Int. J. Curr. Res. Med. Sci. (2022). 8(5): 14-25

International Journal of Current Research in Medical Sciences

ISSN: 2454-5716

(A Peer Reviewed, Indexed and Open Access Journal)

www.ijcrims.com

Original Research Article

Volume 8, Issue 5 -2022

DOI: http://dx.doi.org/10.22192/ijcrms.2022.08.05.003

Standardization of Suringiyathi Chooranam – A siddha herbomineral formulation

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Abstract

Background:

Siddha system of medicine is one of the ancient traditional systems of medicine in southern part of India and practice for treating various diseases including chronic conditions also. In this system there are many Siddha formulations have been mentioned, out of them Suringiyathi Chooranam (SGYC) one of the medicines which is mentioned in Anupoga Vaithya Thevaragasiyum, for the treatment of Sinusitis (Azhal thalainokkadu). Among this, poly herbal and herbomineral formulation has gained great importance and rising global attention recently. Now a days the traditional formulation of medicine need to standardization on the basis of Physiochemical analysis and Phytochemicals analysis. This paper is an attempt to describe the standardization and efficacy of the drug Suringiyathi Chooranam.

Methods:

The drug was screened for physiochemical, phytochemical analysis and HPTLC to estimate the quality of the drug.

Results:

The achieved results of physico-chemical, TLC profiling, HPTLC finger print profiling will be useful as tools for authentication and standardization profile of the herbomineral formulation.

Conclusion:

The reported results will be supportive for standardization and future studies of SGYC.

Keywords: Siddha medicine, Azhal thalainokkadu, Suringiyathi Chooranam, phytochemical activity, HPTLC.

Introduction

Siddha is one of the traditional medicinal systems with an established history of many countries paiticularly in India. This system of Medicine is one of the oldest healing sciences based on vadham, Pitham and Kabam that has been created by siddhars. Though it is curable but this system of medicine is still trying to prove their drug effectiveness through the standardization methods. In this study suringiyathi Chooranam was selected and screened for standardization methods as per the procedure the medicine is composed of 11 herbs such as *Terminalia*

chebula (Flower & Fruit), *Zingiber officinale, Piper longum, Piper nigrum, Terminalia bellerica, Phyllanthus embilica, Solanum surattense, Clerodendrum viscosum, Costus speciosus, Nardostachys jadamasi,* and one mineral drug that is *Sodium chloride impura*(Induppu), these drug possess the anti inflammatory ,anti microbial ,analgesic and antioxidant property. It is effective against in the treatment of sinusitis (Azhal thalinokkadu).It is the inflammation of the sinuses, which are air filled cavities in the skull. It can be acute or chronic. Types of sinuses are maxillary, frontal, ethmoid and sphenoid. Etiology of sinusitis can arise from both infectious and non infectious. Infectious etiologies included viral, bacterial and fungal. Noninfectious etiologies are allergic rhinitis (with either mucosal or polyp obstruction), barotraumas (deep sea diving or air travel), exposure of chemical irritants. Common symptoms of sinusitis include nasal congestion, nasal discharge (thick, purulent or discolored nasal discharge, facial pain or pressure (particularly at supra and infra orbital region) with associated referred pain to the ears and teeth, headache, cough, sneezing, fever, fatigue, nausea, hyposmia or anosmia, halitosis.

Materials and Methods

2.1Selection of drug:

The drug Suringiyathi Chooranam was collected from the classical Siddha literature.

Physio– chemical analysis

Fig 1: Physio– chemical analysis

2.2 Collection and authentication of the drug:

The raw materials included in the formulation are *Terminalia chebula* (Flower & Fruit), *Zingiber officinale, Piper longum, Piper nigrum, Terminalia bellerica, Phyllanthus embilica, Solanum surattense, Clerodendrum viscosum, Costus speciosus, Nardostachys jadamasi, Sodium chloride impura* (Induppu) were procured from the country drug shop at Chennai , Tamilnadu. They were identified and authenticated by the Botanist, Govt. Siddha medical college, Arumbakkam,Chennai-106.

2.3Purification of drug:

The purification of drugs was done by procedures mentioned in Siddha literature. This Suringiyathi Chooranam is herbomineral medicine .the aim of this study was carried out to standardized the drug Suringiyathi Chooranam by evaluating physiochemical properties.

