Research Article

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STANDARDIZATION OF SIDDHA HERBAL FORMULATION "KALLADAIPPUKU KUDINEER CHOORANAM"

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Abstract

Kalladaippuku Kudineer Chooranam is a Siddha herbal formulation traditionally used in the management of urolithiasis. The main aim of this study was to standardize the drug and to evaluate its microbial load and aflatoxin content in accordance with AYUSH protocols and WHO guidelines. Preliminary phytochemical screening revealed the presence of carbohydrates, flavonoids, quinones, and carboxylic acids. Physicochemical analysis indicated an acidic pH, with total ash value, acid-insoluble ash, and water-extractive values of 6.18%, 1.35%, and 11.59%, respectively. HPTLC fingerprinting showed seven peaks at 254 nm and eight peaks at 366 nm in the densitometric chromatogram. Aflatoxin levels were within the permissible limits, and no microbial contamination was detected. The findings of this study contribute to the quality assessment of the formulation and support its therapeutic potential in the management of urolithiasis.

Keywords: *Kalladaippuku Kudineer Chooranam, Siddha Medicine, Urolithiasis, Standardization, HPTLC, Microbial load.*

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Introduction

Siddha medicine is one of the ancient systems of medicine predominantly practiced in South India, adopting a holistic approach to rejuvenate both the body and the mind. According to the Siddha system, diseases occur due to an imbalance of the *Mukkutram*, namely *Vatham*, *Pitham*, and *Kabam*. The affected *dhosam* can be normalized through therapeutic procedures such as *Viresanam*, *Vamanam*, and *Nasiyam*, along with the administration of 32 internal and 32 external medicines.

Kalladaippu Kudineer Chooranam is one such internal medicine mentioned in the classical Siddha text "Agasthiyar Vaithiya Kaaviyam 1500" [1], and it is indicated for the management of urolithiasis. Urolithiasis refers to the formation of calculi or stones in the urinary tract. These calcifications commonly occur in the kidneys or ureters, but may also affect the bladder and urethra. Kidney stones are prevalent worldwide, with an estimated global prevalence of approximately 12%. The condition affects individuals of all ages, sexes, and races, but is more commonly observed in men than women, particularly between 20 and 49 years of age [2].

Kalladaippu Kudineer Chooranam is believed to be effective in the treatment of urolithiasis due to its anti-lithiatic and diuretic properties. However, there is a lack of scientific validation supporting its therapeutic efficacy. Therefore, the present drug requires standardization to ensure its safety profile and efficacy for internal use.

Thus, the present study deals with the preliminary phytochemical screening, physicochemical analysis, powder microscopy, High-performance Thin Layer Chromatography(HPTLC), Microbial load and Aflatoxin analysis based on the AYUSH protocol[3] and WHO guidelines[4]to scientifically validate the siddha formulation "KalladaippukuKudineerChooranam" to be used in the management of urolithiasis in the siddha system of medicine.

Materials and Methods

Collection of Drug

Kalladaippuku Kudineer Chooranam consists of three ingredients, which were procured from a well-reputed indigenous raw drug shop. The drugs were authenticated by the Department of Siddha Pharmacology, Government Siddha Medical College, Chennai. The raw drugs were purified as described in the classical Siddha texts "Sikitcha

Ratna Deepam Enum Vaithiya Nool" [5] and "Marunthu Sei Iyalum Kalaium."

The purified drugs were mildly roasted and then ground into a coarse powder. The powdered ingredients were mixed in equal proportions, and water was added to prepare a decoction, as mentioned in the Siddha literature "Marunthu Sei Iyalum Kalaium" [6].

Table 01: Ingredients of *Kalladaippuku Kudineer Chooranam*

Ingredients	Botanical Name	Ratio	Used Part
Nathaichoori	*Spermacoce Hispida*	Equal quantity	Seed
Naaipagal	*Momordica Dioica*	Equal quantity	Whole plant
Karunjeeragam	*Nigella sativa*	Equal quantity	Seed

Preparation of the Extract

The extract of *Kalladaippuku Kudineer Chooranam* was prepared using the maceration method and subsequently subjected to the following standardization parameters.

