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Research Article

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Phytochemical standardization of poly herbal siddha formulation Soothaga chooranam by HPLTC techniques

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Keywords

Siddha medicine for PCOS,
Soothaga Chooranam(SOOC),
phytochemical activity,
HPTLC for Siddha drugs.

Abstract

Background:
Siddha system of medicine is one of the ancient traditional systems of medicine in India and practiced mostly in its southern part for treating various diseases. Their aim was to create a disease –free society .They have prescribed many medicines for that. Soothaga Chooranam is a Poly herbal formulation originated from the siddha system of medicine .it is mainly used for Poly Cystic Ovarian Syndrome (PCOS).through the individual herbs used in formulation have the previous record of standardisation ,there is no record on the formulation hence the same is aimed. Ingredients were purified individually as per classical literature after which the formulation was prepared. The prepared drug was subjected to analysis. The derived HPTLC finger print profile serve as diagnostic Poly herbal formulation.

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

Results:
Phytochemical analysis revealed the presence of alkaloids, carbohydrate, tannin, steroid, triterpenoid and glycoside. total flavonoids and phenol content was found and concentration of lead ,arsenic , mercury, and cadmium was found to be under the limit.Now-a-days, there's a constant have to be investigate their restorative medicinal uses additionally to conduct phytochemical and bioactivity studies to prove their therapeutic properties. To know any data approximately any medicinal plant, there's a vital to go through all the accessible text of siddha conjointly the past audits from later investigate. Phytochemical examinations and natural audits on the plants will lead to the profitable data which can offer assistance the researchers to know more progressed information almost these plant species. The

result of HPTLC analysis appears phytoconstituents display in each sample has no traces of heavy metals Arsenic and Cadmium, whereas the sample shows the presence of Lead at 5.28 ppm, and Mercury at 0.99 ppm. he sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. It was observed from results of In-vitro anti-microbial assay that the formulation SOOC possesses significant antimicrobial activity against E.coli, Solmonella, Staphylococcus aureus, Pseudomonas aeruginosa.

Conculsion:

From the results of the study, it was apparent that the Siddha formulatingSOOC complies with the standard and may be utilised for clinical administration of Soothaga Vaayu(PCOS). But advance ponders got to be carried out to discover the precise part of phytotherapeutics show within the detailing might mindful for the anticipated pharmacological activity in animals and human as well.

Introduction

Siddha medicines are very effective and have therapeutic value in nature but need of standardization, it is required to create the standardization method. In this study, Soothaga Chooranam was selected and screened for standardization technique as per procedures. Ingredients of SOOC are Mukia maderasapatana, Alternanthera sessilis, Phylanodiflora Gossypium arboreum,Vinga radiata,Cuminum cyminum. Mukia maderasapatana called Madras pea it has anti-hyperglycaemic, anti-hyperlipidaemic activity, (A.J.A. Petrus, et.al. Pharcmacogancy, 2012). Alternanthera sessilis called as Sessile joyweed It has a anti hyperglycaemic activity (Walter TM et.al(2014). Phylanodiflora is called as Frog fruit. it has emmenagogue activity (Irrusappan H et.al(2022). Gossypium arboreumcalled as Asian cotton .It has emmenagogue activity. (Conway GA et.al (1979). Vinga radiata Called as Mung beanhas anti hyperglycaemic activity.(Hithamani G et.al, (2014). Cuminum cyminum Called as zeera, it has anti microbial, anti oxidant activity (Al-Snafi AE et,al. (2016).

Materials and Methods

2.1 Selection of drug

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

2.2 Collection and authentication of the drug

The raw materials included in the formulation are Mukia maderasapatana, Alternanthera sessilis, Phylanodiflora Gossypium arboreum,Vinga radiata, Cuminum cyminum. 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.

2.3 Purification of the drug

The purification process was done according to the procedures mentioned in the classical Siddha literature.

This Abstract Soothaga Chooranam traditional Siddha polyherbal medicine. The aim of this study was carried out to standardize the drug Soothaga Chooranam by evaluating physico chemical properties.

2.4 Preparation of the drug

The whole plant of musumusukkai are air dried, roasted pulverized and taken for about 250 grams Similarly, the rest of the ingredients like ponnangani, poduthalai, paruthi vithai paruppu, siru pairu are pulverized and taken about 250 gramsThen the seeragam are purified and soaked in goat‘s urine for about 3 days. They are dried and pulverized taken about 250 grams.The above pulverized drugs are mixed together in a specific proportion and store in an air tight container.

