Physicochemical and Phytochemical analysis of *Kottai Karanthai Chooranam* (KKC)

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Abstract

Medicinal plants, used to treat a wide range of diseases and ailments, represent the richest natural sources of various phytochemicals. *Sphaeranthus indicus L.* commonly called "*Kottai karanthai*" in Tamil. *Kottai karanthai* chooranam (KKC) is a Traditional Siddha single-herbal formulation. The aim of the present study was to analyze physicochemical parameters and to screen the phytochemicals in KKC. It was identified as a brownish, moderately fine, non-free-flowing powder with a characteristic odor and soft consistency. Its particle size was 75.3 ± 19.95µm. KKC showed solubility in DMSO, ethanol and water. Physicochemical analysis indicated a loss on drying of 6.16 ± 0.208% at 105°C, a total ash content of 0.55 ± 0.02% and no detectable acid-insoluble ash. Water and alcohol-soluble extractable matters were 10.9 ± 0.55% and 8.9 ± 0.65%, respectively, with a pH of 6.51. Phytochemical screening of methanol extracts confirmed the presence of significant bioactive compounds, including alkaloids, flavonoids, saponins, tannins, cardiac glycosides, triterpenoids and total phenolics. The results contribute to its standardization and quality control of KKC and provide scientific support for its traditional use in Siddha Medicine.

Keywords: *Kottai karanthai chooranam*, Phytochemical Screening, Physico-chemical analysis, Siddha Herbal Formula

Introduction

Siddha Medicine is one of the oldest traditional medical systems, particularly among the Tamil people[1].Classical Siddha literatures describe many therapeutic practices and pharmaceutical preparations. Siddha treatment emphasizes both curing diseases and preventing from them, using plant, mineral, and animal based substance[2,3]. The Siddha System includes 64 categories of medicines comprising 32 types internal medicines and 32 types of external medicines[4]. *Chooranam* is one of the internal medicines, the raw materials were individually dried, purified and finely powdered, each powder was sieved through a fine cotton cloth and combined in the prescribed portion[5]. *Sphaeranthus indicus* L. from Asteraceae family commonly called East Indian Globe thistle in English and *Kottai karanthai* in Tamil[6-11]. *Kottai karanthai chooranam* (KKC) is a Traditional Siddha single herbal formulation which is mentioned in Siddha classical text book and used to cure *Vellai* (Leucorrhea), *Ul ranam* (contusion), *Karappan* (Dermatitis), *Kirani* (untreated diarrhea), and *Malaththai velipaduththum* (inducing bowel evacuation)[12]. Phytochemicals are chemical compounds naturally found in plants and that can have either positive or negative effects on health[13]. Standardization of herbal formulations is a requirement in ensuring their quality, purity, safety, and therapeutic efficacy. The establishment of a reliable and reproducible standardization system for each final product is indispensable for maintaining uniformity and scientific validation[14]. Scientific validation of traditional medicine through standardization enables the identification of bioactive constituents and elucidation of their pharmacodynamics and pharmacokinetics, thereby clarifying the mechanisms of drug action[15]. This

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study was mainly conducted to prepare the KKC according to Standard Operating Procedure (SOP) and to evaluate its standardization parameters. Therefore, the objectives of this study is to prepare the *kottai karanthai* chooranam (KKC) as per Standard Operating Procedures (SOP) mentioned in the classical text, and to evaluate physicochemical parameters and screen phytochemicals in KKC.

Materials and methods

Preparation of the drug

Raw drug collection, identification and authentication

Fresh raw materials of *Sphaeranthus indicus* (whole plant) were collected from Jaffna district, Sri Lanka. The plant material was identified and authenticated by experts from the Department of Gunapadam, Siddha Teaching Hospital, Kaithady, Jaffna, Sri Lanka.

Preparation of Kottai karanthai chooranam

The collected plant materials were cleaned, dried, purified, powdered and sieved through a fine cotton cloth. The final product was air tightly packed and labeled as KKC.

Evaluation of standardization parameters

Standardization parameters were assessed according to the guidelines of the Pharmacopoeial Laboratory for Indian Medicine (PLIM)[16].

Organoleptic evaluation

Description

The KKC was placed on a watch glass and its morphological characteristics were observed.

Colour

The KKC was observed against a white background under tube light to determine its colour[16,17].