Preliminary Phytochemical Analysis

The quantitative test for preliminary phytochemical Screening of Kalladaippuku Kudineer Chooranam shows the presence of Carbohydrates, Flavonoids, Quinones and Carboxylic acid [7].

Physicochemical Analysis

Physicochemical parameters such as Total ash, Acid-insoluble value, pH, Loss on drying, Water extractive value, Alcoholic Extractive value, Bulk density were carried out as per the AYUSH protocol [3].

HPTLC Finger Printing

TLC Methodology

Twenty-five grams of *Kalladaippuku Kudineer Chooranam* was macerated with 250 mL of analytical-grade methanol (1:10 w/v) in a clean conical flask, and the mixture was left overnight with intermittent shaking. The macerate was then filtered using Whatman No. 1 filter paper and concentrated on a water bath. Ten microliters (10 µL) of the concentrated extract was applied onto a TLC plate using a CAMAG Linomat 5 applicator and developed using toluene : ethyl acetate : formic acid (7.5 : 2.5 : 0.1) as the mobile phase. After development, the plate was air-dried, and the chromatograms were documented using a CAMAG TLC Visualizer under UV 254 nm and UV 366 nm. The plate was then scanned at 254 nm (absorbance mode) and 366 nm (fluorescence mode), and the fingerprint profiles of the extract were recorded. Subsequently, the plate was sprayed with 5% vanillin–sulphuric acid reagent and heated at 105 °C until colored spots appeared. The developed plate was scanned under UV 520 nm, and the fingerprint profiles of the extract were documented.

Microbial Load Determination

The microbial quality, including the isolation and identification of pathogenic bacteria from commercial and homemade herbal medicines, was tested according to the regulations of the WHO standards (2007). The tests were used to quantify the number of bacteria and fungi isolated that are able to grow aerobically in 1 g of the sample. The samples were homogenized by mixing vigorously with H_2O. 1 gram of sample was transferred to 9 mL of peptone broth, and then serial dilutions were made to achieve an appropriate concentration. All microbial analyses were carried out in triplicate. Briefly, serial dilutions were made, and viability was assessed using the pour plate method on Casein soyabean digest agar and Sabouraud dextrose agar for bacterial counts and fungal identification. All dehydrated media were prepared according to the manufacturer's instructions and seeded and incubated at 37 °C for 24 to 48 hours for bacterial screening and at 25 °C for 48 to 72 hours for fungal screening. At the end of the incubation period, the number of colony-forming unitsper gram (CFU/g) was calculated by multiplying the average number of colonies by the dilution factor. The obtained CFU/g of sample was compared with the WHO standards. Samples that presented bacterial growth greater than 10^5 CFU in 1 g of herbal medicine were considered unsatisfactory or inadequate according to WHO guidelines for aerobic bacteria.

Identification of Bacteria

For the detection of *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, EMB agar, MacConkey agar, deoxycholate citrate agar, cetrimide agar, and mannitol salt agar were used, respectively. After the incubation period, the pathogenic bacterial isolates were preliminarily characterized based on colony morphology, Gram staining, and biochemical tests, including oxidase, gas, and catalase production.

Aflatoxin Test

Aflatoxins are a group of naturally occurring mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, two common mold species. AflaTest is a quantitative method used for the detection of aflatoxins B_1, B_2, G_1, G_2, M_1, and M_2 using an AflaTest fluorometer.

For extraction, 5 g of *Kalladaippuku Kudineer* and 0.4 g of sodium chloride were mixed with methanol containing 2% Tween 20 or phosphate buffer in a 60:40 (v/v) ratio. The mixture was vortexed at high speed for 3 minutes and then filtered through fluted filter paper. Ten milliliters of the filtered extract were transferred to a measuring cylinder, diluted with 20 mL of purified water, and vortexed for 1 minute. The diluted extract was subsequently filtered through a pre-wetted glass microfiber filter (1.5 µm).

