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Research Article

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Standardization of Samuthara chooranam – A siddha herbomineral formulation

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Keywords

Siddha medicine,
Maha vatham,
Samuthara Chooranam,
phytochemical activity,
HPTLC.

Abstract

Background
The Siddha system of medicine is an ancient system of Indian medicine that is practiced in Tamilnadu of Southindia and in other tamil speaking regions of the the world. Siddha system of medicine focuses on addressing the root cause of the diseases rather than treating the diseases and symptoms. There are lot manuscript and sastric medicine preparations are available in this system ,out of them Samuthara Chooranamone of the medicines which is mentioned inKosaye Anuboga Vaithiya BrahmaRagasiyam, for the treatment of Irritable bowel syndrome(IBS)(Maha vatham). 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 Samuthara 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.

Introduction

The Siddha system of medicine is an ancient system that is practiced in Tamilnadu of South India and in other Tamil speaking regions of the world. Siddha system refers some kind of practices and life style habits which can prevent death, and leads to attains divinity. Siddhars consider diseases are the barriers to attain the divinity . So they create medicines for these diseases.Siddha system of medicine focuses on addressing the root cause of the diseases rather than treating the diseases and symptoms. The preparation of medicine for this system, is the combination of herbs , medicinal plants, metals ,minerals , animal products and marine resources go on to make the required drug. These methods are practiced by siddhars not only for prevent the death but also for creates rejuvenation and longitivity of life. These special types of methods like KAYAKARPAM, MUPPU, ATTANGANGAYOGAM, RASAVATHAM.

They create "PINI ANUGA VITHI", which plays important role in preventing diseases and make fast recovery from the diseases. Though it is curable but this system of medicine is still trying to prove their drug effectiveness through the standardization methods. In this study Samuthara Chooranam was selected and screened for standardization methods as per the procedure the medicine is composed of 6 herbs such as *Zingiber officinale* , *Piper longum* ,*Terminalia chebula* , *Carum copticum* ,*Emblica ribed, Ferula asafoitita,* and three mineral drug such as *Sodium chloride impura,* otherwise known *Rock salt(Inthuppu), Sodium chloridum impura (Kalluppu)* , *Potassium carbonas impure (Yavacharam).* These drug possess the anti spasmodic ,anti diarrheal property. It is effective in the treatment of Irritable bowel syndrome (Maha Vatham). Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by abdominal pain or discomfort and altered bowel habit in the absence of detectable structural abnormalities.IBS is a disorder that affects all ages especially women are diagnosed 2-3 times more than men. In Rome III Criteria for the diagnosis of IBS were revised. The Rome III Criteria is, Recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months associated with two or more of the followings

1. Improvement with defecation
2. Onset associated with a change in frequency of stool
3. Onset associated with a change in form (appearance) of stool.

Materials and Methods

Selection of drug:

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

Collection and authentication of the drug:

The raw materials included in the formulation are *Zingiber officinale* , *Piper longum* ,*Terminalia chebula* , *Carum copticum* ,*Emblica ribed* , *Ferula asafoitita,* and three mineral drug such as *Sodium chloride impura* ,otherwise known *Rock salt(Inthuppu)* ,*Sodium chloridum impura (Kalluppu)* , *Potassium carbonas impure (Yavacharam)* 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.

Purification of drug:

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

Preparation of the drug:

Inthuppu , Kalluppu , Yavacharam , Inji, Thippili , Kadukkai,Omam,Perungayam , Vaividangam were taken in the equal quantity and made in to fine powder. The powder were stored in the clean air tight container.

Physicochemical Evaluation

Physiochemical analysis:

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

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°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.

Final Test report

S.No	Parameter	Mean (n=3) SD
1.	*Loss on Drying at 105 •C (%)*	4.5 ± 0.9
2.	*Total Ash (%)*	11.5 ± 0.9539
3.	*Acid insoluble Ash (%)*	0.2233 ± 0.08737
4.	*Water soluble Extractive (%)*	26.73 ± 4.957
5.	*Alcohol Soluble Extractive (%)*	10.8 ± 0.5196

