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# Evaluate the physicochemical parameters and phytochemical screening of *Kodiveli chooranum*

Varuna, V. [1,*], Thayalin, T.[2], and Velayuthamurty, K.[3]

[1]*Faculty of Graduate studies, University of Jaffna, Sri Lanka*

[2]*Faculty of Siddha Medicine, University of Jaffna, Sri Lanka*

[3]*Faculty of Science, University of Jaffna, Sri Lanka*

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**ABSTRACT**

Medicinal plants are vital in traditional medicine due to their secondary metabolites. Standardization is crucial for ensuring the quality, efficacy, and safety of herbal drugs. This study evaluated the physicochemical parameters and phytochemical composition of *Kodiveli chooranum* (KC), a polyherbal formulation used in Siddha Medicine for various skin disorders. Physicochemical parameters, including ash values, pH, loss on drying, total solids, extractive values, swelling index, heavy metal content, and microbial load, were determined according to procedures [1, 2]. Phytochemical screening was performed on aqueous and methanolic extracts of KC and its constituent plants. The total ash content of KC was 6.29 ± 0.09%, with an acid-insoluble ash of 1.66 ± 0.22%. The pH was 6.04 ± 0.08, and the loss on drying was 6.66 ± 0.84%. The total solid content of the aqueous extract was 8.93 ± 0.22%, with alcohol-soluble and water-soluble extractive values of 4.83 ± 0.12% and 9.37 ± 1.36%, respectively. The swelling index was 30.35%. Heavy metal analysis revealed lead at 0.6 mg/Kg, while arsenic, cadmium, and mercury were below detectable limits. Microbial load testing showed a total bacterial count of 1.54 x $10^5$ CFU/g and a fungal count of 6.00 x $10^2$ CFU/g. Phytochemical screening of KC extracts indicated the presence of all tested phytochemicals except flavonoids. These findings suggest that KC's physicochemical parameters are within acceptable limits based on WHO guidelines. The presence of diverse phytochemicals supports its potential use in treating skin disorders. Further studies are warranted to evaluate the antimicrobial properties and to determine the minimum inhibitory concentration of KC to validate its therapeutic efficacy.

**Keywords**: *Kodiveli chooranum*, Standardization, Phytochemical screening.

## 1. INTRODUCTION

Medicinal plants produce secondary bioactive compounds, including alkaloids, flavonoids, terpenes, and phenolic compounds, which serve as potential therapeutic agents for various ailments in traditional and allopathic medicinal systems. The bioactive compounds can interact with biological systems and various physiological actions in the human body [3] and are crucial to treat numerous health conditions including skin ailments, digestive, and respiratory problems, cardiovascular conditions, chronic pain, immune support etc [4]. Around 60 % of the population worldwide uses the conventional medicinal system (Ayurveda, Siddha, and Unani and Siddha Medicine) as their primary health care [5]. However, herbal preparations need to confirm the *quality,* purity, safety, reproducible therapeutic effect, and bio-efficacy due to the increasing demand for herbal preparation [4]. effect, and bio-efficacy due to the

* Corresponding author: varunav@esn.ac.lk

increasing demand for herbal preparation [4]. Herbal preparations are easily available, inexpensive, and have fewer side effects. The drug production process includes collecting raw materials, cleaning impurities, drying, grinding, and packaging. Throughout these processes, contamination, mishandling, storage, and transport affect the quality of the drugs [6]. *Kodiveli chooranum* (KC) is a polyherbal preparation under the 32 types of internal medicine mentioned in Siddha literature *vaithiya pooranam* 205 [7], which is used for *kiranthi* (itching), *soolai* (pain), *vayvu rogam, kuttam* (type of skin diseases), *puzhuveddu* (patches on the scalp), *vandukadi* (insect bite), *silvisham* (poisonous bite), *elikadi* (rat bite) and *moolachchoodu* (heat in anal region). Systematic standardization of *Kodiveli chooranum* is not available. The present study aims to evaluate *Kodiveli chooranam's* physicochemical parameters and qualitative phytochemical screening for its scientific validation.

