STANDARDIZATION OF A SIDDHA HERBOMINERAL FORMULATION KANDAAMALAGA ILAKAM

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ABSTRACT

In recent years scientific validation and standardization of drug is essential for global acceptance. The aim is to do physicochemical and preliminary phytochemical analysis for kandaamalaga ilakam as per the pharmacopoeia laboratory standards of Indian medicine. The drug prepared as per the method mentioned in the Siddha literature. Physicochemical evaluation; ash values, namely total ash, acid-insoluble ash, water-soluble ash, alcohol soluble extractive value, and loss on drying were determined. Phytochemical analysis has been shown that the presence of alkaloids, carbohydrates, glycosides, phenols, flavonoids, diterpenes, fat and fixed oil, quinines. TLC, HPTLC finger printing, Heavy metal analysis, Pesticide residues, Microbial load and specific pathogen contamination were studied as per the pharmacopeial laboratory standards of Indian medicine. The result is shown that the specified drug is containing various phytochemical and is free from microbial contamination and pesticide residues. The heavy metals such as Arsenic, Mercury, Cadmium and Lead are occurs in below detectable limit.

Keywords: Physicochemical analysis, Phytochemical, HPTLC, Kandaamalagaka ilakam.

INTRODUCTION

Siddha system of medicine is one of the ancient systems of medicine, which was founded by Siddhars. Kandaamalaga ilakam is a siddha medicine, which had been chosen from the text Sarabenthirar varthiya muraikai (Paandu kaamalai sikitchai). It has been indicated for Azhal veluppu noi (Iron deficiency anemia). Kandaamalaga ilakam is a herbomineral formulation which contains 9 herbal ingredients and one mineral ingredient Induppu. Standardization of drug is helpful to exhibit conformation of its identity and to determine its purity, safety, potency and efficacy for safer practice and marketing approval. Till date, there is no standard available for kandaamalaga ilakam. Hence, the current study has been carried out to assess its Physicochemical, Phytochemical and other standardization parameters as a part of their scientific validation.

MATERIAL AND METHODS

Identification of raw drugs

The required raw drugs for preparation of Kandaamalaga ilakam were purchased from a well reputed country shop at Chennai. The herbal ingredients were authenticated by Assistant Professor of Botanist, National Institute of Siddha, Chennai (certificate no: NISMB3272018) were deposited in the medicinal Botany laboratory, national institute of Siddha, Chennai, India. Mineral drug was authenticated from head of the department of Gunapadam, National Institute of Siddha, Chennai-47. The raw drugs were purified and medicine was prepared at Gunapadam laboratory, National Institute of Siddha, Chennai-47.

Ingredients of kandaamalaga ilakam

1. Nellikkai (Phyllanthus emblica Linn), 2. Seeragam (Cuminum cyminum Linn), 3. Lavangapathiri (Cinnamomum tamala Buch. Hum) Nees, 4. Kiraambu (Syzygium aromaticum Linn), 5. Venkodivelverppattai (Plumbago zeylanica Linn), 6. Vaividangam (Emblica ribes Burm. f), 7. Sirunagapoop (Mesua nagassarium Burm. f), 8. Elam (Elettaria cardamomum Manton), 9. Athimadhuram (Glycyrrhiza glabra Linn), 10. Induppu (Sodium chloride impura).

Method of Purification

Nellikkai-Fruit-Cleaned in water and removed the seed.

Purification of other raw drugs and mineral drug were done as per the methods given in Siddha text sigichaa ratha deepam.

Method of Drug Preparation

Kandaamalaga ilakam was prepared according to the procedure mentioned in Siddha classical text Sarabenthirar Varthiya Muraikai (Paandu Kaamalai Sigitehai).
Analytical study
The organoleptic characteristics (Appearance, colour, odour, taste and touch), tests for presence of Heavy metals (such as arsenic, mercury, cadmium and lead), Pesticide residues, Microbial load and specific pathogen contamination were studied as per standard operation procedures at VS clinical research and hospitals, Taramani, Chennai. Physicochemical evaluation; pH value ash values, alcohol soluble extractive value, loss on drying, and Preliminary phytochemical screening for the detection of carbohydrates, proteins, flavonoids, saponins, diterpenes, fat and fixed oils, were studied at The Tamil Nadu Dr. MGR Medical University, Anna Salai, Guindy and Chennai-600032.