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

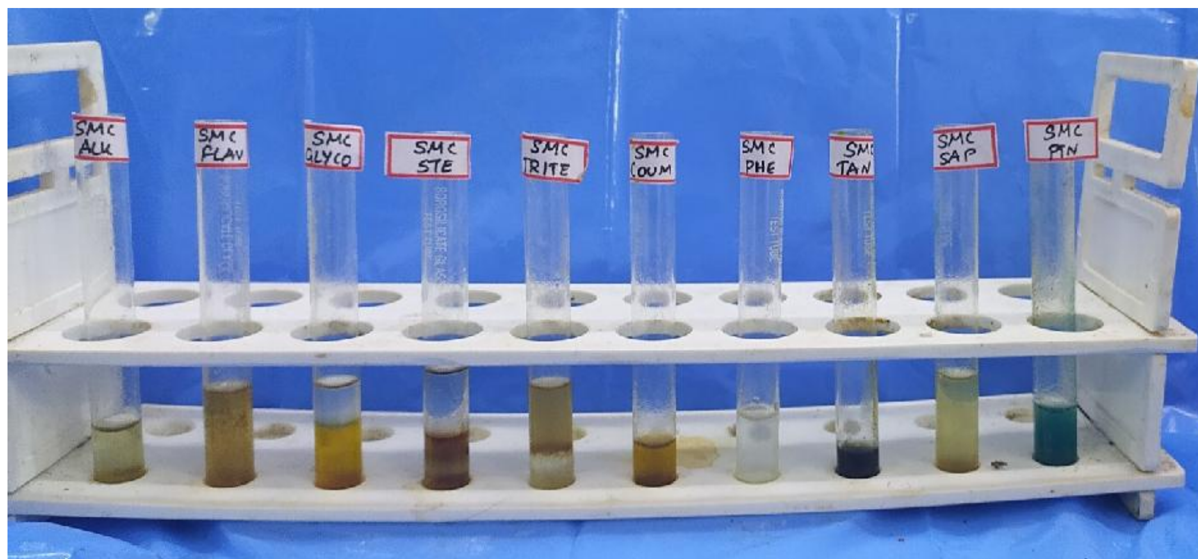
To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

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

Qualitative Phytochemical Investigation



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

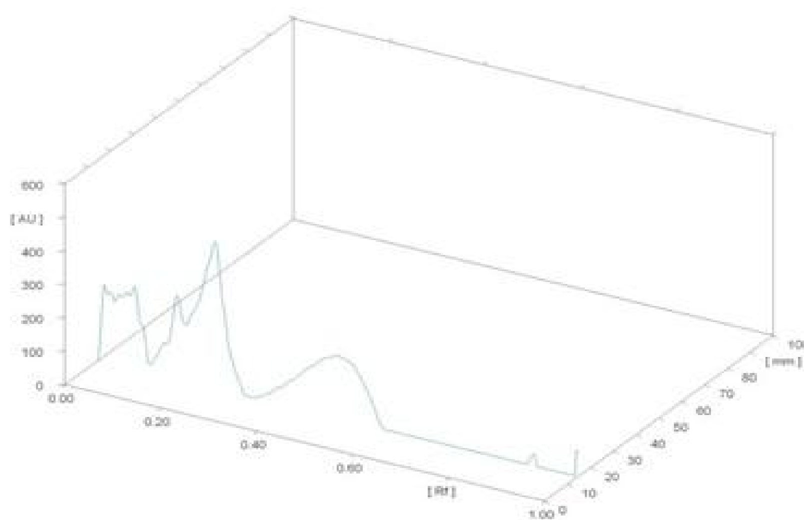
It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

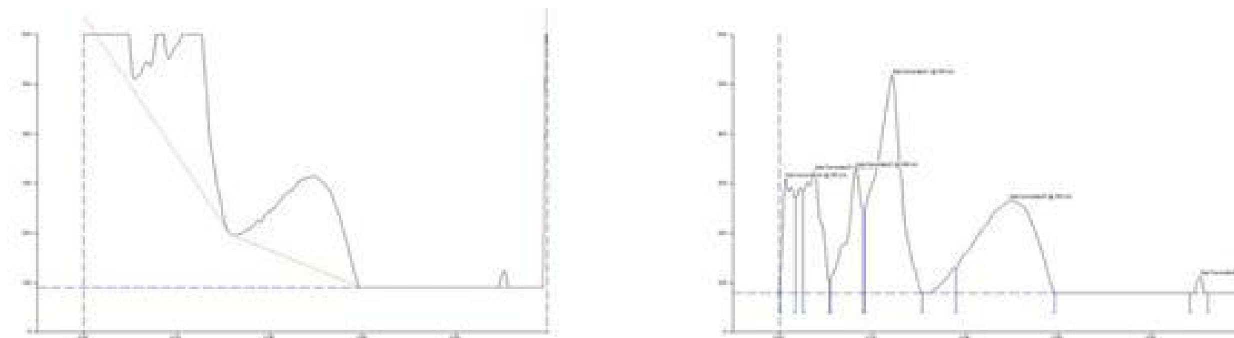
Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