## 2. MATERIALS AND METHODOLOGY

Siddha polyherbal drug *Kodiveli chooranum* is mentioned in Siddha literature *vaithiya pooranam* 205. Table 01 tabulates the ingredients and quantity of the KC.

### 2.1 Preparation of drug *Kodiveli Chooranum*

*Acalypha indica* L. and *Cynodon dactylon* L. were collected from Trincomalee and authenticated in the Royal Botanical Garden, Peradeniya, Sri Lanka. The remaining raw materials were purchased from the authenticated drug dealer *Kailasapathy* Pharmacy, Jaffna. The ingredients of KC were purified according to the purification procedure outlined in the Siddha literature [8,9]. After purification, the ingredients were weighed using an electronic balance and then pulverized individually into a fine powder using the motor and pestle. The powdered samples were sieved with white cloth (*vasthira kayam*) to obtain a fine powder [10]. The fine powder was stored in an airtight container and labeled. The KC was prepared, as shown in Table 01.

### 2.2 Preparation of hot aqueous and cold methanolic extracts of *Kodiveli chooranum* and its ingredients

#### 2.2.1 Preparation of hot aqueous extract

The 10 g powdered ingredients were weighed and boiled separately with 100 mL distilled water at 80ºC for 2 hours. The mixture was then filtered using Whatman filter paper, and the filtrates were stored for qualitative phytochemical screening.

Table 1. Ingredients of *Kodiveli chooranum*

| S. No. | Ingredients - raw materials with botanical name and Tamil name | Quantity | Parts used |
|---|---|---|---|
| 1 | *Plumbago zeylanica* L. (*Venkodiveli*)* | 560 g | Root bark |
| 2 | *Smilax zeylanica* L. (*Parankipattai*)* | 560 g | Yam |
| 3 | *Cynodon dactylon* L. (*Arugu*)* | 560 g | Whole plant |
| 4 | *Acalypha indica* L. (*Kuppaimeni*)* | 560 g | Whole plant |
| 5 | *Psoralea corylifolia* Linn. (*Karpoharisi*)* | 17.5 g | Seed |
| 6 | *Nigella sativa* L. (*Karuncheeragam*)* | 17.5 g | Seed |
| 7 | *Cuminum cyminium* L. (*Natseeragam*)* | 17.5 g | Seed |

*g- gram, * Purified raw drugs, S. No.-Serial number

#### 2.2.2 Preparation of cold methanolic extract

The 10 g powdered ingredients were weighed and macerated separately with 100 mL of methanol for 72 hours. The mixture was then filtered using Whatman filter paper, and the filtrates were stored for qualitative phytochemical screening.

### 2.3 Physicochemical parameters of *Kodiveli chooranum*

All physicochemical analyses (Ash value, loss on drying at 110 ºC, pH value, total solids of aqueous extract, extractive values, swelling index, forming index, heavy metals, and microbial contamination) were analyzed at the Department of Chemistry at the University of Jaffna, Sri Lanka.

### 2.4 Determination of Ash value

#### 2.4.1 Total Ash value

The empty crucible was measured using an electronic balance, and the powder of KC (4 g) was measured with the crucible and incinerated at the furnace (PROTHERM) for 5 hours at 450 ºC. The crucible was taken the following morning and kept in the desiccator for 30 minutes. Finally, the weight of the crucible was measured and recorded. Ash value was calculated using the recorded weights; the procedure was triplicate, and the average value was calculated [2].

$$\text{Percentage of ash value} = \frac{\text{weight of the ash}}{\text{Weight of the sample taken}} \text{ x } 100.$$

#### 2.4.2 Acid-insoluble ash value

The above-prepared ash (0.25, 0.24, 0.25 g) was mixed with 25 mL of 1 N HCl in a beaker, transferred into a conical flask, and heated for 5 minutes at 40 ºC using a hot plate (STUART Scientific, Magnetic stirrer hot plate). The solution was filtered using Whatman filter paper (40 sizes) (A-1, FILTERKING 110 mm), and filter paper with insoluble acid was folded and kept in the weighed crucible. Then, the crucible was incinerated in the furnace (PROTHERM) for 5 hours at 650 ºC and weighed the following day. The weight was recorded, and the acid-insoluble ash value was calculated using the recorded weights. The procedure was triplicate, and the average value was calculated [1].

