big support system.

Conflict of Interest: No conflict of Interest.

Source of Funding: None

Ethical Approval: Approved

REFERENCE

[1] Agathiyar vaithiya valladhi 600, Page no. 137, I edition, Thamarai Noolagam publication.

[2] Shivatare, R. S., Nagore, D. H., Nipanikar, S. U., A review." Journal of Scientific and Innovative Research, 2013.

[3] A Review Report on the Standardization of Herbal Medicines"

[4] Lukasz Komsta, Monika Waksmundzka-Hajnos, Joseph Sherma. Thin Layer Chromatography in Drug Analysis. CRC Press, Taylor and Francis.

[5] Wagner H. Plant Drug Analysis. A thin Layer chromatography Atlas.2nd ed. Heidelberg: Springer-Verlag Belgium; 2002:305, 227.

[6] M. A. Heba, E. S. Basha, Recent Applications of Fourier Transform Infrared Spectrophotometry in Herbal Medicine Analysis

[7] Rod Heu, Sina Shahbaz mohamadi, John Yorston, Patrick Capeder, Target Material Selection for Sputter Coating of SEM Samples

[8] PerkinElmer. (2008). Analytical methods for atomic absorption spectroscopy. PerkinElmer Inc.