Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 14 (1), 2023; 65-70

Phytochemical and Physicochemical analysis of Siddha polyherbal formulation *KaranthaiChooranam*

Research Article

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Abstract

Aim: Standardization of a Siddha Poly herbal compound formulation is essential for establishing its authentication quantity and efficacy. In this present study, an attempt has been made to standardize the *KaranthaiChooranam* (a polyherbal formulation that contains 14 plant materials). Hence standardization of traditional drugs becomes highly essential to explore their potency and efficacy in the global market. It has been increasing public interest and acceptance of traditional medicine. Methods: Physico-Chemical analysis, Phyto-Chemical Screening, Particle Size, Pesticide Residue, and Sterility test were performed as per PLIM (Pharmacopeia Laboratory of Indian Medicine) guidelines. Results: The loss of drying of the tested drug was 105°c .KC contains 0.59% acid-insoluble ash and solubility in the water was 9.63 %. Phytochemical screening *KC* has shown the presence of Alkaloids, Glycosides, Saponins, Phytosterols, Fixed Oil, Resins, Phenols, Flavonoids and Tannins. Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be 115.5 ± 42.83 µm. Conculsion: From this set of parameters, it can be concluded that it is sufficient to evaluate the authenticity of *KaranthaiChooranam* can be used as a reference standard for the preparation of the standardized pharmaceutical product and further quality content research. This study suggests that quality specifications of KC can be developed using parameters described in *Siddha* along with analytical tools available today.

Key Words: *KaranthaiChooranam,* Standardization, Herbal Drug, Physicochemical, Phytochemical analysis.

Introduction

India is a mother hub for the development of *Ayurveda, Unani, Siddha,Homoeopathy* and other natural herbs-based health science (AYUSH). Ayush Pharmaceutical industry is having great potential and opportunities for development in future. Mainly in following herbal medicinal plants and their value-added products well accepted in domestic and international market(1).Siddha system of medicine, which is the traditional heritage science of Tamil origin, renders the application of numerous medicines based on plants, minerals-metals and animal origin(2).

World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles6. If a principle active component is known, it is most logical to quantitate this compound. Where active ingredients contributing to therapeutic efficacy are known botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose (3)

Herbal medication as the major remedy in the traditional system of medicine has been used in medical practices since antiquity. The Siddha system is one of the traditional systems of medicine which is flourished in southern India, especially Tamil Nadu. In the Siddha system besides herbs, metals and mineral drugs were used as medicine in 32 subdivisions (4). The mode of preparation and plant used in traditional medicine varies from place to place.

The traditional system of medicine became significantly more popular all over the globe because of its curative property, less toxicity and minimal side effects. Hence most of the Siddha formulations are herbal and polyherbal formulations. Central Council for Research in Ayurveda and Siddha has provided

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proforma for each category of medicine (5). Standardization of formulation was done based on its macroscopic and microscopic characters, Physico-Chemical Parameters, Phyto Chemical Screening, Fluorescence studies and Thin Layer Chromatography Analysis. Phytochemical screening has shown the presence of Alkaloids, Glycosides, Saponins, Phytosterols, Fixed Oil, Resins, Phenols, Flavonoids and Tannins, Phytochemical(6)

Matrials and Methods

Selection of source of drugs

The raw drugs were procured from the raw drug shop R.N. Rajan and Co, Chennai. After proper authentication by the Medicinal Botanist, Govt Siddha Medical College, Arumbakkam, Chennai, the preparation was made.

Purification of Raw drugs:

All herbal drugs are purified as mentioned in the text *Gunapaadam Mooligai Vaguppu*(7).

Table1: Composition of Polyherbal formulation (8)