### 2.5 pH value

The 5 g of KC was mixed with 50 mL of distilled water, stirred well, and filtered using Whatman filter paper (40 sizes) (A-1, FILTERKING 110 mm), and allowed to rest for 30 minutes. The digital pH meter (HACH) was calibrated with pH solutions of 4, 7, and 10 at room temperature by using the knob and recording the reading. Subsequently, the sample was introduced into the knob, and the reading of the sample was recorded. The procedure was performed in triplicate, and the average value was calculated [2].

### 2.6 Calculation of Loss on drying at 110 ºC

The empty weight of the beaker was measured, and the mixture of the KC (2g) was measured with a beaker and was burnt in the hot air oven (BIOBASE -BOV-T270C) for 5 hours at 110 ºC. Then, it was kept in the desiccator for 30 minutes, and the weight was measured. The procedure was triplicated, and the average value was calculated [1].

### 2.7 Total solids of aqueous extract

2g of KC was added with 50 mL of distilled water in a pre-weighted beaker and heated in the water bath at 20 °C until the water evaporated. The beaker was transferred to an oven and heated at 105°C. The experiment was triplicated, and the average value was calculated [2].

$$\text{Percentage of total solids} = \frac{\text{Weight of the residue}}{\text{Weight of the sample taken}} \text{ x } 100$$

### 2.8 Calculation of extractive values

#### 2.8.1 Calculation of water-soluble extractive

The 4 g of KC was placed into a reagent bottle and macerated with 100 mL of distilled water for 24 hrs, shaking at 30-minute intervals for 6 hrs, and allowed to stand for 18 hrs. Filtered rapidly, taking precautions against loss of solvent, 25 mL of filtrate was taken in a pre-weighted beaker kept in a water bath to evaporate the solvent and placed in a hot oven at 105 °C for 6 hrs, placed in a desiccator to cool, and weighed. The procedure was triplicate, and the average value was calculated [2].

$$\text{Percentage of water} - \text{soluble extractive} = \frac{\text{Weight of extract } 100}{25 \text{ x weight of sample was taken}} \times 100$$

### 2.8.2 Calculation of alcohol-soluble extractive

The 4 g of KC was placed into a reagent bottle, and 100 mL of ethanol (99.9%) was added, shaken at 30-minute intervals for 6 hrs, and allowed to stand for 18 hrs. Filtered rapidly, 25 mL of filtrate was taken in a pre-weighted beaker kept in a water bath to evaporate the solvent and placed in a hot oven at 105°C for 6 hrs, placed in a desiccator to cool, and weighed. The procedure was triplicate, and the average value was calculated [2].

$$\text{Percentage of alcohol} - \text{soluble extractive} = \frac{\text{Weight of extract } 100}{25 \text{ x weight of sample was taken}} \times 100$$

### 2.9 Swelling index

One gram of KC was taken in a measuring cylinder, and 25 mL of distilled water was added and shaken every 10 minutes for 1 hour, and allowed to stand for 24 hours at room temperature. The volume was recorded after 24 hours, and the procedure was triplicated; the average value was calculated [11].

### 2.10 Forming index

One gram of KC was placed into the 500 mL conical flask, 100 mL of water was added, and the mixture was boiled for 30 minutes, allowed to cool, and filtered into a volumetric flask. The filtrate was made up of 100 mL of water, and poured into the 10 test tubes in 10 successive portions of 1 mL, 2 mL, to 10 mL, and the volume was adjusted to 10 mL in each test tube with distilled water. The test tube was shaken for 15 seconds and allowed to stand for 15 minutes. The height of the foam was measured, and the forming index was calculated [11].