Odour

The odour was assessed at two intervals with a gap of two minutes between assessments to nullify the effect of previous smelling[16,17].

Particle size determination

Particle size determination was carried out using the optical microscopic method. The samples were dissolved in sterile distilled water (approximately $1/100^{th}$ dilution). Diluted samples were mounted on a slide and fixed on the stage at the appropriate location. Light microscopic images were obtained using a scale micrometer to determine the average particle size[18].

Physicochemical analysis

Percentage loss on drying (moisture content)

4g of the sample was weighed, placed in a pre-weighed beaker and dried at 105ºC constantly for 5 hours. It was then weighed hourly until two consecutive weights did not differ by more than 0.25%. Constant weight was confirmed after drying and cooling in a desiccator for 30 minutes, until the weight difference was not more than 0.01g[16,17].

Calculation:

Percentage of loss on drying at 105ºC

$$= \frac{\text{Loss in weight of the sample} \times 100}{\text{Weight of the sample taken}}$$

Determination of total ash

The test drug was accurately weighed in a silica dish and incinerated in a furnace at 400ºC until it turned white, indicating the absence of carbon. The percentage of total ash was calculated relative to the air-dried sample[16,17].

Calculation

Percentage of total ash

$$= \frac{\text{Weight of ash} \times 100}{\text{Weight of the sample taken}}$$

Determination of acid insoluble ash

The ash obtained from total ash test was boiled with 25ml of dilute hydrochloric acid for 6 minutes. The insoluble matter was collected in a crucible, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash[16,17].

Calculation

Percentage of Acid – insoluble ash

$$= \frac{\text{Weight of the acid insoluble residue} \times 100}{\text{Weight of the sample}}$$

Determination of alcohol soluble extractive

The sample was macerated with 100 mL of alcohol in a closed flask for twenty-four hours, with frequent shaking during the first six hours and was allowed it to stand for eighteen hours. The solution was filtered rapidly, taking precautions against loss of solvent, 25ml of the filtrate were evaporated to dryness in a tared flat bottomed shallow dish, dried at 105ºC, and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug[16,17].

Determination of water-soluble extractive

The test sample was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, with frequent shaking during the first six hours and was allowed it to stand for eighteen hours. The solution was filtered rapidly, taking precautions against loss of solvent, 25ml of the filtrate were evaporated to dryness in a tared flat bottomed shallow dish, dried at 105ºC, and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug[16,17].

pH determination

10g of the sample was mixed with 90ml of distilled water. The mixture was stirred for three hours using a rotary shaker. Then the pH of the mixture was measured using a pH meter [16,17].

Phytochemical analysis

Phytochemical screening was carried out to identify the presence of alkaloids, flavonoids, glycosides, steroids, triterpenoids, phenols, cardiac glycoside, tannins, saponins, and total phenolics using standard protocols (Table 1).

Table 1: Phytochemical analysis tests

Phytochemical	Test Method	Observation for the presence
Alkaloids	Wagner's method	A reddish-brown precipitate
Flavonoids	Alkaline reagent test	Formation of an intense yellow color
Glycosides	Keller-Kiliani	A pink, red, or violet coloration
Steroids	Liebermann Burchard's	Show a series of colors. starting with pink or red, then turning to violet or purple, and finally to deep green or bluish-green.
Triterpenoids	Salkowski test	A reddish-brown coloration will form at the interface
Phenols	Ferric chloride test	Formation of a colored complex, typically a blue, purple, green, or red-brown color
Cardiac glycosides	Keller-kiliani	A brown ring will form at the interface between the two layers
Tannins	Ferric chloride	A change in color, which can be a greenish-black or brownish-green
Saponins	Foam test	Formation of a stable, persistent foam lasting at least 15 minutes
Total phenolic	Folin-Ciocalteu method	Formation of a blue color solution

Results and observations

The fresh materials and raw materials are shown in Figures 1 and 2.

Fig.1: Fresh raw materials of *Sphaeranthus indicus*

Fig.2: Dried and purified raw materials of *Sphaeranthus indicus*

Standardization parameters of the KKC

Table 2 shows the organoleptic parameters of KKC.

Table 2: Organoleptic parameters of KKC

Parameter	Interpretation
State	Solid
Nature	Moderately fine
Odor	Characteristic
Touch/ Consistency	Soft
Flow Property	Non-Free flowing
Appearance	Brownish

The organoleptic characters of the KKC were brownish, moderately fine, non-free-flowing powder with a characteristic odor and soft consistency.