TLC Visualization of SMC at 366nm

3D-Chromatogram



HPTLC fingerprinting of Sample SMC



Peak table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	24.4	0.01	230.6	16.58	0.03	190.1	3446.9	7.86
2	0.05	207.0	0.08	246.7	17.75	0.11	24.5	5403.2	12.32
3	0.11	27.0	0.17	251.9	18.12	0.18	168.2	5189.1	11.83
4	0.18	169.1	0.24	439.4	31.61	0.31	1.8	15084.9	34.39
5	0.38	49.9	0.50	187.7	13.50	0.59	0.2	14511.9	33.09
6	0.89	0.0	0.91	34.0	2.45	0.92	0.0	225.3	0.51

Report

HPTLC finger printing analysis of the sample reveals the presence of six prominent peaks corresponds to presence of six versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.05to0.89.

Heavy metal analysis by AAS

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 AA240 Series. Inorder 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 ofHNO_3.

Standard reparation

As & Hg- 100 ppm sample in 1mol/L HClCd &Pb-100 ppmsample in 1mol/L HNO_3

Test Report

Name of the Heavy Metal	Absorption Max max	Result Analysis	Maximum Limit
Lead	217.0 nm	5.91PPM	10 ppm
Arsenic	193.7 nm	0.74PPM	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.91PPM	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 metal cadmium, whereas the sample shows the presence of Lead at 5.91 ppm, Arsenic at 0.74 ppm and Mercury at 0.91 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.

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.

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 thechromatogram 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 theorigin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry.Locatethespotsontheplateby examinationunderUVlightat365nm.

Aflatoxin	SampleSMC	AYUSHSpecificationLimit
B1	NotDetected–Absent	0.5ppm
B2	NotDetected–Absent	0.1ppm
G1	NotDetected–Absent	0.5ppm
G2	NotDetected–Absent	0.1ppm

Result: The results shown that there were no spots were being identified in the test sample loaded on TLCplates when compare to the standard which indicates that the sample were free from Aflatoxin B1, AflatoxinB2,AflatoxinG1,AflatoxinG2.

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°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.

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

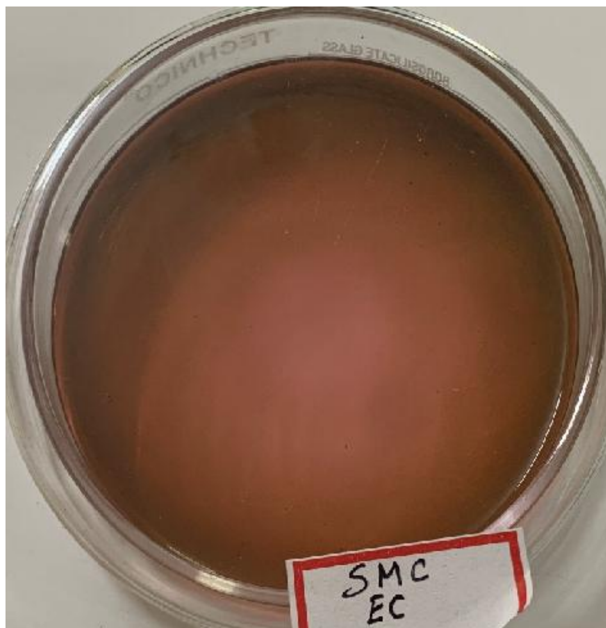
No growth was observed after incubation period. Reveals the absence of specific pathogen