2.4 Preparation of the drug:

Kadukai poo , Chukku , Thippili , Milagu , Kadukaai , Thaandrikai , Nellivatral , Kandangathriver, Kanduparangi,Kostam, Sadamanjil, Indhuppu are taken in the equal quantity and made in to fine powder. The powder were stored in the clean air tight container.

Table 1: Physio– chemical analysis

State	Solid
Nature	Fine
Odour	Characteristic
Touch	Soft
Flow Property	Non Free flowing
Appearance	Brownish

Table 2: Solubility Profile

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

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then weighed.

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.

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.

Determination of Alcohol Soluble Extractive

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

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.

Table 3: Final Test report of Physio– chemical analysis

S.No	Parameter	Mean (n=3) SD
1.	***Loss on Drying at 105 °C (%)***	6.833 ± 0.4933
2.	***Total Ash (%)***	14.07 ± 1.365
3.	***Acid insoluble Ash (%)***	0.01833 ± 0.01185
4.	***Water soluble Extractive (%)***	18.73 ± 2.608
5.	***Alcohol Soluble Extractive (%)***	6.367 ± 0.9504

Phytochemical Analysis

Test for alkaloids:

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, 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 color.

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 color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color 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 few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

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

Test for Cyanins

A. Aanthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. 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 Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

Results

Fig 2: Qualitative Phytochemical Investigation

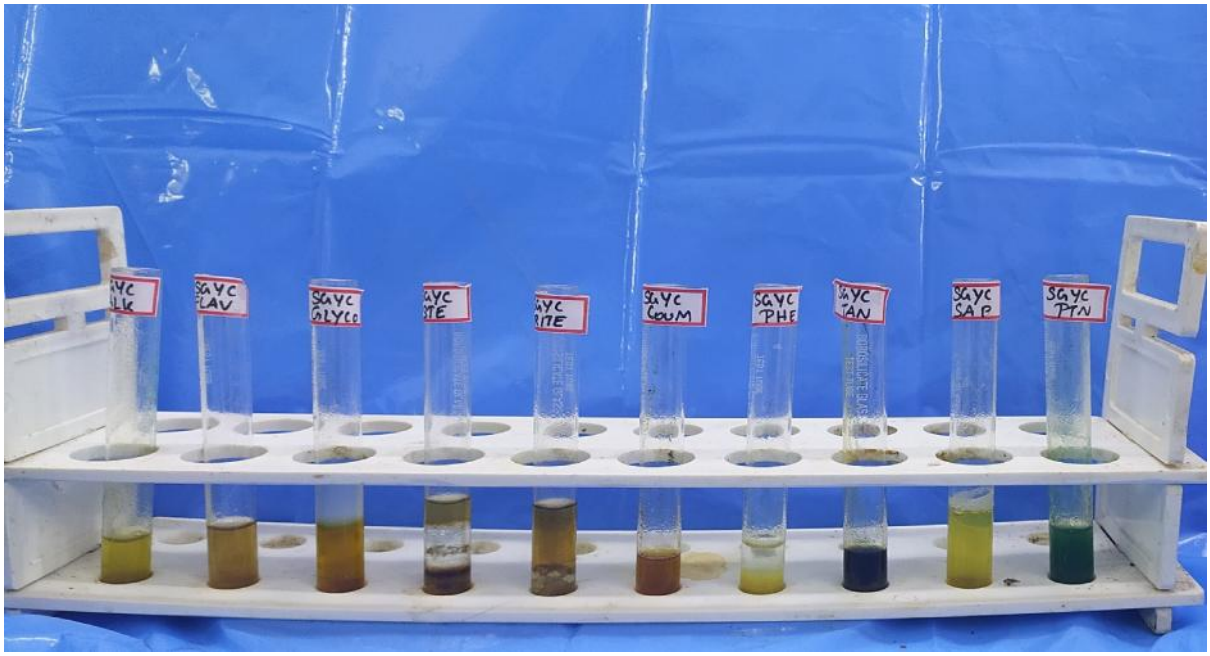


Table 4:Phytochemical Analytical Report

S.NO	Test	Observation
1	ALKALOIDS	+
2	FLAVANOIDS	+
3	GLYCOSIDES	+
4	STEROIDS	_
5	TRITERPENOIDS	+
6	COUMARIN	+
7	PHENOL	+
8	TANIN	+
9	PROTEIN	_
10	SAPONINS	+
11	SUGAR	+
12	ANTHOCYANIN	_
13	BETACYANIN	_

(+) –> Indicates Positive and (-) –> Indicates Negative

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 pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm.

