



Research Article

PHYTO CHEMICAL AND PHYSIO CHEMICAL EVALUATION OF *KALLADAIPPU KUDINEER*

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ABSTRACT

Traditional Siddha medicine offers a vast array of polyherbal formulations used effectively in the treatment of various ailments. One such formulation is *Kalladaippu Kudineer Chooranam*, traditionally used in the management of urinary calculi (*Kalladaippu*). This study aims to evaluate the phytochemical constituents and physicochemical properties of *Kalladaippu Kudineer Chooranam* to scientifically validate its therapeutic potential and ensure quality control. The formulation was prepared according to classical Siddha literature and subjected to preliminary phytochemical screening, which revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds- indicating significant therapeutic potential such as anti-inflammatory, diuretic, and lithotriptic activities. Physicochemical parameters including organoleptic properties, total ash, acid-insoluble ash, water-soluble ash, loss on drying, and extractive values (water and alcohol soluble) were determined using standard methods. The results were within acceptable limits, suggesting the formulation's purity and stability. This dual evaluation ensures the standardization and authenticity of the formulation, supporting its traditional claims with scientific evidence. The presence of multiple bioactive compounds highlights the formulation's potential for further pharmacological and clinical investigations. Thus, the study provides a foundation for the integration of *Kalladaippu Kudineer Chooranam* into evidence-based practice and contributes to the preservation and validation of Siddha medicine.

INTRODUCTION

The Siddha system of medicine is one of the oldest traditional systems practiced predominantly in South India, especially Tamil Nadu. Rooted in Tamil culture, it is based on the concepts of five elements (*Pancha Bootham*), three humors (*Mukkuttram*), and vital life forces (*Uyir Thathukkal*) [1]. Siddha medicine emphasizes holistic healing through herbal, mineral, and animal-based formulations, along with lifestyle modifications, detoxification, and spiritual practices. Urolithiasis or renal calculi, is a common disorder characterized by the formation of stones in the urinary tract due to the crystallization of dietary minerals. It can cause acute flank pain, hematuria, dysuria, and urinary urgency.

In Siddha literature, this condition correlates with *Kalladaippu Noi*, as mentioned in *Yugi Vaithiya Sinthamani* (verse 800)

A classical formulation- *Kalladaippu Kudineer*- has been traditionally used for treating *Kalladaippu Noi* mentioned in the classical Siddha literature '*Therayar vagadam*' [2]. Its ingredients, such as *Aerva lanata*, *Tribulus terrestris*, *Crataeva magna* and *Aerva* have documented diuretic and anti-lithic properties. This study aims to scientifically validate the formulation through phytochemical and physicochemical evaluation.

AIM AND OBJECTIVES

To perform phytochemical and physicochemical evaluation of *Kalladaippu Kudineer*, a Siddha herbal formulation indicated for *Kalladaippu Noi*.

MATERIALS AND METHODS

Source of Raw Drugs

All ingredients were procured from authenticated country drug stores and validated by experts at Government Siddha Medical College, Palayamkottai.

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**Composition of the Formulation**

S.No	Tamil Name	Botanical Name	Parts Used	Quantity
1	<i>Serukan peelai</i>	<i>Aerva lanata</i>	Root	5gm
2	<i>Perum peelai</i>	<i>Aerva javanica</i>	Root	5gm
3	<i>Nerunjil</i>	<i>Tribulus terrestris</i>	Root	5gm
4	<i>Mavilingam</i>	<i>Crataeva magna</i>	Root	5gm
5	<i>Pirai</i>	<i>Streblus asper</i>	Root	5gm

**Method of Preparation**

10gm of *Kudineer chooranam* was boiled with 400ml of water and reduced to 50 ml.

Dosage: 60ml twice daily for 45 days.

**Physiochemical Analysis of Kalladaippu Kudineer****Loss on Drying**

An accurately weighed 2gm of *Kalladaippu kudineer chooranam* formulation was taken in a tarred glass bottle. The crude drug was heated in an oven at 105°C for 6 hours until a constant weight was obtained. The percentage moisture content of the sample was calculated concerning the shade-dried material.

**Determination of Total Ash**

Weighed accurately 2gm of *Kalladaippu kudineer Chooranam* formulation was added in a crucible at a temperature of 600°C a muffle furnace till carbon-free ash was obtained. It was calculated regarding the air-dried drug.