Solubility of KKC is shown in Table 3.

Table 3: Solubility of KKC

Solvent used	Solubility / Dispensability
Chloroform	Insoluble
Ethanol	Soluble
Water	Soluble
Ethyl acetate	Insoluble
DMSO	Soluble

KKC was showed solubility in DMSO, ethanol, water and insoluble in chloroform and ethyl acetate.

Microscopic observation of particle size for the sample KKC is shown in Figure 3.

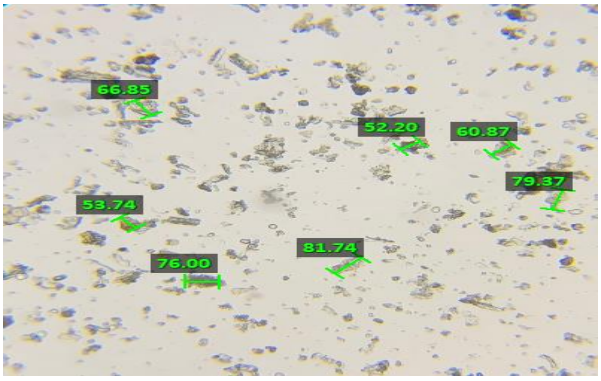

Fig. 3: Microscopic observation of particle size for the sample KKC

Microscopic observation of the particle size analysis revealed that the average particle size of the sample was found to be 75.3 ± 19.95µm further the sample has particle with the size range of lowest 52.20µm to highest 81.74µm.

Physicochemical Evaluation of KKC is shown in Table 4.

Table 4: Physicochemical Evaluation of KKC

Parameter	Mean (n=3) SD	Interpretation
Loss on Drying at 105 °C (%)	6.16 ± 0.208	**Acceptable** – Ideally < 10% to ensure low moisture (prevents microbial growth).
Total Ash (%)	0.55 ± 0.02	**Very good** – Low ash suggests minimal inorganic or contaminant material.
Acid insoluble Ash (%)	0 ± 0	**Excellent** – Indicates absence of silica, dirt, or sand.
Water soluble Extractive (%)	10.9 ± 0.55	**Normal range** – Usually 10–20% depending on formulation.
Alcohol Soluble Extractive (%)	8.9 ± 0.65	**Acceptable** – Often in the 5–15% range for herbal powders.
pH	6.51	**Good** – Near-neutral pH, compatible with oral use

Physicochemical analysis indicated a loss on drying of 6.16 ± 0.208% at 105°C, a total ash content of 0.55 ± 0.02% and no detectable acid-insoluble ash. Water and alcohol-soluble extractable matters were 10.9 ± 0.55% and 8.9 ± 0.65%, respectively, with a pH of 6.51.

Table 5 is shown the phytochemical screening of KKC

Table 5: Phytochemical screening of KKC

Phytochemical	Present / Absent
Alkaloids	Present
Flavonoids	Present
Glycosides	Absent
Steroids	Absent
Triterpenoids	Present
Phenols	Absent
Cardiac glycosides	Present
Tannins	Present
Saponins	Present
Total phenolic	Present

Preliminary phytochemical screening of KKC carried out using standard test methods, demonstrated the presence of alkaloids, flavonoids, triterpenoids, cardiac glycosides, tannins, saponins and total phenolics. In contrast, glycoside, steroids and phenols were not detected.

Discussion

Sphaeranthus indicus Linn., known as *Kottai Karanthai* in Tamil belonging to *Asteraceae* family. It is an annual herb widely distributed across India and Sri Lanka, often found as a weed in wet fields and margins. In Siddha medicine the whole plant and its parts (roots, flowers, leaves, seeds) are used for broad spectrum of ailments[19].

Organoleptic characters of KKC

Kottai Karanthai chooranam (KKC) is a single herbal formulation. Organoleptic characters of KKC are brownish, moderately fine, non-free-flowing powder with a characteristic odor and soft consistency. (Table 2)

Solubility profile

KKC is soluble in DMSO, ethanol, and water. Insoluble in chloroform and ethyl acetate. Water solubility drugs can enhance oral bioavailability and readily absorbed in gastrointestinal tract.

