Research Article

Standardization of Pungampoo Ennai for the Management of Balakarappan

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ABSTRACT

The Siddha system of medicine, an ancient therapeutic practice originating from South India, utilizes various diagnostic methods and treatments. One such treatment is Pungampoo Ennai, a polyherbal formulation described in the Siddha text, particularly used for the management of Bala Karappan, a pediatric skin disorder resembling atopic dermatitis. This study aims to standardize and evaluate Pungampoo Ennai through organoleptic, physicochemical, phytochemical, biochemical, and safety analyses. The formulation was prepared using purified ingredients, including Pungampoo, Pulinaralai Kizhangu, Vasambu, and others. The standardized product was subjected to several tests, including viscosity, refractive index, weight per ml, iodine value, saponification value, acid value, and peroxide value. The HPTLC fingerprinting revealed the presence of various phytochemicals, contributing to the drug's medicinal properties. Toxicological safety was ensured through the absence of heavy metals, pesticides, aflatoxins, and microbial contamination. The findings confirm the identity, purity, and quality of Pungampoo Ennai, providing a comprehensive reference for its safe and effective use in clinical applications. This study supports the use of standardized Siddha formulations as safe and effective therapeutic options in modern medicine.

Keywords: Herbal drug, Pungampoo Ennai, Standardization.

INTRODUCTION

Siddha system is an ancient system of medicine and it is originated from south India. The main advantage of Siddha system is to remove the root cause of the disease. The Eight-Fold Examination method (Envakai Thervukal) is used to determine the diagnosis, etiology, treatment and prognosis of the disease.

Kuzhandhai Maruthuvam is a specialized branch in Siddha system which deals with the treatment of children upto 12 years. Accordingly in Siddha system of medicine the lifetime of the child is divided into various stages of Paruvam (Milestones). As per the Siddha literature, Bala karappan is associated with the symptoms of erythema, oedema, intense pruritis, exudation, crusting and scaling. In the textbook of Kuzhandhai Maruthuvam (Bala vagadam), Bala Karappan is described as one type of Karappan among 18 types. The signs and symptoms of Bala karappan described in Siddha literatures nearly correlated with the signs and symptoms of Atopic dermatitis. The disease presents with eczematous, itchy lesions that show distinct distribution in different pediatric age groups, and episodes of clinical exacerbation called flares or flare-ups. AD affects 5%–20% of children.

Thailam are important group of formulations used by Siddha physicians to treat various types of disease. The principle is to extract the therapeutic compounds into oil. The thailam have the colour, taste and odour of the drugs used and consistency of oil. They are similar to ghirta, but are prepared with oil instead of ghee. Generally, they are used for external applications, some of them are also used internally.

I have chosen polyherbal Siddha formulations (Internal) to treat the above ailment from the authenticated Siddha literatures. The selected trial medicines are Pungampoo Ennai (internal) collectively indicated for all types of Karappan classified in the Siddha literature including Bala Karappan. Pungampoo Ennai is used to treat Balakarappan according to textbook reference of K. S. Murugesa Mudhaliyar, Dr. Pon. Guru sironmani, textbook of Balavagadam published by Indian System of Medicine and Homeopathy 2010 (pg no:373).

Standardization of siddha medicines will help to follow a protocol for usage of siddha medicine in a safe and effective way around worldwide. This present study is to standardize the organoleptic characters, physicochemical, phytochemical, biochemical analysis of Pungampoo Ennai. The outcome of this study may help to use as a standard reference for further studies performed in this study drug.

MATERIALS AND METHODS

The drug pungampoo ennai was prepared in the Gunapadam laboratory of National institute of siddha, Chennai, Tamilnadu, India. The standardization methods are carried out on standard research laboratory in Chennai.

Ingredients of Pungampoo Ennai:

1. Pungampoo
2. Pulinaralai kizhangu
3. Vasambu
4. Vellulli
5. Karunjchirakam
6. Kudasapalai pattai
7. Nannari ver
8. Amanaku Nei
9. Cow's milk

Collection of raw drugs:

The drug used in the preparation of Pungampoo Ennai were purchased from Ramaswamy chettiyar country drug shop kandhaswamy kovil street, Paris, Chennai. Raw drugs were authenticated by the Medical Botanist of National Institute of Siddha, Chennai – 47.