High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics.

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 analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

Fig 3:TLC Visualization of SGYC at 366 nm

Fig 4: 3D – Chromatogram

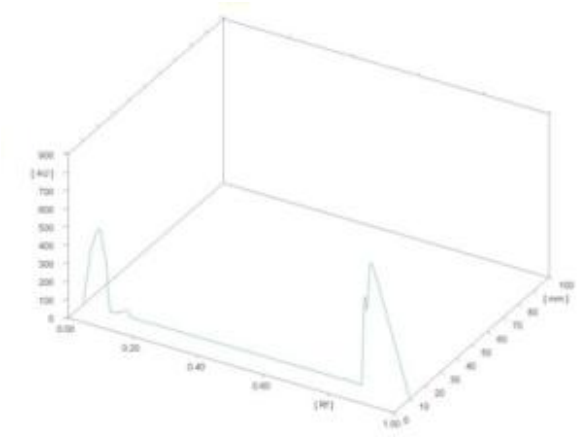


Fig 5: HPTLC finger printing of Sample SGYC

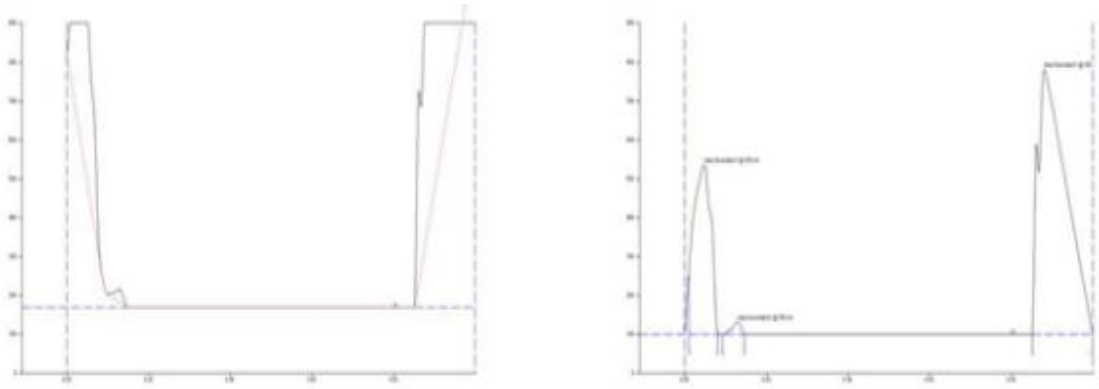


Table 5: HPTLC Peak table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	156.5	0.05	437.4	37.90	0.08	2.2	11826.7	28.35
2	0.09	0.0	0.13	33.7	2.92	0.15	1.1	449.9	1.08
3m	0.85	0.0	0.88	683.0	59.18	1.00	0.0	29434.5	70.57

REPORT:

HPTLC finger printing analysis of the sample reveals the presence of three prominent peaks corresponds to presence of three versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.01 to 0.85 .

Methodology for heavy metals analysis

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 determination 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 HNO3

Standard reparation

As & Hg- 100 ppm sample in 1mol/L HCl
Cd & Pb- 100 ppm sample in 1mol/L HNO3

Table 6: Heavy metals analysis

Name of the Heavy Metal	Absorption MaxΛ max	Result Analysis	Maximum Limit
Lead	217.0 nm	5.21 PPM	10 ppm
Arsenic	193.7 nm	0.72 PPM	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.34 PPM	1 ppm

BDL- Below Detection Limit

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metals Cadmium, whereas the sample shows the presence of Lead at 5.21 ppm, Arsenic at 0.72 ppm and Mercury at 0.34 ppm.

Methodology for pesticide:

Test sample 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 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.

Results:

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.

Methodology of aflatoxin:

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5µL, 7.5 µL 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 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.

Aflatoxin sample KMM AYUSH specifications 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

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, AflatoxinB2, Aflatoxin G1, Aflatoxin G2.

Test Report

Test for Specific Pathogen

Methodology

Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) 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 color with respect to pattern of colony formation in each differential media.