Ten milliliters of the diluted extract were passed through an AflaTest WB column under gentle pressure to maintain a flow rate of 1–2 drops per second. The column was washed with 10 mL of 2% Tween 20, followed by two washes with 10 mL of purified water. Elution was

performed by passing 1 mL of 100% HPLC-grade methanol through the column at a rate of 1 drop per second. The eluate was collected in a sterile VICAM cuvette, to which 1.0 mL of AflaTest Developer was added and mixed thoroughly. The cuvette was immediately placed in the fluorometer (VICAM Series 4EX), and the aflatoxin concentration was recorded after 60 seconds.

Methodology for Powder Microscopic Studies

Five grams of the coarsely powdered sample were weighed and mixed with 50 mL of water in a beaker, followed by gentle warming until the sample was completely dispersed. The mixture was then centrifuged, and the supernatant was decanted. The sediment was washed several times with distilled water, centrifuged again, and the supernatant was discarded. A few milligrams of the sediment were mounted in glycerin for microscopic examination, and a few milligrams were placed in a watch glass. To identify lignified cells, a few drops of phloroglucinol followed by concentrated hydrochloric acid were added, and the material was mounted in glycerin. Photomicrographs were captured using a microscope equipped with a camera. Microscopic examination revealed the following characteristics: the epidermis consisted of thick-walled parenchymatous cells filled with reddish-brown contents; the pigment layer comprised thick-walled cells containing reddish-brown pigment; and the endosperm consisted of moderately thick-walled rectangular to polygonal cells filled with aleurone grains and oil globules (*Karunjeeragam*). Sharp-pointed, multicellular, conical-shaped trichomes measuring up to 250 µm were observed, along with numerous pitted vessels and elongated thin-walled fibers (*Naaipagal*). Thick-walled endocarp cells were observed in surface view, and endosperm cells in surface view were filled with protein bodies. Numerous isolated protein bodies from endosperm cells (*Nathaichoori*) were also noted.

Results

Physicochemical parameters

Table02: Physiochemical Analysis of KKC

S.No.	Test Parameter(s)	Result(s)
1	**Ash** (%w/w)Total ash	6.18 %
2	**Acid-insoluble ash**	1.35 %
3	**pH**	6.02
4	**Loss on Drying**	0.66 %
5	**Water Extractive Value**	11.59%
6	**Alcohol Extractive Value**	18.48 %
7	**Bulk density**	0.5025g/cm^3

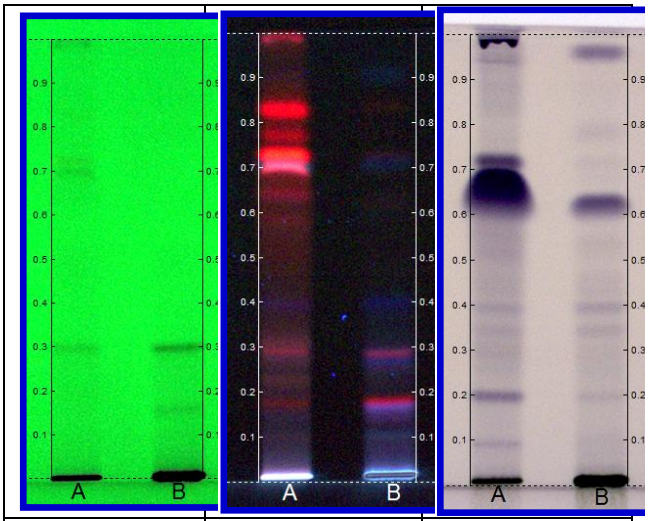
Preliminary Phytochemical Analysis

Table 03: Phytochemical findings of KKC

S.NO	Preliminary Test	Methanol extract	Hydro alcohol extract
1	**Alkaloids** Wagner's test Picric acid test	- -	- -
2	**Carbohydrates** Fehling's test	-	+
3	**Glycosides** Borntrager's test Aqueous NaOH test	- -	- -
4	**Proteins & amino acids** Biuret test Ninhydrin test	- -	- -
5	**Flavonoids** Lead acetate test	+	+
6	**Phenolic compounds** Lead acetate test	-	-
7	**Tannins** Braymer's test 10% NaOH test	- -	- -
8	**Phytosterols** Salkowski's test	-	-
9	**Cholesterol**	-	-
10	**Terpenoids**	-	-
11	**Quinones**	-	+
12	**Anthocyanin**	-	-
13	**Carboxylic acid**	+	-
14	**Gums & mucilage**	-	-
15	**Fixed oil & fat**	-	-