### 2.11 Identification of Heavy Metals

One gram of *Kodivelich chooranum* was measured into a test tube, and 15 mL of $HNO_3$ and 5 mL of perchloric acid ($HClO_4$) were added and allowed to stand overnight in a fume cupboard to dissolve into a homogenous mixture. Then, the mixture was placed in an oven at 90ºC, and the temperature was increased every 2 minutes by 10ºC up to 170ºC. A drop of hydrogen peroxide ($H_2O_2$) was added until the formation of white fumes, and allowed to cool. The deionized water was added to the mixture in a volumetric flask up to 50 mL, and the solution was filtered through Whatman no. 42 filter paper. The results were analyzed using inductively coupled plasma-coupled mass spectrometry (ICP-MS) [12].

### 2.12 Microbial load

Bacteria and fungi counts were determined by the plate count method. Tests for specific organisms were carried out in standard procedures [13].

### 2.13 Qualitative phytochemical screening of KC

The hot aqueous and cold methanolic extracts of KC and its ingredients were analyzed for phytochemicals to identify the presence of secondary metabolites as alkaloids, glycosides, steroids, flavonoids, saponin, reducing sugar, protein, tannin, and terpenoids by using the standard procedure described by [14, 15, 16].

## 3. RESULTS AND DISCUSSION

The Siddha preparation plays a crucial role in these preparations because of their fewer side

Table 2. Physicochemical parameters of *Kodiveli chooranum*

| S. No. | Parameters | Values (mean ± SD) |
|---|---|---|
| **1** | Total Ash (%) | 6.29 ± 0.09 |
| **2** | Acid-insoluble ash (%) | 1.66 ± 0.22 |
| **3** | Alcohol soluble extractive (%) | 4.83 ±0.12 |
| **4** | Water soluble extractive (%) | 9.37 ± 1.36 |
| **5** | pH | 6.04 ± 0.08 |
| **6** | Loss on drying at 110 ºC (%) | 6.66 ± 0.84 |
| **7** | Total solids of aqueous extract (%) | 8.93 ± 0.22 |

*SD- Standard deviation

treating and preventing diseases. Herbal preparations possess antioxidant and antimicrobial activities and are potentially effective for various ailments. People turn to effects, quality, and affordability. Standardizing herbal drugs is crucial to justify their safety, potential, and efficacy to the scientific community.

Table 2 presents the physicochemical parameters of the aqueous extract of the KC. The amount of inorganic substances in the formulation, represented by a total ash value of 6.29, indicates the drug's purity. The acid-insoluble ash value reflects the siliceous matter content. The tested drug value was 1.66, suggesting the superior quality of the drug. Moreover, the European Pharmacopeia (2007) reported that the maximum limit for total ash content is 14%, with 2% for acid-insoluble ash [17]. The pH of the aqueous extract is 6.04 ± 0.0818, which is weakly acidic; acidic drugs are effective and suitable for better absorption in the stomach for human use [18]. The moisture content of the drug may enhance microbial contamination. Furthermore, the moisture content limit for herbal preparations is set at 8%, according to the National Agency for Food and Drug Administration and Control, while the moisture content of KC is 6.66 ± 0.84, which may help prevent bacterial and fungal growth [18]. The swelling index of the KC was 30.35%, indicating the presence of mucilaginous substances in the tested drug that may act as a laxative, while the forming index was absent. The water and alcohol-soluble extractive values indicate the percentage of soluble constituents in the drug. The extractive value for water (9.37 ± 1.36) exceeds that of alcohol (4.83 ± 0.12), indicating that bioactive compounds are more soluble in water than in alcohol [19].

Table 3. Heavy metals analysis of *Kodiveli Chooranum*

| S. No. | Parameters | Result (mg/Kg) |
|---|---|---|
| **1.** | Arsenic | Not detected |
| **2.** | Cadmium | Not detected |
| **3.** | Lead (Pb) | 0.6 |
| **4.** | Mercury (Hg) | Not detected |

Table 3 shows the heavy metals in the *Kodiveli chooranum*, arsenic, cadmium, and mercury, were not detected, and lead in 0.6 mg/Kg. According to WHO, the permissible limit of Lead in herbal preparations is 10 ppm [20].

