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Evaluation of analytical specifications of siddha formulatory drug Karappan poosu ennai (Medicated oil)

Dr. M. Krishnayini[1], Dr. D.K. Soundararajan[2]

[1]PG Scholar, Department of Kuzhanthai Maruthuvam, GSMC, Palayamkottai, Tamil Nadu, India
[2]Professor and Head of The Department, Department Of Kuzhanthai Maruthuvam, GSMC, Palayamkottai, Tamil Nadu, India

Keywords

Siddha's system, Analytical, Karappan Poosu Ennai, external medicine,

Abstract

Siddha's system of medicine has unique ideas and principles towards disease and its management. It also has specific diagnostic and prognostic parameters. Siddha formulations mainly based on three major forms of medicinal products which are herbs, minerals, and animal products. For evidence purposes, there is an urge to perform scientific validation of our Siddha literature and medications. It will help to standardize Siddha formulations. For that, the analytical specification of Karappan Poosu Ennai which is one of the forms of external medicine (medicated oil) as per PLIM guidelines was evaluated and documented here.

1. Introduction

During childhood, children suffers a lot due to skin diseases. In our siddha literature the skin disease is described as the term called karappan. Our classical paediatric text Balavagadam classified it into 18 major types based on site and characterization of lesion. It affects children, when exposed to allergic agents whether it may be environment or food items. It is described as skin manifestations present in the form of erythema, wheals, papules, pustules, vesiculation, scaling and oozing.

One of the external application for karappan is Karappan Poosu Ennai (Medicated Oil). According to PLIM guidelines specific analytical parameters for medicated oil are available. In order to standardize the drug, those parameters are evaluated here.

2. Materials and Methods

2.1 Drug selection

The siddha formulation drug Karappan Poosu Ennai selected from the siddha paediatric literature Pillai Pini Maruthuvam PartII and this medication is applied externally for treating skin disease called karappan (all 18 types).

2.2 Ingredients of Karappan poosu ennai

This poly herbal formulation contains one fresh herb, raw drugs, the ingredients of the drug and its quantity are listed below in **Table 1.**

Table 1

S.No	Name	Botanical name	Family	Part used	Quantity
1.	Milagu	*Piper nigrum*	Piperaceae	Fruit	*8 g*
2.	Velluli (vellaipoondu)	*Allium sativum*	Alliaceae	Bulb	*8 g*
3.	Vasambu	*Acorus calamus*	Araceae	Rhizome	*8 g*
4.	Vengayam	*Allium cepa*	Alliaceae	Bulb	*17.5 ml*
5.	Nellivatral	*Phyllanthus emblica*	Euphorbiaceae	Fruit	*1 pidi*
6.	Poovarasam poo suttasaambal	*Thespesia populnea*	Malvaceae	Flower	*1 pidi*
7.	Aadutheendapaalai	*Aristolochia bracteolata*	Aristolochiaceae	Leaves	*Required amount*
8.	Gingelly oil				*500 ml*

2.3 Collection of plant materials

Plants are collected near Tirunelveli town and the raw drugs were brought from a well reputed raw drug store in Tirunelveli Town.

2.4 Identification and authentication of the drug

The herbarium of plants specimen and raw drugs were identified and authenticated by the Head of Department of Postgraduate Department of Gunapadam, Government Siddha Medical College, Palayamkottai. The sample specimen of each raw material and herbarium is stored in the PG Department of Gunapadam for future reference.

2.5 Purification of the raw drugs

Purification of raw drugs were done as per classical Siddha literature.

2.6 Preparation of the drug

Make the raw drugs into paste with the extract of *Aristolochia bracteolata* (Aadutheenda paalai) and mix it with gingelly oil and then boiled in the pan until the watery fractions gets evaporated. It is then filtered and then packed according to packaging requirement.

Picture 1 - Sample Description

2.7 Administration of the drug

Form of the medicine: Medicated Oil (Ennai)
Route of administration: External Application
Indication: 18 types of karappan

2.8 Analytical specifications of medicated oil

As per the guidelines of PLIM **(**The Pharmacopoeial Laboratory For Indian Medicine), Analytical Specifications Of medicated oil (ennai or thailam) includes Physiochemical Description, Test For Heavy Metals, Sterility Test (detecting microbial contamination), Identifications TLC/HPTLC, Test For Specific Pathogen , Pesticide Residue and Test For Afla Toxins.