Thin Layer Chromatography

Table 04: Solvent System: Toluene: Ethyl acetate: Formic acid (7.5: 2.5: 0.1)



UV – 254 nm	UV – 366 nm	V - S Reagent

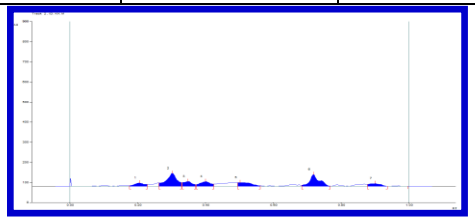


Fig 01: HPTLC fingerprint at 254 nm in Methanol extract (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.18 Rf	3.1 AU	0.21 Rf	16.3 AU	7.44 %	0.23 Rf	9.1 AU	402.3 AU	7.20 %
2	0.26 Rf	16.5 AU	0.30 Rf	64.5 AU	29.49 %	0.33 Rf	18.0 AU	1657.8 AU	29.68 %
3	0.33 Rf	18.1 AU	0.35 Rf	24.1 AU	11.04 %	0.37 Rf	8.5 AU	530.2 AU	9.49 %
4	0.37 Rf	8.6 AU	0.40 Rf	23.2 AU	10.61 %	0.43 Rf	10.5 AU	605.6 AU	10.84 %
5	0.50 Rf	18.4 AU	0.50 Rf	18.7 AU	8.56 %	0.56 Rf	2.2 AU	631.8 AU	11.31 %
6	0.68 Rf	5.5 AU	0.72 Rf	58.6 AU	26.80 %	0.77 Rf	1.0 AU	1379.4 AU	24.69 %
7	0.88 Rf	9.4 AU	0.90 Rf	13.3 AU	6.06 %	0.94 Rf	0.3 AU	379.3 AU	6.79 %

Fig 02:R_f values at 254 nm in Methanol extract (Absorbance mode)

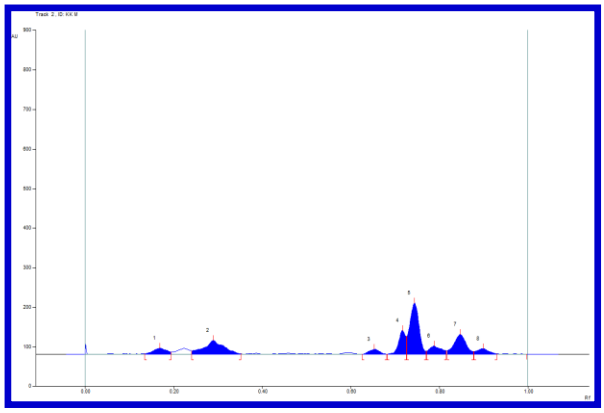


Fig 03:HPTLC fingerprint at 366 nm in Methanol extract (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.13 Rf	1.0 AU	0.17 Rf	15.2 AU	4.50 %	0.19 Rf	6.1 AU	381.6 AU	5.55 %
2	0.24 Rf	8.9 AU	0.29 Rf	35.5 AU	10.50 %	0.35 Rf	1.0 AU	1306.9 AU	19.02 %
3	0.63 Rf	0.2 AU	0.65 Rf	12.4 AU	3.66 %	0.68 Rf	0.5 AU	241.2 AU	3.51 %
4	0.68 Rf	0.5 AU	0.72 Rf	60.2 AU	17.84 %	0.73 Rf	45.0 AU	779.8 AU	11.35 %
5	0.73 Rf	45.9 AU	0.74 Rf	129.4 AU	38.34 %	0.77 Rf	7.4 AU	2244.6 AU	32.67 %
6	0.77 Rf	7.7 AU	0.79 Rf	20.8 AU	6.16 %	0.82 Rf	8.9 AU	469.1 AU	6.83 %
7	0.82 Rf	8.9 AU	0.85 Rf	49.8 AU	14.75 %	0.88 Rf	6.3 AU	1138.5 AU	16.57 %
8	0.88 Rf	6.4 AU	0.90 Rf	14.3 AU	4.24 %	0.93 Rf	1.3 AU	309.4 AU	4.50 %

