International Journal of Recent Advances in Multidisciplinary Topics
Volume 3, Issue 3, March 2022
https://www.ijramt.com | ISSN (Online): 2582-7839

Standardization of Siddha Herbomineral Formulation Irunelli Karpam

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***Abstract*: Siddha system of medicine is a renowned holistic system of traditional medicine emphasizing curative and preventive measures. The medicines used in siddha are of plant origin, metals, minerals and animal products. It is one of the major Indian systems of Medicine. The people were well in the physical and mental health with the Siddha medicines. Till date, lesser studies have seen conducted on standardization of such preparations. Irunelli Karpam, a traditional siddha herbo - mineral drug was prepared as per the procedure mentioned in siddha literatures. In the present study an attempt has been made to standardize Irunelli Karpam. According to PLIM guidelines, standardization of drugs conforms identity, determination of quality, purity and detection of adulteration by various parameters. The review article will help to provide details of information about physiochemical Screening, biological and pesticide screening (Aflatoxins, Sterility test by pour plate method test for specific pathogen).**

***Keywords*: Gandhagam (Sulphur), herbo mineral formulation, Irunelli Karpam**

1. Introduction

Siddha system of medicine is one of the oldest one from Dravidian culture. This system is mainly focused on food as medicine. 'Kaya Karpa' medicines find a special place in Traditional Siddha Medicine. Kayakarpam is also called as elixir science is unique and treasure of the siddha system. Kayam means body karpam means stone also known as life span of Brahma according to Hindu mythology. Hence, this medicine is one which makes human body as stone and not affected by any diseases or aging. These kinds of medicines are available from herbal preparation, metals and from animal products also. Many of siddhars such as sage Agathiyar and Bohar are written about in various literature. These medicines are preventive as well as cure the disease. The kayakarpam prevent the aging process as one of the actions is antioxidant property. The Irunelli karpam (INK) is made up of Indian gooseberry (nellikai) and sulfur (nelikkai ghanthakam) in equal quantity and prepared by grinding then drying the finished product. Iru nelli karpam, a herbo-mineral medicine containing Sulphur (Kanthagam) processed with the juice of Phyllanthus emblica (Nellikai) is widely used for treating skin disorders. Nellikai kanthagam and Nellikai are the two ingredients used in this preparation and hence the name, 'Iru Nelli Karpam'. Theriyar compares Kanthagam with a mother nurturing her child. Nellikai, one among the 'Thiripala' is a well-known Karpam. This is one of the kayakarpam medicines used for various types of skin diseases number. Sirangu (Scabies) is one of the important skin diseases being countered in day-to-day clinical practice. Scabies occurs worldwide regardless of age, sex, race, socio-economic status or standards of personal hygiene. Cyclical epidemics occur at intervals of 10 to 15 years. Outbreaks may frequently occur in childcare centres and kindergartens, and are also reported in nursing homes and institutions. Scabies is more likely to spread in situations of overcrowding. The Siddha medicine, 'Iru Nelli Karpam' has been advocated for the treatment of Scabies often with very good results. The preparation of medicine on the basis of narrated in the siddha literature – The Siddha formulary of India part I first edition (English version) page no 6.

2. Materials and Methods

The siddha drug Irunelli Karpam was selected from a classical Siddha literature.

Table 1
Ingredients of Irunelli Karpam

Drug	Chemical name/botanical name	Quanitity
Purified Gandhagam	Sulphur	1 part
Nellikai saru	*phyllanthus emblica*	2 parts

- Dosage: 200 mg, twice a day.
- Vehicle/ adjuvant: Ghee

The literature evident shows the INK medicines used for treat various types of skin diseases especially Sori, Sirangu.

1) Collection, Identification and Authentification of the drug

The required herbo mineral drugs were purchased from a well reputed Siddha drug store. The drugs are identified and

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authenticated by Department of Gunapadam, Government Siddha Medical College & Hospital, Palayamkottai.

2) Methods of Purification and Preparations

Sulphur purified as per the evidence mentioned in the yaagobu vaiththiyam. The preparation of medicine on the basis of narrated in the siddha literature – The Siddha formulary of India part I first edition (English version) page no 6. The trail drug is prepared from the grind Purified Gandhagam with Nellikai saru in small quantities. Dry and powder. Finally, end material store in air tight glass container.