In this article, all the analytical specifications except TLC/HPTLC are evaluated.
The analysis was done by Noble research solutions Pvt. Ltd., Chennai, India.

2.9 Physicochemical analysis of karappan poosu ennai

Physiochemical analysis includes Sample Description, Solubility Test, Determination Of Iodine Value, Determination Of Saponification Value, Acid, Determination Of Viscosity Value, Determination Of Refractive Index, Determination Of Weight Per ml, Acid Value, Peroxide Value. The analysis were done at Noble research solutions Pvt. Ltd., Chennai, India.

3. Results and Discussion

3.1. Sample Description

Table 2

State	Liquid
Nature	Slightly viscous
Odor	characteristic
Touch / Consistency	Greasy
Flow Property	Free flowing
Appearance	Dark yellowish brown

3.2 Solubility Profile

Table 3

S.No	Solvent Used	Solubility / Dispersibility
1	Chloroform	Soluble
2	Ethanol	Insoluble
3	Water	Insoluble
4	Ethyl acetate	Soluble
5	DMSO	Insoluble

3.3 Determination of Iodine value

About 20 gm of test sample was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 minutes. Followed by this about 25 ml of Wiji's solution was added in the same flask and shaken well. The flask was allowed to stand for 30 mins and refrigerated for an hour. About 10 ml of KI solution was added to this and titrated against 0.1 N Sodium thiosulphate solutions until the appearance of yellow colour. 1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue colour indicates end point. Repeat the above procedure without taking sample and note the corresponding reading for blank titration.

3.4 Determination of saponification value

About 2 gm of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added to the round bottomed flask. Repeat the same procedure without taking the sample for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.

3.5 Determination of Viscosity value

Viscosity determination were been carried out using Ostwald viscometers. Measurement of viscosity involves the determination of the time required for a given volume of liquid to flow through a capillary. The liquid is added to the viscometer, pulled into the upper reservoir by suction, and then allowed to drain by gravity back into the lower reservoir. The time that it takes for the liquid to pass between two etched marks, one above and one bellow the upper reservoir, is measured.

3.6 Determination of Refractive Index

Determination of RL was carried out using Refractometer.

3.7 Determination of Weight per ml

Weight per ml was determined using the comparative weight calibration method, in which the weight of 1ml of the base of the formulation was calculated and then weight of 1 ml of finished formulation were been calculated. The difference between weight variations of the base with respect to finished formulation calculated as an index of weight per ml.

3.8 Acid Value

Accurately 5 g of test sample was weighed and transferred into a 250 mL conical flask. To this, a 50 mL of neutralized alcohol solution was added. This mixture was heated for 10 min by heating mantle. Afterwards, the solution was taken out after 10 min and 1 or 2 drops of phenolphthalein indicator was added. This solution was titrated against KOH solution from the burette. The appearance of pink colour indicated the end point.

The volume of consumed KOH solution was determined and the titration of test sample was carried out in triplicate and the mean of the successive readings was used to calculate the acid-value of the respective sample by following expression.

Acid value = Titer Value X 0.00561X 1000 / Wt of test sample (g)

3.9 Peroxide value

5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Peroxide value = 10 (a-b)/w

The test report of the above analysis are mentioned in **Table 4**.

Table 4

S.No	Parameter	KPE
1	Viscosity at 50^{o}C (Pa s)	64.7912
2	Refractive index	1.66
3	Weight per ml (gm/ml)	0.08
4	Iodoine value (mg I2/g)	94.34
5	Saponification Value (mg of KOH to saponify 1gm of fat)	142.36
6	Acid Value mg KOH/g	1.0659
7	Peroxidase Value mEq/kg	6.16

3.10 Test for heavy metals

Heavy Metal Analysis evaluated by **Atomic Absorption Spectrometry (AAS).** It is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA240 Series in order to determine the heavy metals such as mercury, arsenic lead and cadmium concentrations in the test item**.**

Sample Digestion

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO_3.