TLC and HPTLC finger printing were studied as per the WHO standards at in Regional Research Institute of Unani Medicine (RRIUM), Rayapuram, Chennai-600013\(^3\). The results found were discussed below.

Organoleptic study
Organoleptic characters for various sensory characters like colour, taste, odour etc., were carefully noted and the interpretation illustrated in Table 1.

Colour
The kandaamalaga ilakam was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour
The kandaamalaga ilakam was smelled twice individually with an interval of 2 minutes.

Taste
Small amount of kandaamalaga ilakam was kept over the tip of the tongue.

Physico chemical analysis
Loss on drying
An accurately weighed 2 gram of kandaamalaga ilakam formulation was taken in a tarred glass bottle. The crude drug was heated at 105\(^\circ\)C for 6 hours in an oven till a constant weight. The percentage moisture content of the sample was calculated with reference to the shade dried material.

Determination of total ash
Weighed 2 gram of kandaamalaga ilakam formulation was added in crucible at a temperature 600\(^\circ\)C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

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

Determination of water soluble ash
Total ash 1 gram was boiled for 5 minutes with 25 ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450\(^\circ\)C in a muffle furnace. The amount of soluble ash is determined by drying the filter.

Determination of water soluble extractive
5 gram of air dried drug, coarsely powdered Kandaamalaga ilakam was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently. Solution was filtered ad 25 ml of filtrate was evaporated in a tarred flat bottom shallow dish, further dried at 100\(^\circ\)C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

Determination of alcohol soluble extractive
2.5 gram of air dried drugs; coarsely powdered kandaamalaga ilakam was macerated with 50 ml alcohol in closed flask for 24 hours. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10 ml of filtrate was evaporated in a tarred flat bottom shallow dish, dried at 100\(^\circ\)C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

Determination of Total Sugar
1 mg/ml of concentrated drug to be tested was prepared by dissolving it in distilled water. To this 3 ml of 52 % perchloric acid, 0.1 ml of 80 % phenol and 5 ml conc. H\(_2\)SO\(_4\) were added, cooled for a few minutes after which the absorbance was measured at 490 nm. The same procedure was repeated with sugar solution in place of the drug. After plotting a standard graph with absorbance on y-axis and concentration on x-axis the concentration of sugar present in the drug was calculated.

Determination of reducing sugar
1 mg/ml concentration of required drug was prepared by dissolving it in distilled water, pipette out in different test tubes and made up to 3 ml using distilled water. After adding 3 ml of DNS reagent, boiled in water bath for 5 minutes along with which 1 ml 40 % Rochelle salt solution was added. Later cooled at room temperature and absorbance reading were taken at 510 nm. The same procedure was repeated by replacing the drug with glucose and plotted the standard graph using OD measurement on Y-axis and concentration on X-axis. The amount of reducing sugar present in the sample was calculated from the standard graph.

Determination of fat content
5-6 gram of crushed air-dried drug was accurately weighed and transferred to the extraction thimble, extracted with Solvent ether (or petroleum ether, b. p. 40\(^\circ\) to 60\(^\circ\)) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. The extract was then filtered into a tarred evaporating dish, evaporated off the extract using anhydrous sodium sulphate and removed off the solvent under vacuum at 40\(^\circ\)C. Later the percentage of fat content was calculated with reference to the air-dried drug was calculated\(^{1}\).

Microbial Contamination
10 gram of the preparation being examined was dissolved properly in a buffered sodium chloride- peptone solution, pH 7.0 with no antimicrobial activity. Later the volume was made up to 100 ml using the same media chosen, maintaining the pH. For determining bacterial and fungal count petri dishes 9 to 10 cm in diameter was plated with 15 ml of liquefied casein soybean digest agar and Sabouraud dextrose agar with antibiotics at not more than 45\(^\circ\) along with 1 ml of the pretreated preparation.
Alternatively, it was also spread plated with the pretreated preparation on the surface of solidified medium in a Petri dish of the same diameter. The drug of preparation was diluted so that a colony count of not more than 300 could be expected. Two petri pates were plated at same dilution and incubated at 30° to 35°C for 5 days for bacteria and 20° to 25°C for 5 days for fungus until a more reliable count was obtained in a shorter time. After incubation the results were chosen from the plate with greatest number of colonies but not more than 300 colonies.