Result

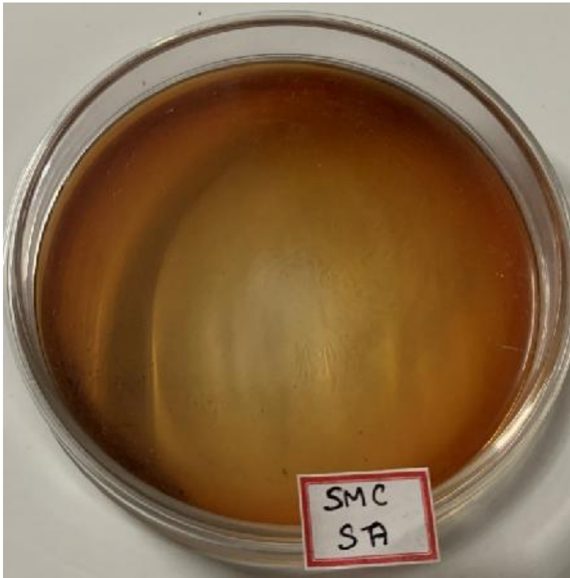
No growth / colonies were observed in any of the plates inoculated with the test sample.

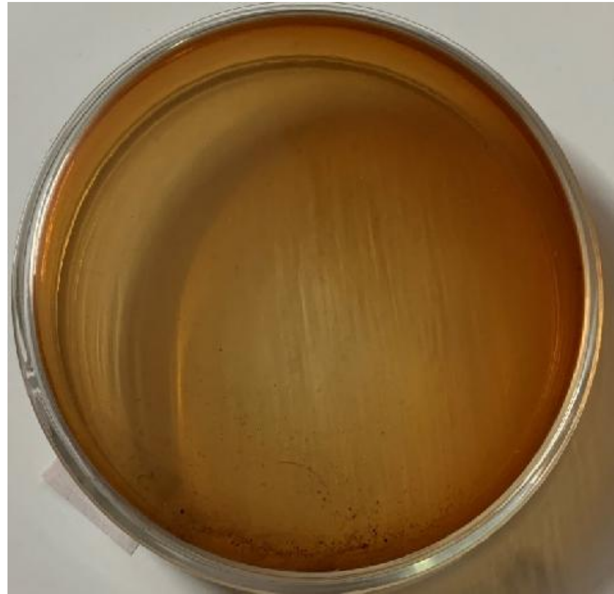
Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

Culture plate with E-coli (EC) specific medium



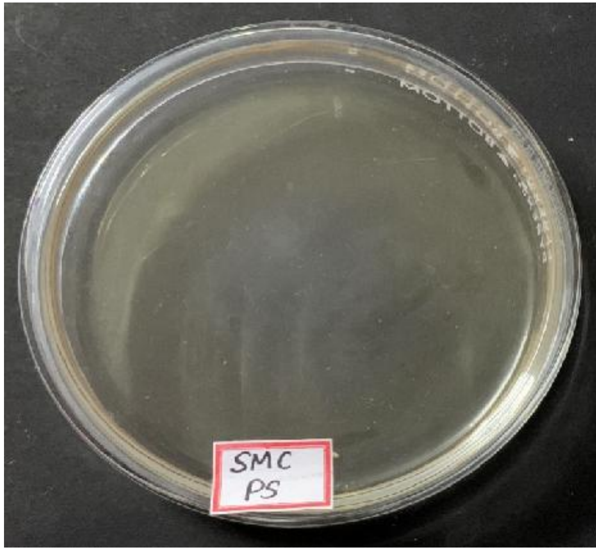
Culture plate with Salmonella (SA) specific medium