The analysis report is detailed in **Table 5.**

Table 5

Name of the Heavy Metal	Absorption Max Λmax	Result Analysis
Lead	217.0 nm	24.56
Arsenic	193.7 nm	6.35
Cadmium	228.8 nm	1.24

BDL-Below Detection Limit

3.11 Sterility test (test for microbial contamination)

It was done by Pour Plate Method. Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{o}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^{o} C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU. The result shown in **Table 6** and it was observed that no growth/colonies in any of the plates inoculated with test sample.

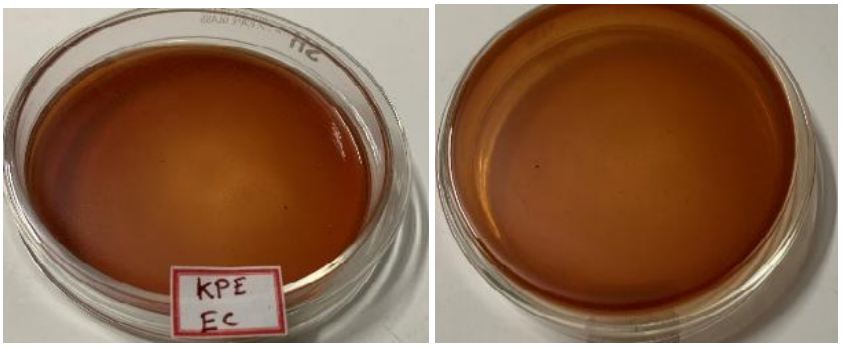
Table 6

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

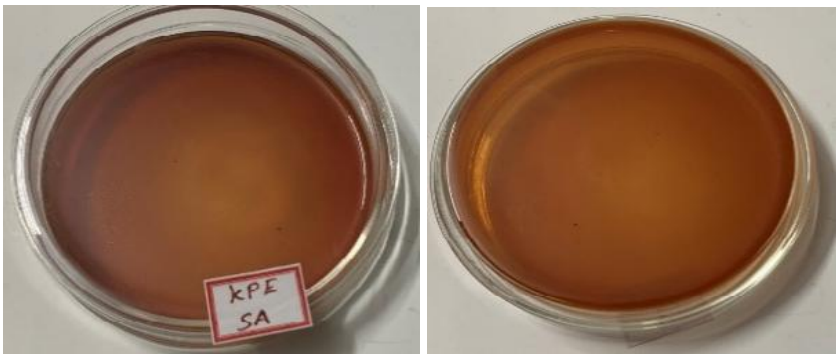
3. 12 Test for specific pathogen

Test sample was directly inoculated in to the specific pathogen medium (Eosin Methylene Blue Agar- *E.coli*, Deoxycholate Agar - *Salmonella*, Mannitol salt Agar-*Staphylococcus aureus*, Cetrimide Agar- *Pseudomonas aeruginosa*) by pour plate method. The plates were incubated at 37^{o}C for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media. It was observed that there is no growth after inoculation reveals that the absence of specific pathogen (shown in picture 1.1, 1.2, 1.3, 1.4)

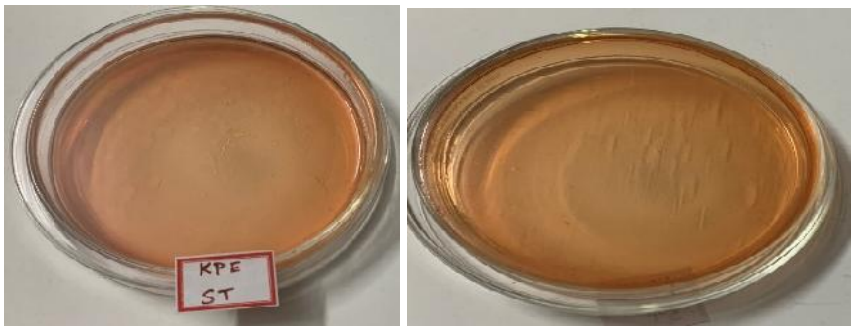
1.1 Culture plate with *E. coli* (EC) specific medium