Physicochemical analysis

Standardization plays a vital role in the evaluation of herbal formulations as it ensures the quality, purity, and safety of the drugs through various physicochemical parameters. In the present study, parameters such as moisture content, ash values, extractive values and pH analyze for *Kottai karanthai chooranam* to establish its quality profile[11].

Moisture content

The loss on drying test is a crucial indicator of moisture and volatile matter content, which in turn influences the stability and susceptibility of herbal drugs to microbial growth and deterioration. The percentage of loss on drying for *Kottai karanthai chooranam* is 6.16 ± 0.208% (normal range: 1–20%). This relatively low value indicates minimal moisture content, suggesting a higher degree of stability and reduced microbial contamination[20].

Ash values

Ash value determination is another important parameter in assessing the quality of herbal drugs. The total ash content reflects the presence of inorganic matter, while acid-insoluble ash indicates contamination with earthy materials such as sand and silica. In this study, the total ash value of *Kottai karanthai chooranam* is 0.55 ± 0.02% (normal range: 1–25%), and the acid-insoluble ash value is 0 ± 0 (normal range: 0.1–10%). Both values are low, thereby indicates the absence of significant contamination, substitution, or adulteration. This also supports the purity and authenticity of the formulation[20].

Extractive values

The extractive values are indicative of the number of active constituents that can be extracted in specific solvents, thereby reflecting the quality and strength of the formulation. The alcohol-soluble extractive value of *Kottai karanthai* is 8.9 ± 0.65%, while the water-soluble extractive value 10.9 ± 0.55%. The higher alcohol-soluble extractive content suggests that the bioactive constituents of the formulation are predominantly alcohol soluble, which is important for therapeutic efficacy and aligns with the nature of the ingredients used[20].

pH

pH was 6.51 and it was compatible with oral use.

Physicochemical parameters analyzed in this study confirm the quality and stability of *Kottai karanthai chooranam*. The low moisture and ash contents indicate purity and minimal contamination, while the extractive values highlight the presence of active constituents responsible for its pharmacological activity. These findings provide scientific validation and quality assurance for the traditional use of this formulation.

Phytochemical screening

Phytochemical analysis is a vital step in assessing the therapeutic potential of medicinal plants, as it enables the identification of bioactive compounds responsible for their pharmacological and biological activities[21]. The phytochemical analysis of the KKC reveals the presence of several bioactive compounds, such as alkaloid, flavonoid, triterpenoids, cardiac glycosides, tannins, saponins, and total phenolic, each of which contributes to its pharmacological activities.

Tannins are well documented for their antimicrobial properties, inhibiting the growth of bacteria, fungi, yeasts, and viruses. Their activity supports the traditional use of the plant in treating skin diseases and preventing secondary infections. They use to treat diarrhea, stomach ulcer and prevent wounds from infections[20,22]. Flavonoids are recognized for their antioxidant and hypolipidemic effects, they use to treat diseases that associated with oxidative stress[22,23]. Their presence may explain, at least in part, the plant's potential role in regulating lipid metabolism. Similarly, saponins known to exhibit antibiotic and anti-inflammatory effects and possess diverse biological properties, including hemolytic activity, hypocholesterolemic properties and a characteristic bitter taste[22,24]. Alkaloids, another major group of secondary metabolites detect, are well known for their cytotoxic properties, analgesic, antispasmodic, and antibacterial activities[24]. Cardiac glycosides are another compound present in KKC, it has beneficial effect on heart health and they enhance cardiac muscle contractility by inhibiting Na^+/K^+ ATPase enzyme, which increases intracellular calcium levels and improves the efficiency of the heart's pumping action[24].

The presence of these secondary metabolites highlights the pharmacological significance of the drug. Their combined effects contribute to the traditional uses and justify further investigations into the drugs bioactivity and therapeutic potential.

Conclusion

The present study confirms that *Kottai karanthai chooranam* prepared as per classical Siddha guidelines, meets essential physicochemical standards including low moisture content, minimal ash values, suitable extractive values, and near neutral pH indicating its purity, stability, and safety for therapeutic use. KKC contains significant bioactive phytochemicals such as alkaloid, flavonoid, triterpenoids, cardiac glycosides, tannins, saponins, and total phenolics. However, further comprehensive studies are required to establish its complete standardization and to validate its efficacy through advanced scientific approaches.

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