**Determination of Acid Insoluble Ash**

Ash above obtained, was boiled for 5 min with 25ml of 1M hydrochloric acid and filtered using an ashless filter paper. Insoluble matter retained on filter paper was washed with hot water, and the filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

**Determination of Water-Soluble Ash**

Total ash 1g was boiled for 5 mins with 25ml water, and insoluble matter collected on an ashless

filter paper was washed with hot water, dry and ignite the residue at a temperature not exceeding 450°C in a muffle furnace for 15 minutes. the amount of soluble ash is measured by evaporating the liquid (filtrate) and drying.

**Determination of Water-Soluble Extractive**

Five grams of air-dried, coarsely powdered *Kalladaippu Kudineer Chooranam* was macerated with 100ml of distilled water in a closed flask for 24 hours, with frequent shaking during the process. Determination of alcohol soluble extractive the solution was first filtered, and then 25ml of the clear filtrate was placed into a pre-weighed (tarred) shallow dish. This was evaporated and then dried at 100°C to remove all moisture. The remaining solid (the water-soluble extractive) was weighed, and its amount was used to calculate the percentage of water-soluble substances in relation to the weight of the original air-dried plant drug.

**Determination of Alcohol soluble Extractive**

2.5 g of air-dried drugs, coarsely powdered *Kalladaippu kudineer Chooranam* was macerated with 50ml of alcohol in a closed flask for 24 hrs with frequent shaking, and it was filtered rapidly, taking precaution against loss of alcohol. 10ml of the filtrate was placed in a tarred flat-bottom shallow dish, evaporated, dried at 100°C, and then weighed. The percentage of alcohol soluble extractive was calculated regarding the air-dried drug.

**Physiochemical Parameters**

Parameters	Result of Analysis
Description	Light yellow colour, coarse powder
Loss on drying at 105°C	0.22%
Total ash	7.87%
Acid insoluble ash	1.86%
Water-soluble extractive	14.46%
Alcohol soluble extractive	6.07%
pH (5% Solution)	5.87%

## Phytochemical Screening

### Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

- Mayer's Test:** Mayer's reagent (Potassium Mercuric Iodide) was added to the filtrates. Formation of yellow colored precipitate indicates the presence of alkaloids.
- Wagner's Test:** The presence of alkaloids was indicated by a brown or reddish precipitate formed after adding Wagner's reagent (iodine in potassium iodide) to the filtrates.
- Dragendorff's Test:** The filtrates were reacted with Dragendorff's reagent, and a red precipitate formed, confirming the presence of alkaloids.
- Hager's Test:** The presence of alkaloids was confirmed by the appearance of a yellow precipitate after adding Hager's reagent (saturated picric acid) to the filtrates.

### Detection of Carbohydrates

Each extract was dissolved in 5ml of distilled water and filtered. The filtrates were analysed for presence of carbohydrates

- Benedict's Test:** Filtrates were treated with Benedict's reagent and gently heated. An orange red precipitate is a positive test for reducing sugars.

### Detection of Glycosides

Extracts were hydrolyzed with diluted HCL and then subjected to test for glycosides.

- Modified Borntrager's Test:** Extracts were treated with ferric chloride solution and then immersed in boiling water for approximately 5 minutes. Equal volumes of benzene were used to extract the cooled mixture. The benzene extract was then treated with ammonia solution. The appearance of a rose-pink color in the ammoniacal layer confirms anthranol glycosides.
- Cardiac Glycoside (Keller-Killiani Test):** The Extract was shaken with distilled water (5ml). To this, glacial acetic acid (2ml) containing a few drops of ferric chloride was added, followed by  $H_2SO_4$  (1ml) along the side of the test tube. Cardiac glycosides are confirmed by a brown ring at the junction of the layers, occasionally with a violet ring forming beneath it.

### Detection of Saponins

- Froth Test:** The extracts were diluted with distilled water to a volume of 20ml and shaken vigorously in a graduated cylinder for 15 minutes. Presence of saponins is confirmed by the development of a 1cm foam layer.
- Foam Test:** A 0.5gm sample of the extract was mixed vigorously with 2ml of water; the formation of foam lasting ten minutes confirms saponins.

### Detection of Phytosterols

- Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of Conc. sulphuric acid, shaken and allowed to stand. Appearance of a golden yellow color indicates the presence of triterpenes.

### Detection of Phenols Ferric Chloride Test

Extracts were treated with 3-4 drops of ferric chloride solution. The formation of a bluish black colour indicates the presence of phenols.