3. Physiochemical Screening

Table 2
Physicochemical analysis of Irunelli Karpam

Parameters	Total ash	Values
Ash value	Water soluble ash	9.90±0.020
	Acid insoluble ash	1.80±0.020
Extractive value	Water soluble extractive value	9.90±0.200
Loss on drying	Loss on drying at 110˚C	1.20±0.500
Ph		7.10

Colour: Green

1. Loss on drying at 1050C Nil
2. Total ash 92.38%
3. Water soluble ash 33.51%
4. Acid soluble ash 46%

1) Percentage loss on drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105oC for 5 hours and then weighed.

2) Determination of total ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

3) Determination of acid insoluble ash

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

4) Determination of water-soluble extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and 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 weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

5) Determination of pH

About 5 g of test sample INK will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and then subjected to pH evaluation.

4. Biological and Pesticide Residue Screening

Sterility Test by Pour Plate Method

1) 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).

2) 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. (Above 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.

3) Examination of the sample

Determine the total aerobic microbial count in the substance being examined by any ofthe standard methods

4) Plate count

For bacteria– Using Petridishes 9 to 10c mindia meter,add to each disha mixture of 1 ml of the pretreated preparation and about 15 ml of liquefied casein soyabean digestagar at not more than 45º. Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30º to 35º for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300colonies per plate as the maximum consistent with good evaluation.

For fungi – Proceed as described in the test for bacteria but use Sabouraud dextrose agar with antibiotics in place of casein soyabean digest agar and incubate the plates at 20ºto 25º for5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than100 colonies.

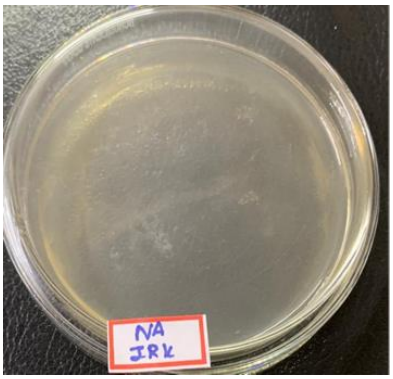

Fig. 1. Sterility Test

5) Observation

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

6) Result

No growth / colonies were observed in any of the plates

inoculates with the test sample.

Table 3
Sterility Test (Total Microbial Load Specification & Result).

Test	Result	Specification	As per ayush/who
Total Bacterial Count	Absent	NMT 105CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 103CFU/g	

B. Limits for ASU products

Table 4
Standard Limits of Microbial screening

Parameter	Specifications
Total Bacterial count	$1x10^5$CFU/gm (CFU-ColonyFormingUnit)
Total fungal count	$1x\ 10^3$CFU/gm

C. Test for specific pathogen

1) Methodology

Test sample was directly inoculated in to the specific pathogen medium (EMB, DDC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37°C for 24 – 72 hrs for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation in each differential media.

2) Test for Specific Pathogen

Table 5
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

3) Observation

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

5. Result

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

Table 6
Test For Specific Pathogen (Specification & Result)