Table 04 shows the total bacterial and fungal count as $1.54x10^5$ and $600x10^{2,}$ respectively. Zamir (2015)[20] reported that the European Pharmacopoeia Edition (2013) (microbiological quality of herbal medicinal products for oral use and extracts used in their preparation) mentioned that the microbial limits for the herbal preparation are $5x10^5$ (Total aerobic microbial count) and $10^4$ (Total combined yeast and mold count).

KC's aqueous and methanolic extracts contained all the tested phytochemicals except the flavonoids in the aqueous extract. Conversely, flavonoids were absent in both extracts of *C. dactylon*, *A. indica,* and *S. china*. Methanolic extracts of *C. cyminum, P. corylifolia, N. sativa,* and *S. china* contained glycosides, were not present in the aqueous extract. Furthermore, the aqueous extracts of *N. sativa* and *S. china* did not contain phenol.

Table 4. Microbial assessment of *Kodiveli Chooranum*

| S. N. | Organism | In colony-forming units/mL (cfu/mL) |
|---|---|---|
| **1** | Total bacterial count | $1.54x10^5$ |
| **2** | Total fungal count | $6.00\ x10^2$ |
| **3** | *E. coli* | Not detected |
| **4** | *Salmonella* spp | Not detected |
| **5** | *Aspergillus niger* | Not detected |

Table 5. Preliminary phytochemical screening of *Kodivelich chooranum* and its ingredients

| S. N. | Phytochemicals | Aqueous extract | | | | | | | | Methanolic extract | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | KC | *P. zeylanica* | *C. dactylon* | *A. indica* | *C. cuminum* | *P. corylifolia* | *N. sativa* | *S. china* | KC | *P. zeylanica* | *C. dactylon* | *A. indica* | *C. cuminum* | *P. corylifolia* | *N. sativa* | *S. china* |
| 1 | Saponin | + | + | + | + | + | + | + | + | + | + | + | - | + | - | + | - |
| 2 | Glycosides | + | + | - | + | - | - | - | - | + | + | + | + | + | + | + | + |
| 3 | Flavonoids | - | + | - | - | + | + | + | - | + | + | - | - | + | + | + | - |
| 4 | Tannin | + | + | - | + | + | + | + | - | + | + | + | + | + | + | + | - |
| 5 | Terpenoid | + | + | + | + | - | + | - | + | + | + | + | + | + | + | + | + |
| 6 | Alkaloids | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 7 | Phenols | + | + | + | + | + | + | - | - | + | + | + | + | + | + | + | + |

+: Present, -: Absent

The phytochemical screening confirmed the presence of alkaloids, steroids, flavonoids, protein, tannin, and terpenoids as preliminary phytochemicals in the *Kodiveli chooranum* and its ingredients. Alkaloids possess antibacterial properties and inhibit the growth of microorganisms by damaging the cell wall, disturbing protein synthesis and nucleic acid synthesis, modifying the bacterial cell membrane permeability, and inhibiting metabolism and efflux pumps [21]. Neumann (2022)[22] reported that the scientific society focused on tannins and flavonoid-rich foods due to their pharmacological activity against bacteria [22]. Tannins and flavonoids interfere with biofilm formation and protein synthesis. Additionally, tannin treats minor skin inflammation and dryness [23]. Furthermore, terpenoids have remarkable medicinal values as antibacterial, hyperglycemic activities, anti-tumor, anti-inflammatory, anti-viral, antimalarial, and promote transdermal absorption, and prevent cardiovascular disease [21].

## 4. CONCLUSION

The present study revealed that the values of all the tested parameters of KC were within the limits as per the standard guidelines of the WHO. The presence of bioactive components indicates its potential use in treating skin diseases. Future studies are needed to screen the antimicrobial properties and determine the minimum inhibitory concentration to confirm the effectiveness of this drug.

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