Fig 04: R_f values at 366 nm in Methanol extract (Absorbance mode)

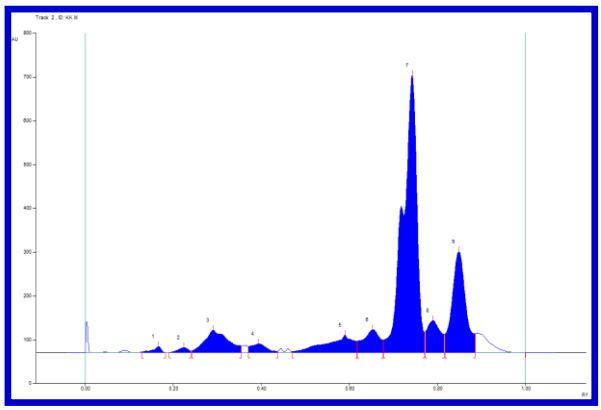


Fig 05: HPTLC finger print at 366 nm in Methanol extract (Fluorescence mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.13 Rf	1.2 AU	0.17 Rf	13.5 AU	1.20 %	0.18 Rf	0.0 AU	213.4 AU	0.68 %
2	0.19 Rf	0.1 AU	0.22 Rf	11.4 AU	1.02 %	0.24 Rf	3.7 AU	215.7 AU	0.68 %
3	0.24 Rf	3.8 AU	0.29 Rf	51.2 AU	4.55 %	0.36 Rf	15.4 AU	2257.9 AU	7.17 %
4	0.37 Rf	14.1 AU	0.39 Rf	19.6 AU	1.74 %	0.44 Rf	2.9 AU	581.9 AU	1.85 %
5	0.47 Rf	2.3 AU	0.59 Rf	40.0 AU	3.56 %	0.62 Rf	25.9 AU	2024.3 AU	6.43 %
6	0.62 Rf	26.0 AU	0.65 Rf	52.3 AU	4.65 %	0.68 Rf	28.7 AU	1606.2 AU	5.10 %
7	0.68 Rf	28.8 AU	0.74 Rf	632.9 AU	56.30 %	0.77 Rf	46.1 AU	16773.5 AU	53.25 %
8	0.77 Rf	46.9 AU	0.79 Rf	72.9 AU	6.49 %	0.82 Rf	41.9 AU	1859.4 AU	5.90 %
9	0.82 Rf	42.3 AU	0.85 Rf	230.4 AU	20.49 %	0.89 Rf	42.9 AU	5969.1 AU	18.95 %

Fig 06: R_f values at 366 nm in Methanol extract (Fluorescence mode)

Microbial load

Tab 05: Microbial load determination

S. No.	Parameters	Results
1	Total Bacterial Count (TBC)	$1.2x10^4$cfu/g
2	Total Fungal Count (TFC)	Less than 10 cfu/g
3	Enterobacteriaceae	Absent
4	*Escherichia coli*	Absent
5	Salmonella Spp	Absent
6	*Staphylococcus aureus*	Absent
7	*Pseudomonas aeruginosa*	Absent