S.No	Ingredients (Botanical Name)	Family	Part Used	Quantity (grams)
1	*Spheranthus indicus* L.	Asteraceae	*Dry Flower*	1*Balam (35 grams)*
2	*Nigella sativa* L.	Ranunculaceae	Seed	1 *Balam (35 grams)*
3	*Terminalia bellirica* (Gaertn.) Roxb.	*Combretaceae*	Seed	1 *Balam (35 grams)*
4	*Acorus calamus*	Acoraceae	Root	1 *Balam (35 grams)*
5	*Piper longum* L.	Piperaceae	Fruit	1 *Balam (35 grams)*
6	*Piper nigrum* L.	Piperaceae	Fruit	1 *Balam (35 grams)*
7	*Celastrus paniculatus* Willd.	Celastraceae	Fruit	1 *Balam (35 grams)*
8	*Rock Salt*		Salt	1 *Balam (35 grams)*
9	*Costus speices*	Zingiberacae	Root	1 *Balam (35 grams)*
10	*Zingiber officinale* Roscoe	Zingiberacae	Rhizome	1 *Balam (35 grams)*
11	*Clerodendrum serratum* (L.) Moon.	Verbenaceae	Rhizome	1 *Balam (35 grams)*
12	*Psoralea corylifolia* L.	Leguminosae	Seed	1 *Balam (35 grams)*
13	*Plumbago zeylanica* L.	Plumbaginacea e	Root	1 *Balam (35 grams)*
14	*Terminalia chebula* Retz	Combretaceae	Seed	1 *Balam (35 grams)*

SOP for *KaranthaiChooranam* procedure:

All the raw drugs were purified as per traditional text and powdered followed by the procedure *Vasthrakayam* to get a fine powder. The final product was stored in an air-tight container.

Physico-chemical analysis

Organoleptic properties are one important parameter for drug standardisation. Organoleptic characteristics like state, appearance, nature, odour and flow properties were observed.

Physico-chemical evaluation

Percentage Loss on Drying (11)

The test drug was accurately weighed in an evaporating dish. The sample was dried at 105° C for 5 hours and then weighed.

Determination of Total Ash (12)

The test drug was accurately weighed in a silica dish and incinerated in the furnace at a temperature of 400 ºC until it turns white which indicates the absence of carbon. The percentage of total ash was calculated concerning the weight of the air-dried drug.

Determination of Acid-Insoluble Ash

The ash obtained by the total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in the crucible and will be washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash will be calculated concerning the weight of air-dried ash.

Determination of Water-Soluble Ash

The ash obtained by the total ash test will be boiled with 25 ml of water for 5 mins. The insoluble matter collected in the crucible will be washed with hot water and ignited for 15mins at a temperature not exceeding 450ºC. The weight of the insoluble matter will be subtracted from the importance of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash concerning the air-dried drug.

Determination of Alcohol Soluble Extractive (12-13)

The test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, frequently shaking for six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dry at 105ºC, to constant weight and weight. Calculate the percentage of alcohol-soluble extractives regarding the air-dried drug.

Determination of Water Soluble Extractive (12-13)

The test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, frequently shaking for six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dry at 105ºC, to constant weight and weight.

Calculate the percentage of water-soluble extractives with reference to the air-dried drug.

Phytochemical analysis (14-15)

Test for Alkaloids: Mayer's Test: To the test sample, 2ml of Mayer's reagent was added, and a dull white precipitate revealed the presence of alkaloids.

Test for Coumarins: To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

Test for Saponins: To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for Tannins: To the test sample, ferric chloride was added, formation of a dark blue or greenish-black colour showed the presence of tannins.

Test for Glycosides- Borntrager's Test drug is hydrolyzed with concentrated hydrochloric acid for 2 hours in a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, the chloroform layer is separated and 10% ammonia solution is added to it. The pink colour indicates the presence of glycosides.

Test for Flavonoids: To the test sample about 5 ml of dilute ammonia solution were been added followed by the addition of a few drops of conc. Sulphuric acid. The appearance of yellow colour indicates the presence of Flavonoids.

Test for Phenols: Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for Steroids: To the test sample, 2ml of chloroform was added with a few drops of conc. Sulphuric acid (3ml), and shake well. The upper layer in the test tube turned red and the sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids Liebermann–Burchard test: To the chloroform solution, a few drops of acetic anhydride was added and then mixed well. 1 ml of concentrated sulphuric acid was added from the sides of the test tube, the appearance of a red ring indicates the presence of triterpenoids.

Test for Cyanins - Aanthocyanin: To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. The formation of bluish-green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test: To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated in a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test): To extract 1% solution of copper sulphate was added followed by a 5% solution of sodium hydroxide, the formation of violet purple colour indicates the presence of proteins.