Table 7: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***

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 8: Absence of 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	

Fig 6:*Culture plate with E-coli (EC) specific medium*

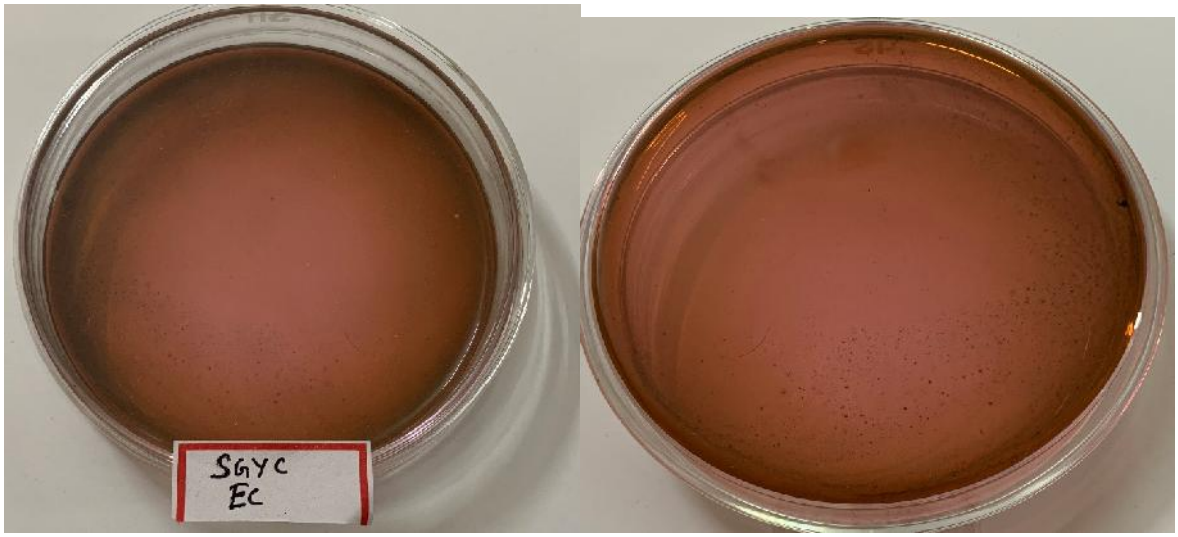


