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PHYTOPHYSICO – BIOCHEMICAL (PPB) PROFILING OF SIDDHA HERBAL FORMULATION "KUNDALADHI KULAMBU (K^2)" FOR QUALITY EVALUATION

Dr. J. Monisha[1]*, Dr. A. Ganesan[2], Dr. B. Dharshini Megha[3]

*[1,3]PG Scholar, Department of Gunapadam (Siddha Pharmacology), Government Siddha Medical College, Chennai – 106, Tamil Nadu, India.

[2]Guide, Principal, Prof., HOD, Department of Gunapadam (Siddha Pharmacology), Government Siddha Medical College, Chennai – 106, Tamil Nadu, India.

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***Corresponding Author**
Dr. J. Monisha
PG Scholar, Department of Gunapadam (Siddha Pharmacology), Government Siddha Medical College, Chennai – 106, Tamil Nadu, India.

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ABSTRACT

Kundaladhi Kulambu (K^2), a productive Siddha formulation, has been traditionally prescribed for the management of jaundice and liver-related disorders. Despite its wide therapeutic use, scientific validation and standardization are required to ensure quality, safety and consistency. The present study aimed to standardize K^2 through phytochemical screening, physicochemical evaluation and biochemical analysis. The formulation was prepared as per Siddha literature and standard protocols were followed to assess pH, ash values, extractive values and biochemical parameters. Phytochemical analysis revealed the presence of alkaloids, tannins, and flavonoids while biochemical tests confirmed essential therapeutic components. Physicochemical parameters were within acceptable ranges, supporting stability and reproducibility. These findings provide baseline standardization data for K^2, reinforcing its potential as a safe and effective traditional medicine for jaundice and liver disorders and laying the groundwork for further pharmacological and clinical investigations.

KEYWORDS: Siddha medicine, Kundaladhi Kulambu (K^2), Phytochemical, Physico-chemical, Biochemical analysis.

INTRODUCTION

The liver is a vital organ responsible for detoxification, metabolism and maintenance of homeostasis. Disorders affecting liver function, such as jaundice, remain a significant health burden in many parts of the world. In traditional *Siddha* medicine, liver ailments are understood as disturbances in Azhal (pitta) and several formulations are documented for their hepatoprotective potential. *Siddha* herbal medicines, in particular, occupy a special place due to their natural origin, lesser side effects, holistic approach, accessibility, rich in healing compounds and clinical application. K^2, a minimalistic yet powerful formulation is traditionally prescribed for jaundice and related hepatic dysfunctions. While its therapeutic benefits are acknowledged in practice, there is limited systematic evidence defining its quality standards. The formulation was prepared as per Siddha literature – "***Agathiyar vaithiya valladhi 600***" and standard protocols were followed to assess its efficacy. Preliminary standardization through phytochemical screening, physicochemical evaluation and biochemical analysis is essential to establish reproducibility and ensure safety. Such approaches not only provide insight into the phytoconstituents and mineral composition of the drug but also generate baseline data for future pharmacological and clinical investigations.

AIM

To scientifically validate and standardize *Kundaladhi Kulambu (K^2),* a traditional Siddha formulation, through phytochemical, physicochemical, and biochemical analyses, ensuring its quality, safety and consistency for therapeutic use in jaundice and liver disorders.

OBJECTIVES

1. To prepare *Kundaladhi Kulambu* (K^2) in accordance with classical Siddha literature – *Agathiyar Vaithiya valladhi 600.*
2. To evaluate physicochemical parameters (pH, ash values, extractive values) for assessing quality and stability.
3. To perform phytochemical screening for identifying major classes of secondary metabolites such as alkaloids, tannins, and flavonoids.
4. To carry out biochemical analysis to confirm the presence of essential therapeutic components.

5. To generate baseline standardization data that supports the reproducibility, safety, and therapeutic potential of K^2 for further pharmacological and clinical investigations.