Electron microscopic observation of particle size for the test - KC (16-17)

Test for pesticide residue (18-19)
Organochlorine pesticides
Organophosphorus pesticides
Pyrethroids

Test samples were extracted with 100 ml of acetone and followed by homogenization for a brief period. Further filtration was allowed and the subsequent addition of acetone to the test mixture. Heating of the test sample was performed using a rotary evaporator at a temperature not exceeding 40°C until the solvent has almost completely evaporated. To the residue add a few millilitres of toluene R and heat again until the acetone is completely removed. The resultant residue will be dissolved using toluene and filtered through a membrane filter.

Sterility test by Pour plate methods

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / unsterile sample (formulation) when coming in contact with the nutrition-rich medium promotes the growth of the organism and after the stipulated period of incubation, the growth of the organism was identified by a characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

Methodology

The test sample was admixed with sterile distilled water and the mixture were been used for the sterility evaluation. About 1ml of the test sample was inoculated in a sterile Petri dish to which about 15 ml of molten agar 45°C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (About 10 minutes). Plates were then inverted and incubated at 37°C for 24-48 hours. Grown colonies of the organism were then counted and calculated for CFU.

Results and discussion

The macroscopic characterization of the *KaranthaiChooranam* (Table.1) was done for authenticfication and to assess the quality of this poly herbal sample.

Table 2: Shows the Organoleptic characteristic of *KaranthaiChooranam*

State	Solid
Appearance	Pale green
Nature	Moderately coarse powder
Odour	Characteristic
Flow property	Non- Free flowing

Solubility of the drug is the basic requirement for the absorption of the drug in the Gastrointestinal tract. The solubility of *KaranthaiChooranam* shows that it is soluble in Chloroform, ethanol, water and ethyl acetate (Table 2).

Table 3: Shows the solubility of K*aranthaiChooranam*

Solvent used	Solubility/dispensability
Chloroform	Soluble
Ethanol	Soluble
Water	Soluble
Hexane	insoluble
Ethyl acetate	Soluble
DMSO	Partially soluble

Table 4: Shows the physicochemical analysis of *KaranthaiChooranam*

Parameter	Mean (N=3) SD
Loss on Drying at 105 °C (%)	1.33 ± 0.15
Total Ash (%)	15.2 ± 0.72
Acid insoluble Ash (%)	0.59 ± 0.08
Alcohol Soluble Extractive (%)	16.67 ± 0.89
Water soluble Extractive (%)	9.63 ± 0.58

The Phytochemical screening of *KaranthaiChooranam* reveals the presence of Alkaloids, Steroids, Triterpenoids, Coumarin, Phenol, Tannin, Saponins, Sugar and Betacyanin (Table 5).

Table 5: Shows the Phytochemical screening of *KaranthaiChooranam*

TEST	OBSERVATION
Alkaloids	+
Flavonoids	-
Glycosides	-
Steroids	+
Triterpenoids	+
Coumarin	+
Phenol	+
Tannin	+
Protein	-
Saponins	+
Sugar	+
Anthocyanin	-
Betacyanin	+

Alkaloids, Steroids, Triterpenoids, Coumarin, Phenol, Tannin, Saponins, Sugar, Betacyanin.

Figure 1 shows Electron Microscopic Observation of Particle Size for the Test Sample- KC

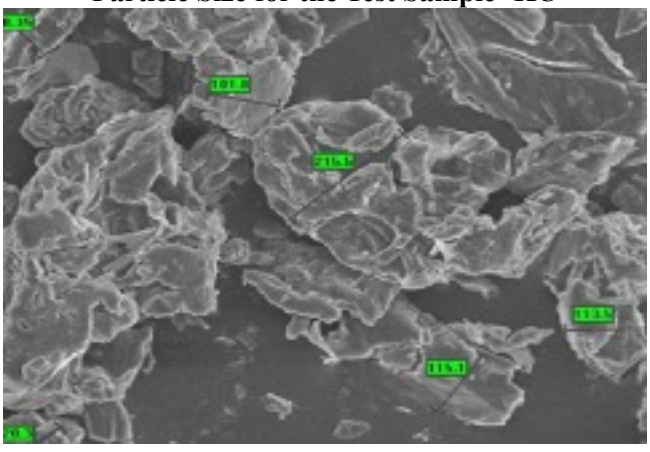


Table 6: Shows the particle size analysis of *KaranthaiChooranam*

Mean	115.5 µm
Std. Deviation	42.83 µm
Std. Error	15.14 µm

Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be 115.5 ± 42.83 µm.