### Detection of Tannins Gelatin Test

The extract is dissolved in 5ml of distilled water, and 2ml of 1% solution of gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

### Detection of Flavonoids

- Alkaline Reagent Test:** Extracts were treated with a few drops of sodium hydroxide solution. Formation of an intense yellow color, which becomes colourless on the addition of dilute acid, indicates the presence of a flavonoid.
- Lead Acetate Test:** Extracts were treated with a few drops of lead acetate solution. Formation of a yellow color precipitate indicates the presence of flavonoids.

### Detection of Proteins and Amino Acids

- Xanthoproteic Test:** The extracts were treated with a few drops of Conc. nitric acid. The formation of yellow color indicates the presence of proteins.
- Ninhydrin Test:** The extract, 0.25% w/v ninhydrin reagent, was added and boiled for a few minutes. Formation of blue color indicates the presence of an amino acid.

### Detection of Diterpenes Copper Acetate Test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. The formation of emerald green color indicates the presence of diterpenes.

### Gum and Mucilage

To 1ml of extract, add 2.5ml of absolute alcohol and stir constantly. Then the precipitate is dried in air and examined for its swelling properties. Swelling was observed, which indicates the presence of gum and mucilage.

### Test for Fixed Oils and Fats

- Spot Test:** A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

### Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of quinones.

**Phytochemical Analysis**

S.No	Name of Phyto Constituents	Aqueous Extract of <i>Kalladaippu Kudineer Chooranam</i>	Ethanollic Extract of <i>Kalladaippu Kudineer Chooranam</i>
1	Alkaloids	Positive	Positive
2	Carbohydrates	Negative	Negative
3	Reducing sugar	Negative	Negative
4	Glycosides	Positive	Positive
5	Cardiac glycosides	Positive	Positive
6	Flavonoids	Positive	Positive
7	Tannin	Negative	Positive
8	Phloba tannins	Negative	Negative
9	Terpenoids	Negative	Positive
10	Triterpenoids	Negative	Positive
11	Lignins	Positive	Positive
12	Proteins & amino acids	Negative	Negative
13	Phenolic compounds	Positive	Positive
14	Saponins	Negative	Negative
15	Phyto sterol	Negative	Positive
16	Quinones	Negative	Negative
17	Anthraquinones	Negative	Negative

**DISCUSSION**

The physicochemical parameters of *Kalladaippu Kudineer Chooranam* provide essential insights into the formulation's quality, purity, and stability.

- Loss on drying (0.22%) indicates minimal moisture content, suggesting good shelf stability and reduced risk of microbial growth.
- Total ash (7.87%) reflects the total inorganic content, which includes both physiological and non-physiological ash. This value is within acceptable limits, ensuring absence of excessive extraneous matter.
- Acid insoluble ash (1.86%) assesses the presence of siliceous material (e.g., sand or soil). A low value indicates minimal contamination.
- Water soluble extractive (14.46%) suggests the presence of water-soluble bioactive constituents such as alkaloids, glycosides, and flavonoids.
- Alcohol soluble extractive (6.07%) reveals the presence of compounds like phenols, flavonoids, and phytosterols that are more soluble in alcohol.
- pH (5.87) shows a mildly acidic nature, which may be suitable for internal consumption and compatible with physiological pH levels of urine, potentially aiding in urinary stone dissolution.

The phytochemical screening further strengthens the pharmacological potential of the formulation. It confirmed the presence of:

- Alkaloids, flavonoids, glycosides, cardiac glycosides, phenolic compounds, and lignins in both aqueous and ethanolic extracts.
- The ethanolic extract additionally revealed tannins, terpenoids, triterpenoids, and phytosterols- all of which are known for their anti-inflammatory, diuretic, and anti-urolithiatic actions.
- The herbal components of *Kalladaippu Kudineer*, such as *Aerva lanata*,<sup>[4-6]</sup> *Aerva javanica*<sup>[7]</sup> and *Tribulus terrestris*<sup>[8,9]</sup>, are extensively cited in literature for their diuretic and lithotriptic activities. The combination of phytoconstituents and physicochemical stability affirms the traditional claim of this formulation in managing *Kalladaippu Noi* (urolithiasis).

**CONCLUSION**

*Kalladaippu Kudineer* demonstrated significant physicochemical stability and rich phytochemical content. These preliminary findings support its traditional use in *Kalladaippu Noi*. Further pharmacological and clinical studies are required to establish its therapeutic efficacy and safety.

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