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

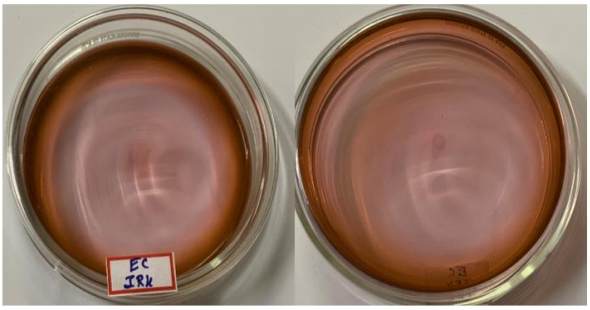

Fig. 2. Culture Plate With E-Coli (EC) Specific Medium

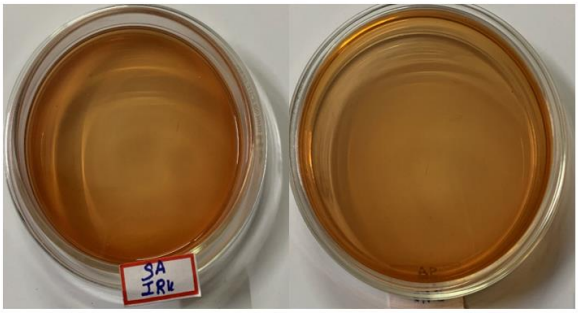

Fig. 3. Culture plate with Salmonella (SA) specific medium

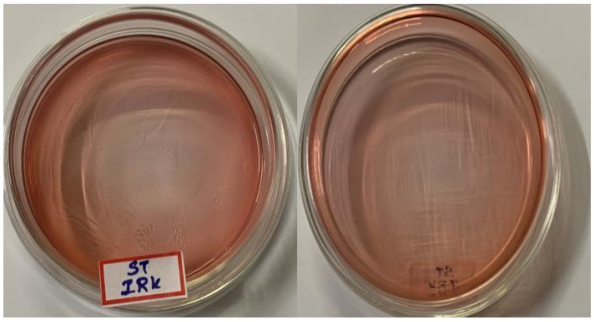

Fig. 4. Culture plate with Staphylococcus Aureus (ST) specific medium

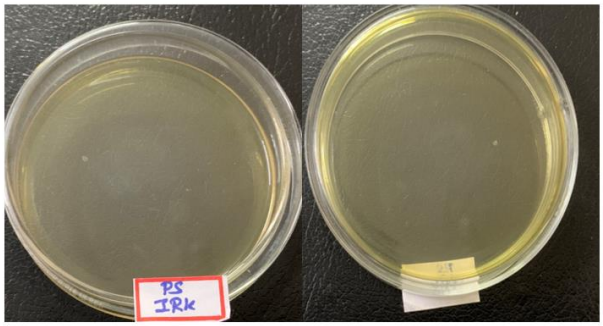

Fig. 5. Culture plate with Pseudomonas Aeruginosa (PS) specific medium

A. Test for aflatoxins

1) Standard

- Aflotoxin B1
- Aflotoxin B2
- Aflotoxin G1
- Aflotioxin G2

2) Solvent

Standard samples was 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 aflotoxin B1and aflotoxin G1 and 0.1µg per ml each of aflatoxin B2 and aflotoxin G2.

3) Procedure

Standard aflotoxin was applied on tothesurface to precoatedTLCplate in the volume of 2.5 µL, 5 µL, 7.5µLand 10 µL. Similarly, the test sample eas 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 (85:10:50) 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 7
Aflatoxin Of the Irunelli Karpam (INK)
Sample – ID:Irunelli Karpam – INK

Aflatoxin	Sample Ink	Ayush Specification Limit
B1	Not Detected – Absent	0.5 ppm
B2	Not Detected - Absent	0.1 ppm
G1	Not Detected - Absent	0.5 ppm
G2	Not Detected - Absent	0.1 ppm

4) Result

The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

B. Limits of ASU product

Table 8

Aflatoxin	**Specifications**
B1	0.5 ppm
B2	0.1 ppm
G1	0.5 ppm
G2	0.1 ppm

C. Pesticide residue screening

1) Test for pesticides: Quantitative analysis

2) Parameters:

- Organochlorine pesticides
- Organophosphorus pesticides
- Organo carbomates
- Pyrethroid insecticides

3) Extraction

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 evaporater at a tempreture not exceeding 40oC until the solvent has almost completely evoporated. 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 throught membrane filter.

Table 9
Standard Limits of Pesticides as per AYUSH

4) LIMITS According to WHO

Parameter	**Specifications**
Quinolphos	0.01ppm
DDE	1.00 ppm
Aldrin	0.05 ppm
Dieldrin	0.05 ppm
DDT	1.00 ppm
DDD	1.00 ppm
HCH	0.30 ppm

Table: Standard Limits of Pesticides as per WHO

5) Test for pesticide residue

Sample – ID: Irunelli Karpam – INK

Table 10
Test Result Analysis of The Sample INK

Pesticide Residue	**Sample**	**AYUSH Limit**
I.Organo Chlorine Pesticides	**KSSP**	**(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.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
IV. Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

D. Result

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

6. Conclusion

The achieved results of physiochemical, Biological Screening, sterility test, test for specific pathogen, aflatoxin and pesticide residue will be useful tool for standardization and quality control assessment of the hero mineral formulation. It proves that the above trial drug was safety drug even though it contains mineral compounds to use as inter- nal medicine. furthermore, pharmacological studies accomplish the medicinal value of the drug.

Acknowledgement

Researcher sincere thanks to supervisor and head of the department and colleagues who were guide me in this research work.

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