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]

Phytochemical screening procedures[2&3]

Test for alkaloids

Mayer's Test: To the test sample, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

Test for saponins

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids

Alkaline reagent test- Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

Test for phenols

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turned into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

Anthocyanin

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

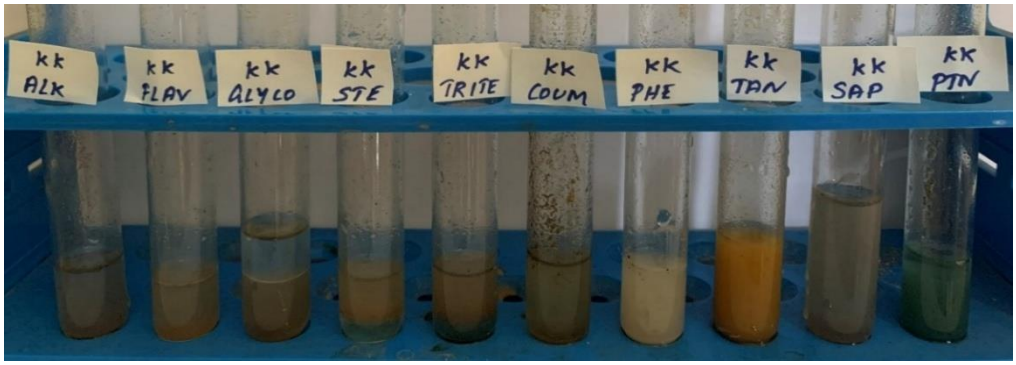
Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

Qualitative Phytochemical Investigation



Physicochemical Analytical and evaluating methods[2&3]

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400°C until it turns white in colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water

and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o}C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water-soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o}C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

pH determination

Required quantity of test sample was admixed with distilled water and the subjected to screening using pH meter.

Biochemical analysis (standard qualitative tests)[2&3]

Analytical Investigation on Test for Acid Radicals

S. No	Test for Specific Acid Radical	Indication / Observation	Inference	Results
1.	**Test for Carbonates** To 1 ml of the test solution about 1 ml of concentration (conc.) HCL was added.	Formation of brisk effervescence indicates the presence of carbonates	Presence of brisk effervescence Absence of brisk effervescence	Positive Negative
2.	**Test for chlorides** To 2 ml of test solution, about 1 ml of silver nitrate solution was added.	Appearance of White precipitate indicates the presence of chlorides.	Presence of White precipitate Absence of White precipitate	Positive Negative
3.	**Test for sulfates** To 1 ml of the test sample add diluted H2SO4 till effervescence ceases followed by this about 1 ml of barium chloride solution was added.	Appearance of white precipitate indicates the presence of sulfates.	Presence e of white precipitate Presence e of white precipitate	Positive Negative
4.	**Test for sulfides**	Formation of colorless gas	Presence of rotten	Positive

	To 1 ml of the test sample about 2 ml of HCL was added with slight warming the mixture.	with the smell of rotten egg indicates the presence of sulfides.	egg smell Absence of rotten egg smell	Negative
5.	**Test for phosphates** To 2 ml of test solution treated with 2 ml of ammonium molybdate solution followed by addition of 2ml of concentrated nitric acid	Formation of yellow precipitate Indicates the presence of phosphates	Presence of yellow precipitate Absence of yellow precipitate	Positive Negative
6.	**Test for Fluoride and Oxalate** To 2 ml of the test solution about 2 ml of dil. acetic acid and 2ml of calcium chloride solution was added	Formation of white precipitate Indicates the presence of Fluoride/ Oxalate	Presence of white precipitate Absence of white precipitate	Positive Negative
7.	**Test for Borates** 2ml of the test solution was added with sulfuric acid and 95% alcohol followed by exposure to flame	Appearance of green flame Indicates the presence of Borates	Presence of green flame Absence of green flame	Positive Negative
8.	**Test for Nitrates** 0.5 ml of test solution heated with copper turning followed by addition of sulfuric acid	Appearance of reddish-brown gas Indicates the presence of Nitrates	Presence of reddish-brown color Absence of reddish-brown color	Positive Negative