Purification of raw drugs:

All the ingredients will be purified as per the Siddha literature Sigicha Rathana Deepam, Published by ISM&H, Chennai, 2007. (edition 1) Purification of raw drugs and preparation of drug was done at the Department of Gunapadam, National Institute of Siddha, Chennai-47. Purification of raw drug was done as mentioned in Table 1.

Preparation of medicated oil:

All Purified the seven ingredients to be taken 10 gm each and grind with cow's milk to become as paste. 320 ml of cow's milk and 320 ml of castor oil mixed together in a vessel after that the already grounded paste to be added and boiled well to obtain the medicated oil form then filtered and stored in airtight container.

Dosage:

7 - 12 years - 16 ml (Uchikarandi) Indigation: Karappan

Organoleptic character

Colour, odour, taste and texture were noted, organoleptic evaluation of Pungampoo Ennai was carried out using standard techniques.

Physiochemical parameters:

All the physio- chemical parameters were carried out as per the methods mentioned in standard books. The parameters are as follows.

Determination of specific gravity

Fill the dry sp. gravity bottle with prepared samples in such a manner to prevent entrapment of air bubbles after removing the cap of side arm. Insert the stopper, immerse in water bath at 50OC and hold for 30 min. Carefully wipe off any substance that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side and quickly weigh. Calculate the weight difference between the sample and reference standard.

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 have been calculated. The difference between weight variations of the base with respect to finished formulation calculated as an index of weight per ml.

Determination of Refractive Index

Determination of RL was carried out using Refractometer.

Determination of Viscosity value

Viscosity determination have 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 below the upper reservoir is measured.

Determination of pH

One gram of the test drug was taken into a 100ml graduated cylinder containing about 50 ml of water. The cylinder was shaken vigorously for two minutes, and the suspension was allowed to settle for hours at 25ºC to 27ºC, then 25 ml of the clear aqueous solution was transferred into a 50 ml beaker and tested for pH using digital pH meter.

Determination of Iodine value

About 20 gm weight equivalent 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.

Determination of saponification value

About 2 gm weight equivalent of test sample was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions were 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 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.

Determination of Acid Value

Accurately 5 g of test sample was weighed and transferred into a 250 mL conical flask. To this, a 50ml 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 pink appearance 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 = Titter Value X 0.00561X 1000 / Wt. of test sample (g)

Determination of 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.

Calculation: Peroxide value = 10(a-b)/w

High Performance Thin Layer Chromatography Analysis (HPTLC)

HPTLC method is a sophisticated and automated selection technique derived from TLC. Pre coated HPTLC graded plates and auto sampler were used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. HPTLC is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. It offers high degree of selectivity, sensitivity and rapidity combined with single step sample preparation. In addition, it is a reliable method for the quantitation of nano grams level of samples. Thus, this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprints of phytochemicals which is suitable for confirming the identity purity of medicinal plant raw materials.

Chromatogram development

It was carried out in CAMAG twin trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366 nm. The data obtained from scanning was brought into integration through CAMAG software. Chromatographic fingerprint ewes developed for the detection of Phyto constituents present in each extract and Rf value were tabulated.

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencils. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro litre by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system.

Toluene: Ethyl Acetate: Acetic Acid (1.5:1:0.5). After the run plates are dried and were observed using visible light Short-wave UV light 254nm and light long-wave UV light 365nm

Heavy metal analysis:

Standard: Hg, As, Pb and Cd – Sigma

Methodology

Atomic Absorption Spectrometry (AAS) 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 AA 240 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.

Standard reparation

As & Hg- 100 ppm sample in 1mol/L HCl Cd & Pb- 100 ppm sample in 1mol/L HNO_3

Sterility test by plate method:

Objective

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

Methodology

Test sample was inoculated in 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 and further extended for 72 hrs for fungal growth observation. Grown colonies of organism were then counted and calculated for CFU.