**Test for specific pathogen**

10 gram of the preparation being examined was dissolved properly in a buffered sodium chloride- peptone solution, pH 7.0 with no antimicrobial activity. Later the volume was made up to 100 ml using the same media chosen, maintaining the pH.

**Escherichia coli**

10 ml of pretreated drug preparation mixed with 50 ml of nutrient broth, placed in a screw capped container shaken properly and allowed to stand for 1 hour. Later the cap was loosen and incubated at 37°C for 18-24 hours. As a primary test for *E. coli* 5 ml of Mac Conkey broth mixed well with 1 ml of enrichment culture in a centrifuge tube, incubated in water bath at 36-38°C for about 2 days. As the acid and gas formation was not found it indicates that the drug to be tested was free from *E. coli*.

**Salmonella spp**

10 ml of pretreated drug preparation along with 1 gram of the product was added with 100 ml of nutrient broth in a sterile screw capped jar, shaken properly and, allowed to stand for 4 hours. Later the cap was loosen and incubated at 35° to 37°C for 24 hours; as a primary test 1.0 ml of enrichment culture was added to 2 tubes containing 10 ml of selenite F broth and tetrathionate bile-brilliant green broth, incubated at 36° to 38°C for 48 hours. From each of these two cultures sub culturing was done by two of the following four agar media like bismuth sulphate agar, brilliant green agar, desoxycholatecitrate agar and xylose-lyxose- desoxycholate agar then incubated at 36-38°C for 18 to 24 hours. As the colonies were not found it indicates that the drug to be tested was free from *Salmonella* spp.

**Pseudomonas aeruginosa**

The pretreated preparation was inoculated in 100 ml of fluid soybean-casein digest medium along with 1 gram of preparation being examined, mixed well and incubated at 35° to 37°C for 24 to 48 hours. On examination of media if growth was found it indicates that the drug to be tested was free from *Pseudomonas aeruginosa*.

**Staphylococcus aureus**

The pretreated preparation was inoculated in 100 ml of fluid soybean-casein digest medium along with 1 gram of preparation being examined, mixed well and incubated at 35° to 37°C for 24 to 48 hours. If growth occurs, coagulase test was conducted by transferring the colonies from the agar surface of media to individual tubes each containing 0.5 ml of mammalian preferably rabbit plasma with or without additives incubated in water-bath at 37°C and examined at 3 hours and subsequently at suitable intervals up to 24 hours. As coagulation was not found it indicates that the drug to be tested was free from *Staphylococcus aureus*.

**Phytochemical analysis**

The preliminary phytochemical screening test was carried out for each extracts of kandaamalaga ilakam as per the standard procedure.

**Detection of alkaloids**

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

**Hager’s test**

Filtrates were treated with Hager’s reagent (saturated picric acid solution). presence of alkaloids confirmed by the formation of yellow coloured precipitate.

**Detection of carbohydrates**

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to the presence of carbohydrates.

**Molisch’s test**

To 2 ml of plant sample extract, two drops of alcoholic solution of alpha naphthol are added. The mixture is shaken well and a few drops of concentrated sulphuric acid are added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

**Benedict’s test**

Filtrates were treated with benedict’s reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

**Detection of glycosides**

Extract were hydrolysed with dil. HCL, and then subjected to test for glycosides.

**Modified Borntrager’s test**

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose colour in the ammionic layer indicates the presence of anthranol glycosides.

**Detection of Phenols Ferric chloride test**

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

**Detection of flavonoids**

Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
Lead acetate test
Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and amino acids
Xanthoproteic test
The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin test
To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of Diterpenes Copper Acetate test
Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

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 quinines
Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of quinines. The preliminary phytochemical studies of aqueous extract of kandaamalaga ilakam were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of kandaamalaga ilakam.