Analytical Investigation on Test for Basic Radicals

S No	Test for Specific Basic Radical	Indication / Observation	Inference	Results
1.	**Test for Lead** 1 ml of the test solution added with 2 ml of potassium chromate solution.	Formation of yellow precipitate indicates the presence of lead.	Presence of yellow precipitate. Absence of yellow precipitate	Positive Negative
2.	**Test for Arsenic** 1 ml of the test solution added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution.	Formation of brownish red precipitate indicates the presence of Arsenic	Presence of brownish red precipitate Absence of brownish red precipitate	Positive Negative
3.	**Test for Mercury** 1 ml of the test solution added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution.	Formation of yellow precipitate indicates the presence of mercury.	Presence of yellow precipitate Absence of yellow precipitate	Positive Negative
4.	**Test for Copper** 1 ml of the test solution added with 1 ml of Ammonium hydroxide (NH4OH) solution	Formation of blue precipitate indicates the presence of copper.	Presence of blue precipitate Absence of blue precipitate	Positive Negative

5.	**Test for Ferric** To 1 ml of test solution, about 2 ml of potassium ferrocyanide was added.	Formation of blue precipitate indicates the presence of ferric.	Presence of blue precipitate Absence of blue precipitate	Positive Negative
6.	**Test for Ferrous** To 1 ml of test solution, about 1 ml of potassium ferric cyanide solution was added.	Formation of blue precipitate indicates the presence of ferrous.	Presence of blue precipitate Absence of blue precipitate	Positive Negative
7.	**Test for Zinc** 1 ml of the test solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears.	Formation of white precipitate indicates the presence of Zinc.	Presence of white precipitate Absence of white precipitate	Positive Negative
8.	**Test for Silver** 1 ml of the test solution was added with 1 ml of conc. HCL followed by appearance of curdy white precipitate. Boil the precipitate with water. It does not dissolve. Add NH4OH solution in it and add 1 ml dilute HNO_3.	Formation of curdy white precipitate indicates the presence of silver.	Presence of curdy white precipitate Absence of curdy white precipitate	Positive Negative
9.	**Test for Magnesium** 1 ml of the test solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears.	Formation of white precipitate indicates the presence of Magnesium.	Presence of white precipitate Absence of white precipitate	Positive Negative

RESULTS

Phytochemical Profile

S No	Test	Observation
1.	ALKALOIDS	+
2.	FLAVANOIDS	+
3.	GLYCOSIDES	-
4.	STEROIDS	+
5.	TRITERPENOIDS	+
6.	COUMARIN	+
7.	PHENOL	+
8.	TANNIN	+
9.	PROTEIN	-
10.	SAPONINS	-
11.	SUGAR	+
12.	ANTHOCYANIN	-
13.	BETACYANIN	+

(+) -> Indicates Positive and (-) -> Indicates Negative

Physicochemical Evaluation

State	Semi Solid
Nature	Moderately fine
Odor	Characteristic
Touch / Consistency	Greasy
Flow Property	Non-Free flowing
Appearance	Brownish

Solubility Profile

S No	Solvent Used	Solubility / Dispensability
1.	Chloroform	Insoluble
2.	Ethanol	Soluble
3.	Water	Soluble
4.	Ethyl acetate	Insoluble
5.	DMSO	Soluble

S No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	5 ± 0.2
2.	Total Ash (%)	0.793 ± 0.03
3.	Acid insoluble Ash (%)	0.05 ± 0.035
4.	Water soluble Extractive (%)	11.13 ± 0.56
5.	Alcohol Soluble Extractive (%)	9.6 ± 0.173
6.	pH	7.21

Biochemical findings

Test for Acid Radicals	
Specific Radical	**Test Report**
Test for carbonates	Positive- Indicates Presence
Test for chlorides	Negative - Indicates Absence
Test for sulphates	Negative - Indicates Absence
Test for sulphides	Negative - Indicates Absence
Test for phosphates	Negative - Indicates Absence
Test for Fluoride and Oxalate	Negative - Indicates Absence
Test for Borates	Negative - Indicates Absence
Test for Nitrates	Negative - Indicates Absence