Test for Specific Pathogen

Methodology

Test sample was directly inoculated into the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37°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

Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	*EC*	*EMB Agar*
Salmonella	*SA*	*Deoxycholate agar*
Staphylococcus Aureus	*ST*	*Mannitol salt agar*
Pseudomonas Aeruginosa	*PS*	*Cetrimide Agar*

Test for Organochlorine pesticide, organophosphorus pesticide and pyrethroids

About 10 g of test substance were extracted with 100 ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating 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 R and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Aflatoxin Assay by TLC Standard:

Aflatoxin B1

Aflatoxin B2

Aflatoxin G1

Aflatoxin G2

Solvent

Standard samples were dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Test solution: Concentration 1 µg per ml

Procedure

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5µL, 5 µL, 7.5 µL and 10 µL. Similarly, the test sample was placed and allowed 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 15 cm from the origin. Remove the plate from the

developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

Table 1: Purification of drugs

Pungampoo	Leaf stalk, calyx, pollen to be removed and the petals are taken.
Pulinaralai kizhangu	Wash well and remove the outer coat and the nerve.
Vasambu	Burn and make into charcoal.
Velluli	Remove the waste outer coating and remove the basal plate.
Karuncheeragam	It will be purified by removing sand particle and dried under sunlight.
Kudasapalai pattai	Clean and remove the outer coat.
Nannari vear	It will be washed by purified water and dried it.
Amanakku Ennai	One part of castor oil and two parts of tender coconut water is mixed and then boiled.

Table 2: Organoleptic characters

State	Liquid
Nature	Viscous
Odour	Characteristic
Touch	Greasy
Flow Property	free flowing
Appearance	Reddish Orange

Table 3: Physio-Chemical Evaluation

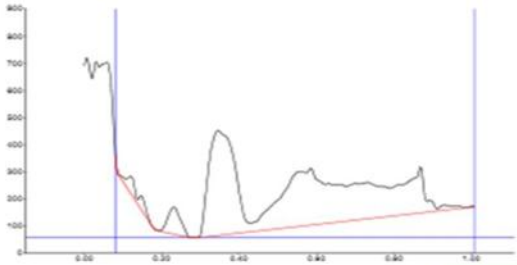
Parameter	PPE
Viscosity at 50°C (Pa s)	109.16
Refractive index	1.52
Weight per ml (gm/ml)	0.933
Iodine value (mg I2/g)	93.98
Saponification Value (mg of KOH to saponify 1gm of fat)	183.27
Acid Value mg KOH/g	1.009
Peroxidase Value mEq/kg	3.96

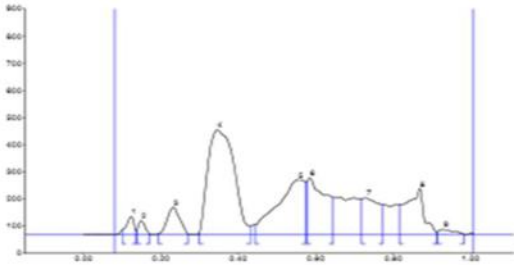
Table 4: Heavy metals/toxic metals results

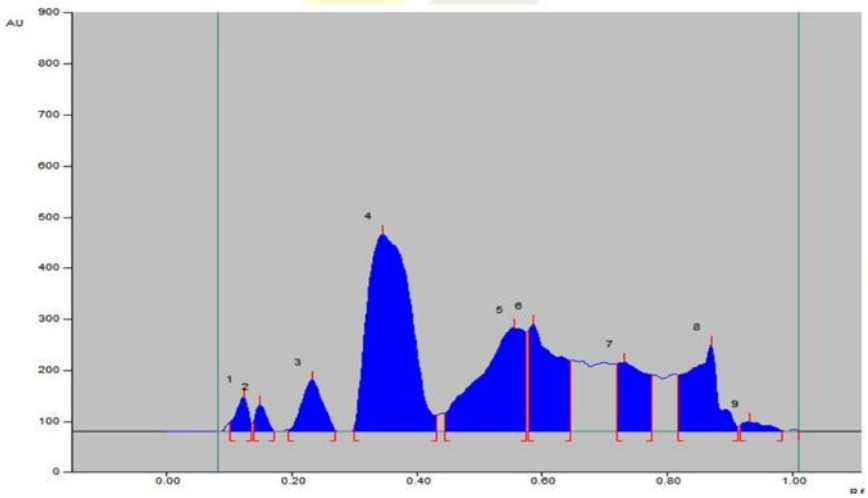
Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	0.660	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

HPTLC:

HPTLC finger printing of PPE







Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.10	18.7	0.12	67.3	5.02	0.14	14.0	592.1	1.81
2	0.14	17.6	0.15	51.5	3.84	0.17	0.8	405.2	1.24
3	0.19	2.9	0.23	101.1	7.54	0.27	1.8	1511.2	4.62
4	0.30	7.0	0.35	386.1	28.78	0.43	31.0	12029.5	36.81
5	0.44	35.5	0.56	202.0	15.06	0.58	194.1	6694.8	20.49
6	0.58	195.9	0.59	209.2	15.59	0.65	138.4	4599.9	14.08
7	0.72	132.2	0.73	134.8	10.05	0.78	111.7	2848.0	8.71
8	0.82	110.4	0.87	170.1	12.68	0.91	8.4	3655.5	11.19
9	0.92	9.4	0.93	19.3	1.44	0.98	1.9	344.8	1.06

REPORT

HPTLC finger printing analysis of the sample reveals the presence of nine prominent peaks corresponds to the presence of versatile phytocomponents present with in it. The major Rf value of the peaks ranges from 0.10 to 0.92.

TLC Visualization of PPE at 366 nm

3D – Chromatogram

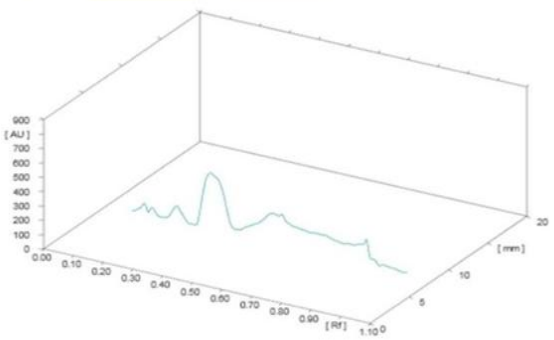


Table 5: Sterility test for pour plate method

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	

Observation: No growth was observed after incubation period reveals the absence of specific pathogen

Result: No growth / colonies were observed in any of the plates inoculates with the test sample.

Table 6: Test for specific pathogen:

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

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

Result: No growth / colonies were observed in any of the plates inoculated with the test sample.

Table 7: Pesticide residue results

Pesticide Residue	Sample PPE	AYUSH Limit (mg/kg)
II. Organo Phosphorus 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. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III. Pyrethroid		
Cypermethrin	BQL	1mg/kg

Table 8: Aflatoxin

Aflatoxin	Sample PPE	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
B2	Not Detected - Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected - Absent	0.1 ppm (0.1mg/kg)

DISCUSSION

The preclinical standardization of Pungampoo Ennai as mentioned in siddha textbook shows that the drug was reddish orange in colour, greasy consistency, The viscosity is 109.16c/pas, The acid value is used to quantify the substance, which is free fatty acid in fat due to enzymatic activity. The acid value of Pungampoo Ennai was found to be 1.009, The peroxide value is found to be 3.96 it indicates the good quality of the oil and the oil has long shelf life.

The refractive index is found to be 1.52 it interprets that there is no adulteration in the sample**.** The index of weight per ml is 0.933 gm/ml. The iodine value is 93.98 (mg I2/g) So

the trial drug Pungampoo Ennai is rich in Poly Unsaturated Fatty Acids (PUFA) which is helpful in reducing LDL cholesterol

The saponification value is 183.27 mh KOH/g to neutralize the fatty acids resulting from the complete hydrolysis of 1gm of sample. So, the saponification value is high percentage of Short Chain Fatty Acids (SCFA) in the sample drug which may improve colonic health and get easily absorbed and digested.

HPTLC finger printing analysis of the sample reveals the presence of nine prominent peaks corresponds to the presence of versatile phytocomponents present with in it. The major Rf value of the peak's ranges from 0.10 to 0.92. Further the peak 1 occupies the major percentage of area 40.10% and peak 5 occupies the second major percentage of area 20.63% which denotes abundant existence of such compound.

This study also reveals that the drug was sterile and free of bacteria, fungi and specific pathogen like Salmonella, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and pesticide residues. In heavy metal analysis there are no traces of Mercury and Arsenic. lead and cadmium in the sample seems to be Below Quantification limit.

There were no spots of Aflatoxin like B1, B2, G1, G2.

CONCLUSION

The result obtained from this study providing the fingerprint of the drug formulation Pungampoo Ennai ensures the identity, purity and quality of the prepared medicine. The result can be used as reference for further research in this drug formulation.

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