Fig 7:*Culture plate with Salmonella (SA) specific medium*

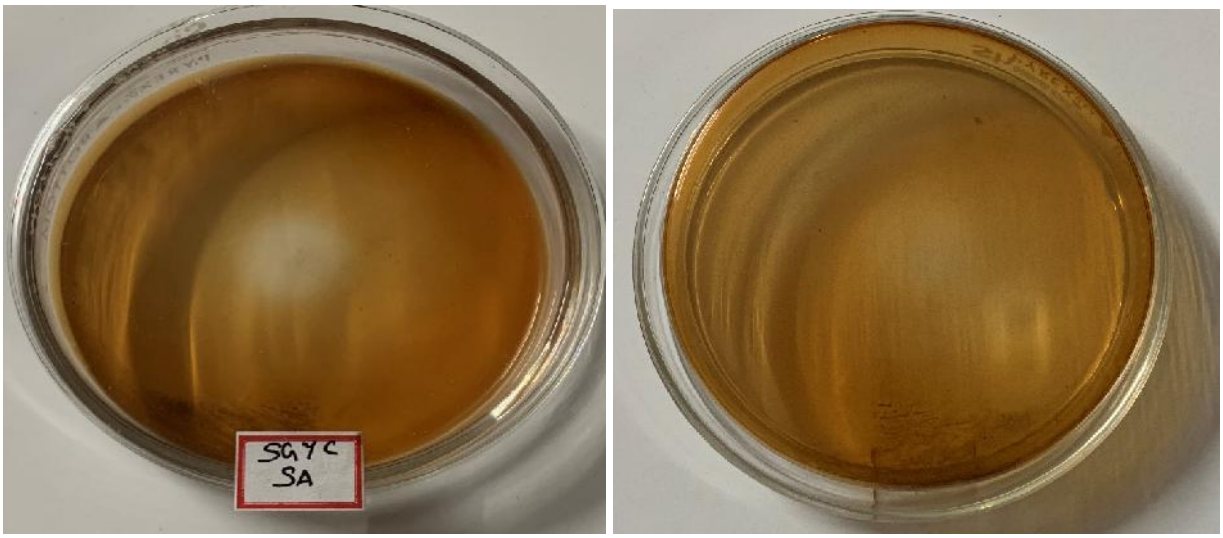


Fig 8:*Culture plate with Staphylococcus aureus (ST) specific medium*

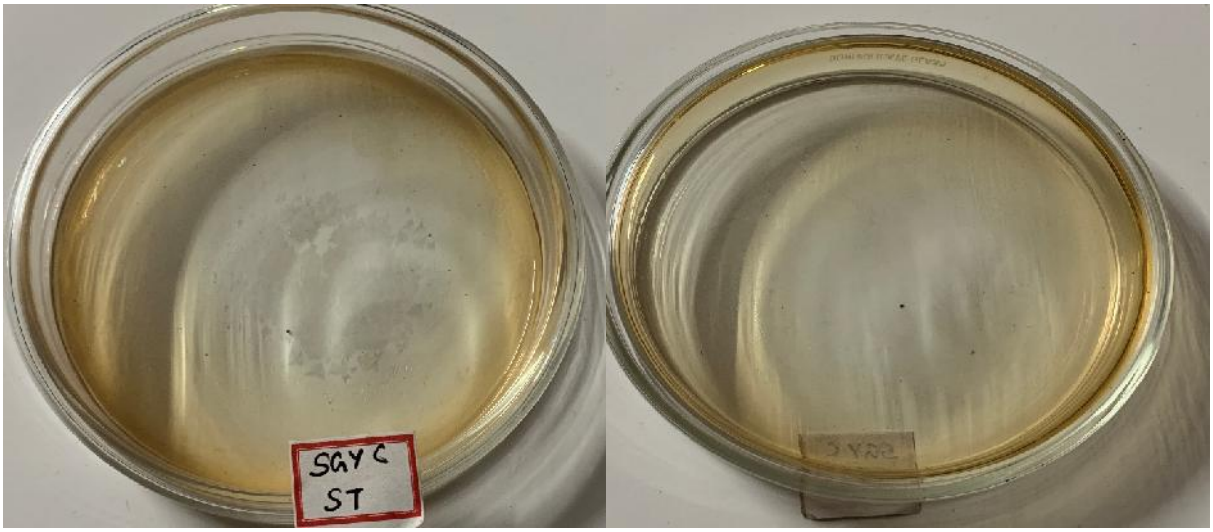
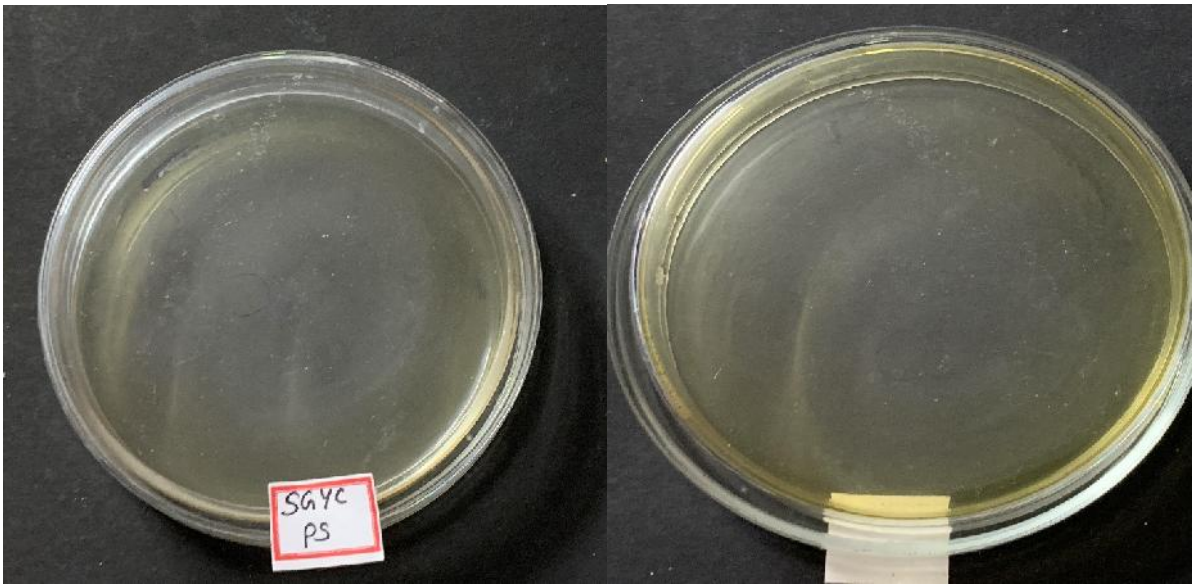