Test For Basic Radicals	
Specific Radical	**Test Report**
Test for Lead	Positive- Indicates Presence
Test for Arsenic	Negative - Indicates Absence
Test for Mercury	Negative - Indicates Absence
Test for Copper	Negative - Indicates Absence
Test for Ferric	Negative - Indicates Absence
Test for Ferrous	Negative - Indicates Absence
Test for Zinc	Negative - Indicates Absence
Test for Silver	Negative - Indicates Absence
Test for Magnesium	Negative - Indicates Absence

DISCUSSION

The standardization of *Kundaladhi Kulambu* (K^2) was carried out through phytochemical, physicochemical and biochemical evaluations to validate its traditional claims and ensure quality, safety and consistency. The phytochemical analysis confirmed the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, phenols, steroids, triterpenoids, coumarins, sugars and betacyanin. These secondary metabolites are known for their hepatoprotective, antioxidant and anti-inflammatory activities, which collectively justify the therapeutic application of K^2 in the management of jaundice and liver-related disorders. Alkaloids and flavonoids enhance liver enzyme activity and reduce oxidative stress, while phenols and tannins protect hepatic cells from free radical damage. Steroids and triterpenoids provide membrane-stabilizing and anti-inflammatory effects and coumarins support detoxification and bile secretion.

The physicochemical analysis revealed that K^2 is a semi-solid, moderately fine and greasy formulation with a characteristic odour and brownish appearance, consistent with classical Siddha preparations. The neutral pH (7.21) indicates physiological compatibility and safety for internal administration. The low moisture content (5%) ensures good stability and minimal microbial contamination, while the total ash (0.793%) and acid-insoluble ash (0.05%) values confirm the presence of essential minerals and the absence of extraneous impurities. Water-soluble (11.13%) and alcohol-soluble (9.6%) extractive values demonstrate a balanced composition of hydrophilic and lipophilic components, reflecting the formulation's chemical uniformity.

Biochemical analysis revealed the presence of carbonates and purified lead, with no traces of toxic metals such as arsenic, mercury or silver. The presence of carbonates indicates beneficial mineral content that may aid in maintaining physiological pH balance and support

hepatic detoxification. The detection of purified lead aligns with traditional Siddha preparation methods, where detoxified metals enhance therapeutic potency. The absence of harmful radicals confirms that the formulation is free from toxic impurities and safe for therapeutic use. Overall, these findings confirm that K^2 is a chemically stable, pharmacologically active, and safely prepared Siddha formulation. The combined phytochemical, physicochemical and biochemical data establish baseline parameters for standardization and provide a scientific foundation for its traditional hepatoprotective use. Further pharmacological and clinical studies are recommended to substantiate its efficacy and promote its evidence-based application in modern healthcare.

CONCLUSION

The present study was undertaken to standardize K^2, a classical Siddha formulation traditionally indicated for the management of jaundice and liver disorders, through comprehensive phytochemical, physicochemical and biochemical evaluations. The phytochemical screening confirmed the presence of major secondary metabolites such as alkaloids, flavonoids, tannins, phenols, steroids, and triterpenoids which are known to possess hepatoprotective, antioxidant and anti-inflammatory properties. The Physicochemical parameters, including ash values, extractive values, moisture content and pH, were found to be within acceptable limits, indicating the formulation's stability, purity, and reproducibility. The biochemical analysis revealed the presence of carbonates and purified lead, with the absence of toxic heavy metals such as arsenic, mercury and silver, ensuring the safety of the preparation. These findings collectively establish baseline quality control standards for K^2 and scientifically substantiate its traditional claims. The results demonstrate that the formulation possesses desirable chemical and physical characteristics, confirming its safety and therapeutic potential as a hepatoprotective agent. Further pharmacological and clinical studies are warranted to validate its efficacy and to promote its integration into evidence-based Siddha practice.

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