Powder Microscopy

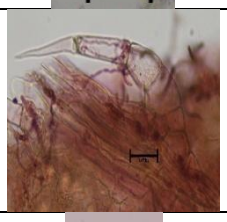
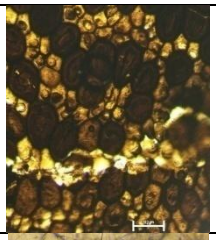
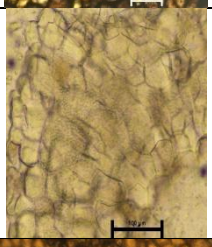
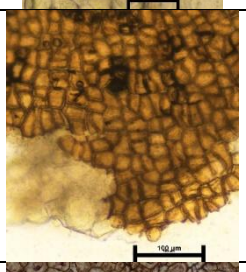
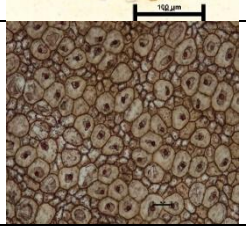
Fig 07: Naaipagal	Pitted vessel	
	Trichomes (Uniseriate)	
	Fibres	

Fig 08: Karunjeeragam	Epidermal cells	
	Endoderm cells	
	Pigmented layer	
Fig 09: Nathaichoori	Epidermal cells of the seed coat/ Endocarp	

Discussion

Kalladaippuku Kudineer Chooranam was coarsely powdered and dark brown in color. The plant extract was prepared by the maceration method for analysis. Standardization of the formulation was carried out to document preliminary phytochemical constituents, physicochemical parameters, HPTLC fingerprinting, microbial load, powder microscopy, and aflatoxin analysis, in accordance with AYUSH and WHO guidelines.

Nigella sativa possesses antioxidant, hypolipidemic, anti-inflammatory, and nephroprotective activities. Phytochemical screening of *Nigella sativa* revealed the presence of alkaloids, flavonoids, and steroids. *Momordica dioica* was found to contain fatty acids and triterpenes and is reported to exhibit antidiabetic properties. The phytoconstituents of *Spermacoce hispida* include flavonoids, proteins, and glycosides, which are responsible for its antioxidant activity. Despite the availability of scientific evidence supporting the individual efficacy of the ingredients of *Kalladaippuku Kudineer Chooranam*, the polyherbal formulation comprising all three ingredients was analyzed collectively for pharmacopeial standards using standard procedures.

The total ash value of 6.18% was within permissible limits, indicating the presence of micro- and macronutrients in the drug. The loss on drying was found to be 0.66%, which is comparatively low and suggests better stability and shelf life. The acid-insoluble ash value (1.35%) indicates minimal siliceous matter and confirms the absence of foreign inorganic contaminants. The water-soluble extractive value (11.59%) suggests the suitability of the drug for aqueous preparations, while the alcohol-soluble extractive value (18.48%) indicates the presence of a substantial amount of active phytoconstituents. The bulk density of the formulation was 0.5025 g/cm^3, demonstrating its suitability for herbal pharmaceutical formulations.

Quantitative preliminary phytochemical screening revealed the presence of carbohydrates, quinones, flavonoids, and carboxylic acids. The quality and consistency of the formulation were further assessed by HPTLC analysis. HPTLC fingerprinting showed seven prominent peaks with Rf values ranging from 0.12 to 0.82 at 366 nm, indicating the presence of multiple phytochemicals.

The presence of these bioactive phytoconstituents supports the safety, quality, and therapeutic potential of the trial drug *Kalladaippuku Kudineer Chooranam*, suggesting its effectiveness in the management of urolithiasis and other renal disorders.

Conclusion

Based on the results obtained from the present study, it can be concluded that *Kalladaippuku Kudineer Chooranam* possesses potent biologically active constituents that may contribute to its therapeutic potential in the management of various diseases. The present investigation has generated evidence-based data regarding the purity, physicochemical characteristics, phytochemical profile, and microbial load of the formulation (KKC), thereby supporting its quality, safety, and suitability for further therapeutic evaluation and regulatory acceptance.

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Conflict of Interest

None

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Self- Funded

Ethical Approval

Approved

Author Contribution

Dr. K. Sudhamathi Pushparaj designed and supervised the project. Dr. S. Swathi collected and analyzed the data and prepared the manuscript. Dr. K. Menaka and Dr. U. Chithra reviewed and revised the final version of the manuscript. All authors read and approved the final manuscript.

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