Fig 9:*Culture plate with Pseudomonas aeruginosa (PS) specific medium*



Sterility test by pour plate method

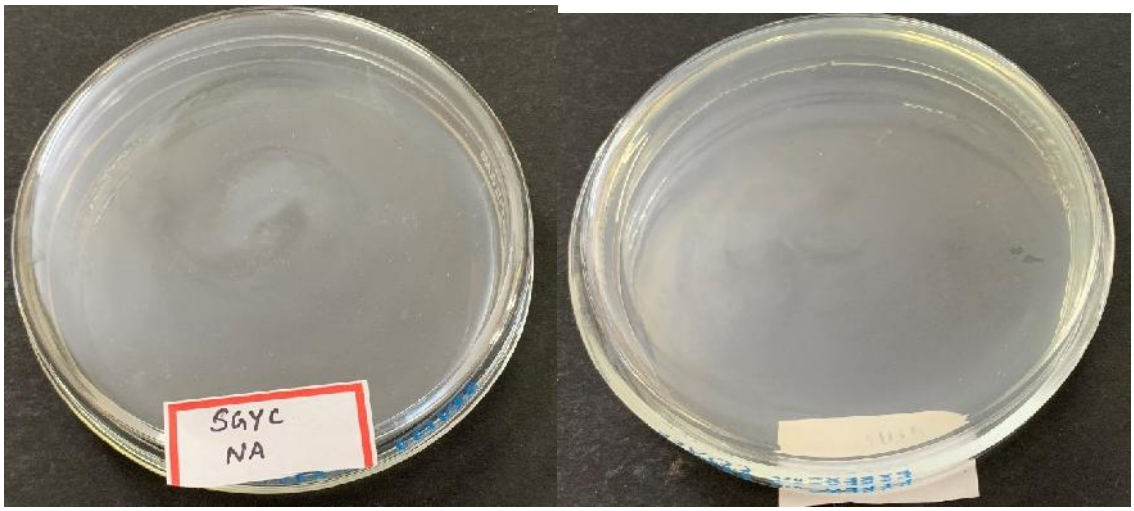
Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile 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^{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.

Fig 9: Sterility test by pour plate method



Observation

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

Result

No growth / colonies was observed in any of the plates inoculates with the test sample.

Table 9: 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

The results obtained from standardization and physiochemical analysis clearly reveals that the loss on drying value was 6.83%, total ash value was 14.07%, and acid insoluble ash is 0.018%.The alcohol soluble extractive value was 6.37%and water soluble extractive was 18.73%.we need to study the medicinal uses and phytochemical and bioactivity analysis to prove their therapeutic properties. The phytochemical analysis indicates the formulation of Suringiyathi Chooranam contains alkaloids, flavanoids, coumarins, saponins, tannins, glycosides, phenol, carbohydrate. HPTLC finger printing analysis of the sample reveals the presence of three prominent peaks corresponds to presence of three versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.01 to 0.85 . The sample has no traces of heavy metals metals Cadmium, where as the sample shows the presence of Lead at 5.21 ppm, Arsenic at 0.72 ppm and Mercury at 0.34 ppm. 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. 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. It was observed form the results of In-vitro anti-microbial assay that the formulation SGYC possesses significant antimicrobial activity against E.coli, Solmonella. Staphylococcus aureus, Pseudomonas aeruginosa. Hence this paper is an attempt that was made to evaluate & standardize surigiyathi chooranam by identifying the ingredients with using chemical parameters such as physico-chemical parameters, preliminary phytochemical analysis, TLC photo documentation & HPTLC finger printing profile. It is also useful in further reference of medicinal plants by going through the Siddha texts and related articles.

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How to cite this article:
Jayasri JM, Chitra U, Anbu N. (2022). Standardization of Suringiyathi Chooranam – A siddha herbomineral formulation. Int. J. Curr. Res. Med. Sci. 8(5): 14-25.
DOI: http://dx.doi.org/10.22192/ijcrms.2022.08.05.003