RESULTS AND DISCUSSION

Table 1: Organoleptic Parameters of kandaamalaga ilakam

| Parameters     | Result       |
|----------------|--------------|
| Colour         | Dark brown.  |
| Odour          | Halwa like odour |
| Taste          | Sweet       |
| Touch          | soft to touch |
| Appearance     | semisolid   |

Table 2: Physicochemical interpretation results

| Parameters              | Percentage |
|-------------------------|------------|
| Loss on drying          | 1.54%      |
| Total ash value         | 2.01%      |
| Acid insoluble ash      | Less than 1% |
| Water soluble ash       | 1.72%      |
| Water soluble extraction| 65.19%     |
| Alcohol soluble extraction| 28.14%    |

Table 3: Interpretation of results

| Tests performed | Result       |
|-----------------|--------------|
| Total sugar     | 530 µg/mg    |
| Reducing sugar  | 260 µg/mg    |
| Fatty Oil Estimation | 11.17 % |
All the physicochemical parameters like loss on drying, total ash value, water soluble ash, water soluble extraction, alcohol soluble extraction, total sugar, reducing sugar, fatty oil estimation indicate that the better quality of the drug preparation.

Table 4: Interpretation of Microbial load results

| Tests performed                  | Result          |
|----------------------------------|-----------------|
| Microbial contamination          |                 |
| Total bacterial count            | 5800 cfu/ml     |
| Total fungal count               | 75 cfu/ml       |
| Test for specific pathogen E. coli | Absent per g  |
| Salmonella spp. S. aureus        | Absent per g    |
| Pseudomonas aeruginosa           | Absent per g    |

Microbial load indicates that the quality standard of finished product. The total bacterial and fungal count was within the permissible limit.

Table 5

| Phytochemicals | Test name                  | H₂O extract |
|----------------|---------------------------|-------------|
| Alkaloids      | Hager’s test               | +ve         |
| Carbohydrates  | Molisch’s test             | +ve         |
| Glycoside      | Modified Bontrager’s test  | +ve         |
| Phenols        | Ferric chloride test       | +ve         |
| Flavonoids     | Alkaline reagent test      | +ve         |
|                | Lead acetate test          | +ve         |
| Diterpenes     | Copper acetate test        | +ve         |
| Fat and fixed oil | Spot test                | +ve         |
| Quinones       | NAOH extract               | +ve         |

The phytochemical analysis results also give additional support to its usage in clinical trial with potent antioxidant action.

Thin Layer Chromatography

Methanol Extract

![HPTLC finger print of methanol extract at 254 nm (Absorbance mode)](image-url)
HPTLC finger print revealed that the possible active phyto constituents, which is responsible for the therapeutic action of the drug.

\[ R_f \text{ values alcohol methanol at 254 nm (Absorbance mode)} \]

\[ R_f \text{ values methanol extract at 366 nm (Absorbance mode)} \]
The heavy metal analysis revealed that the kandaamalaga ilakam was below the WHO/FDA permissible limits of heavy metals and drug is safe for clinical trial.

The drug free from pesticide residues and it is safe for consumption.

CONCLUSION

In the current scenario drug standardization is vital for even centuries old traditional Siddha formulations for its global acclimatization. Evaluation of parameters such as ash value, loss on drying, HPTLC studies are determined, which signifies standard parameters to ensure the purity and quality of the drug. Preliminary phytochemical analysis is shown that the presence of alkaloids, carbohydrates, glycosides, phenols, flavonoids, diterpenes, fat and fixed oil, quinines. The results are shown that the specified drug is containing various phytochemicals and is free from microbial contamination and pesticide residues. The heavy metals such as arsenic, mercury, cadmium and lead are occurs in below the WHO/FDA permissible limit and safe for consumption of kandaamalag ilakam. HPTLC finger print of kandaamaga ilakam is responsible for expression of its biological and clinical action. The present study concludes that standardization of kandaamalaga ilakam have exhibited significant results.

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