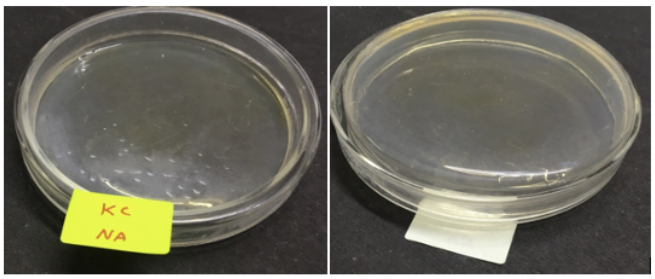
Table 7: Shows the Pesticide Test Result Analysis of the KC

Pesticide Residue	Sample KC	AYUSH Limit
I.Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below quantification Limit

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus and pyrethroids in the sample KC.

Figure 2 and 3 shows the Result of Sterility Test by Pour Plate Method



Observation Sterility Test

No growth was observed after incubation period. Reveals the absence of specific pathogen

Result Sterility Test

No growth / colonies were observed in any of the plates inoculates with the test KC.

Table 8: Shows of Result Sterility Test

Test	Result	Specification	As per AYUSH/ WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

Discussion

Traditional wisdom has claimed that herbal preparations are safe to use, and more and more people throughout the world are consuming them. It is critical to standardise herbal formulations in order to evaluate the drug's effectiveness, safety, and purity. India is recognized as a larger nation for its significant contribution to the production and standardization of the medicinal effects in herbal formulations. India is conducting more research into the use of herbal medicines, and this can only be done by evaluating and analysing herbal goods utilising a contemporary standardized process (20). The WHO also emphasized the significance of medicinal plants in the field of public health care, which directly contributes to the development of a country, and the origin of the guidelines for aiding state representatives in developing national policies in the area of traditional medicine for researching potential uses, including evaluation, safety, and efficacy (21).

The *KaranthaiChooranam* Organoleptic test revealed that it was pale green in colour, had a distinct odour, and taste. Touch exposes physical traits like the fineness and comparatively coarse powder of *Chooranam.*

Solubility is the primary requirement for a medication to be absorbed in the gastrointestinal system. According to studies on its solubility, *KaranthaiChooranam* is soluble in ethanol, water, chloroform, and ethyl acetate.

The alkalinity and acidity of a formulation are indicated by the pH value in a physico-chemical analysis. It is essential for medication absorption. Loss on drying of KC is 1.33 0.15 (%) at 105°C. 15.2 + 0.72 grams of ash were found overall. The ash that was insoluble in acid in KC was 0.59 0.08. Extractive with Solubility in Alcohol (%) = 16.67 ±0.89 this result indicates that there was no external contamination during manufacturing, which could have affected the drug's absorption. 9.63 ±0.58 is the water soluble extractive (%) (22).

The Phytochemical examination of *KaranthaiChooranam* reveals the presence of Alkaloids, Steroids, Triterpenoids, Coumarin, Phenol, Tannic Acid, Saponins, Sugar, And Betacyanin. Each of those is important in human medicine as well as an organism's natural defence (23). Recent developments in the research of conventional medications and their potential for drug discovery have both contributed to an upsurge in interest in bioactive natural compounds (Ethno pharmacology) (24). The size of the particles might affect how quickly a material dissolves. Smaller particles dissolve more quickly because they have a smaller surface area(25). This might apply to about one-sixth of the medications that are currently being developed (26, 27).The average particle size of the sample was determined to be 115.5 42.83 m by microscopic observation of the KC Particle Size Analysis. As a result of these practises, pesticide residues from agricultural fumigations, soil treatments, and pre- or post-harvest periods accumulate on medicinal plants. Organo chlorine, Organo phosphorus, and Pyrethroid residues were not detected in the sample KC, according to the findings.

Conclusion

From the above analysis, it can be concluded that the Physico-Chemical analysis, Phyto-Chemical Screening, Particle Size, Pesticide Residue, and Sterility test were results in evaluation of *KaranthaiChooranam*can be used is the reference standard for quality control/quality assurance purpose. It can also serve as an important source of information to ascertain the identity and to determine the quality and purity of the formulation in future studies.

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