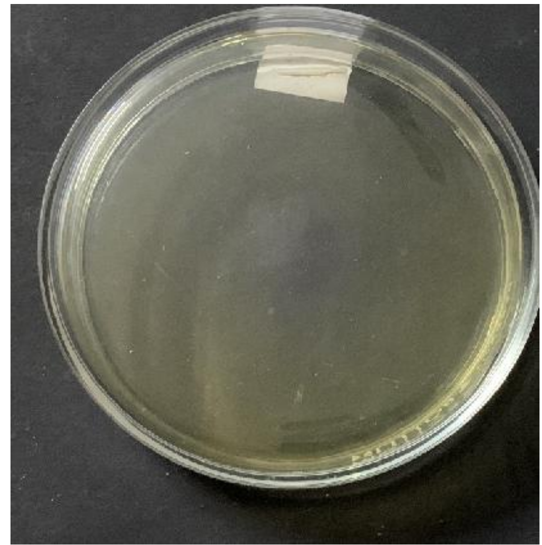




Culture plate with Staphylococcus aureus (ST) specific medium

Culture plate with Pseudomonas aeruginosa (PS) specific medium





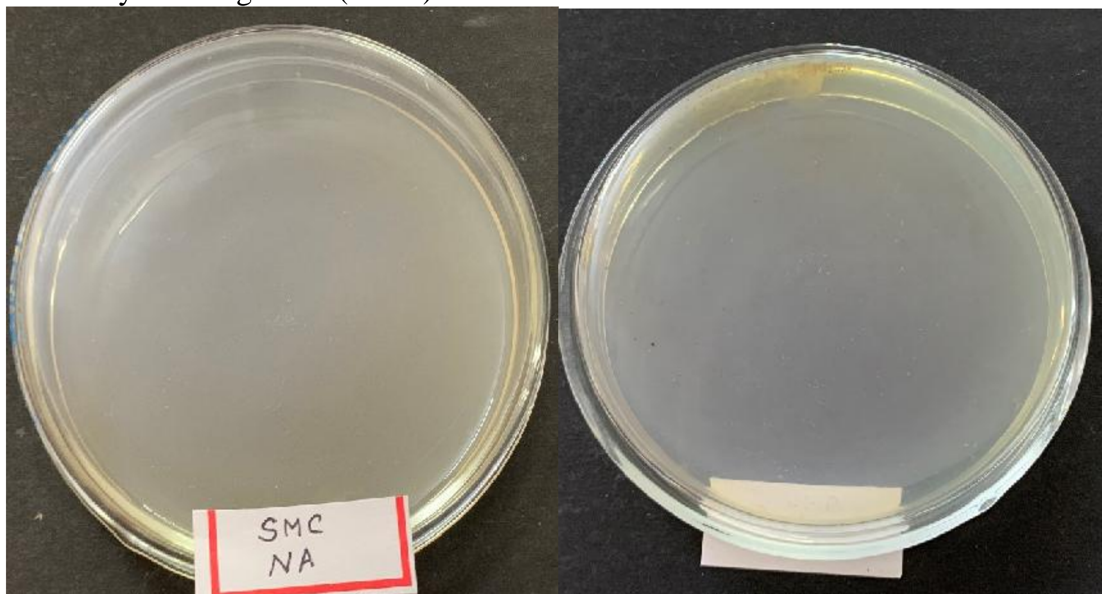
Sterility test by pour plate method

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°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 was then counted and calculated for CFU.



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.

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 4.5%, total ash value was 11.5%, and acid insoluble ash is 0.223%.The alcohol soluble extractive value was 10.8%and water soluble extractive was 26.73%.we need to study the medicinal uses and phytochemical and bioactivity analysis to prove their therapeutic properties. The phytochemical analysis indicates the formulation of Samuthara Chooranam containsalkaloids, flavonoids, steroids, triterpenoids, coumarin, phenol,tanins ,saponins, sugar . HPTLC finger printing analysis of the sample reveals the presence of six prominent peaks correspondsto presence of six versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.05to0.89.Results of the heavy metal analysis have clearly shows that the sample has no traces of heavy metal cadmium, whereas the sample shows the presence of Lead at 5.91 ppm, Arsenic at 0.74 ppm and Mercury at 0.91 ppm. The results shown that there were no spots were being identified in the test sample loaded on TLCplates when compare to the standard which indicates that the sample were free from Aflatoxin B1, AflatoxinB2, AflatoxinG1, AflatoxinG2.

Theresultsshowedthattherewerenotracesofpesticidesresiduessuchas Organochlorine,Organophosphorus,Organocarbamates and pyrethroids in the sample provided for analysis. It was observed form the results of In-vitro anti-microbial assay that the formulation SMC possesses significant antimicrobial activity against E.coli, Salmonella. Staphylococcus aureus, Pseudomonas aeruginosa. Hence this paper is an attempt that was made to evaluate & standardize Samuthara 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.

Conclusion

The achieved results of physico-chemical, preliminary phytochemical tests, qualitative inorganic tests, TLC profiling, HPTLC finger print profiling will be useful as tool for authentication, standardization profile and quality control assessment of the herbomineral formulation

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