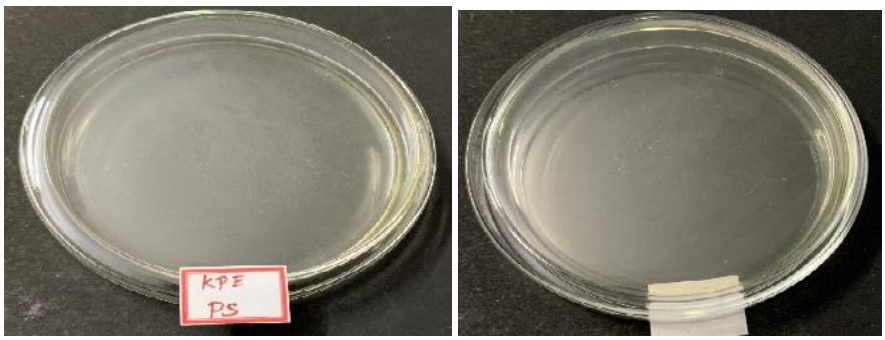
1.2 Culture plate with Salmonella (SA) specific medium



1.3 Culture plate with *Staphylococcus aureus* (ST) specific medium



1.4 Culture plate with *Pseudomonas aeruginosa* (PS) specific medium



3.13 Pesticide residue

Test sample were extracted with 100ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter. Result analysis of drug detailed in **Table 7** and it showed that there were no traces of pesticides residues such as Organochlorine, Organophosphorus, Organocarbamates and pyrethroids in the sample provided for analysis.

Table 7

Pesticide Residue I. Organochlorine Pesticides	Sample KPE	AYUSH Limit (mg/kg)
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. Organophosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2mg/kg
Dichlorovos	BQL	1mg/kg
III. Organocarbamates		
Carbofuran	BQL	0.1mg/kg
III. Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL-Below Quantification Limit

3.14 Test for aflatoxins

Standard aflatoxin was applied on to the surface to precoated TLC plate in the volume of 2.5 µL, 5µL, 7.5µL and 10 µL. Similarly, the test sample was placed and allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85:10:5) until the solvent front has moved not less than 15mm from the origin. Remove the plate from the developing chamber, mark the solvent form and allow the plate to air dry. Locate the spots on the plate by examination under UV light at 365 nm. The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compared to the standard which indicates that the sample free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 and it is detailed in **Table 8.**

Table 8

Aflatoxin	Sample KPE	AYUSH Specification Limit
B1	Not Detected-Absent	0.5ppm
B2	Not Detected-Absent	0.1ppm
G1	Not Detected-Absent	0.5ppm
G2	Not Detected-Absent	0.1ppm

4. Conclusion

Karappan Poosu Ennai satisfies most of the Analytical Specifications of The Medicated Oil as per PLIM guidelines on evaluation. It confirms the safety and quality of the drug. Evaluation of those analytical parameters with the help of modern analytical tools helps in standardization of siddha formulatory drugs. The information collected in this study will be the evidence for future reference.

5. References

1. India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
2. Indian standard methods of sampling and test for oils and fats Indian standard institution New Delhi 47-50. 1964.
3. Lohar. D.R. Protocol for testing of ASU medicines. Pharmacopoeial Laboratory for Indian Medicines, Ministry of AYUSH, 2007.
4. Luciana de CASTRO determining aflatoxins B1 B2 G1 and G2 in maize using florisil clean up with Thin Layer Chromatography and Visual and Densitometric Quantification. Cienc Tecnol Aliment Vol.21 No.1 Campinas 2001
5. Pharmacopeial Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems. Department AYUSH, Ministry of Health & Family Welfare, Govt. of India
6. Pillaipini Maruthuvam part II Department of Indian medicine and Homeopathy. First edition.
7. Pon. Guru Sironmani, Kuzhanthai Maruthuvam- Balavagadam, Dept Of Indian Medicine And Homeopathy
8. WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues. WHO Geneva. 2007.

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