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.

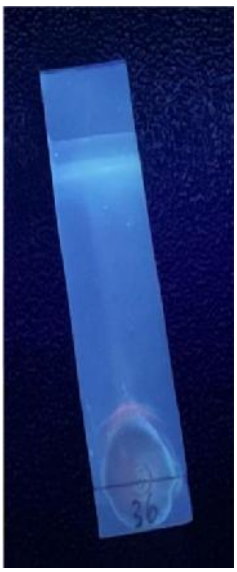
Chromatogram Development

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.

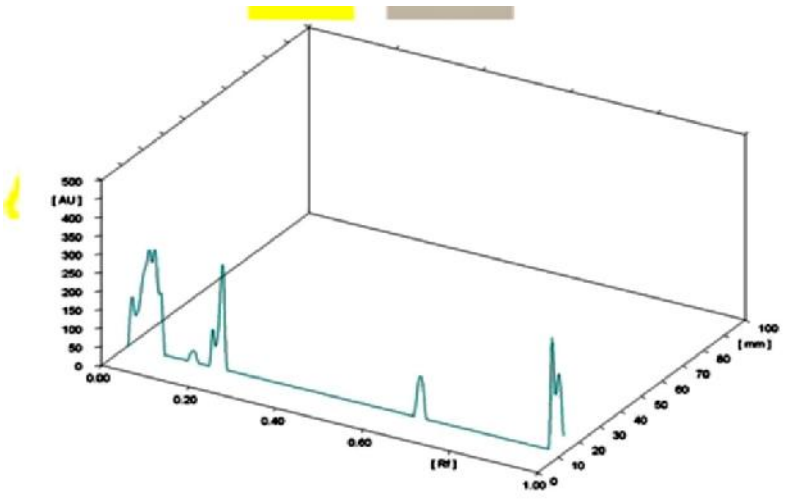
Scanning

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 SOOC at 366 nm



3D - Chromatogram



HPTLC finger printing of Sample SOOC

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1m	0.00	0.0	0.01	133.3	11.67	0.02	89.3	856.4	6.08
2	0.02	86.7	0.06	278.5	24.38	0.09	0.0	6452.2	45.82
3	0.13	0.0	0.15	30.4	2.66	0.17	0.0	251.1	1.78
4	0.18	0.0	0.22	283.6	24.83	0.24	0.0	2531.9	17.98
5	0.66	10.2	0.67	111.9	9.79	0.70	0.0	1050.9	7.46
6m	0.96	0.0	0.97	304.8	26.68	1.00	0.0	2939.5	20.87

PEAK

Report

HPTLC finger printing analysis of the sample reveals the presence of six prominent peaks corresponds to presence of six versatile phytocomponents present within it. Rf value of the peaks ranges from 0.02 to 0.96**.**

Heavy Metal Analysis

Methodology for heavy metal 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 Preparation

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

Name of the heavy metal	Absorption Max^max	**Result Analysis**	**Maximum Limit**
Lead	217.0nm	5.28ppm	10ppm
Arsenic	193.7nm	BDL	3ppm
Cadmium	228.8nm	BDL	0.3ppm
Mercury	253.7nm	0.99ppm	1ppm

BDL- Below Detection Limit

Report and Inference

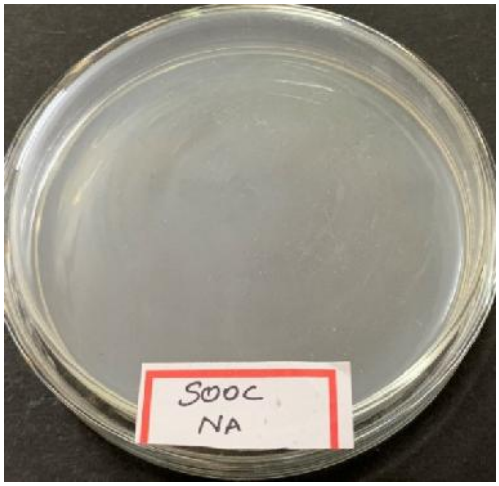
Results of the present investigation have clearly shows that the sample has no traces of heavy metal cadmium, whereas the sample shows the presence of Lead at 7.94 ppm, Arsenic at 0.51ppm and Mercury 0.59 ppm.

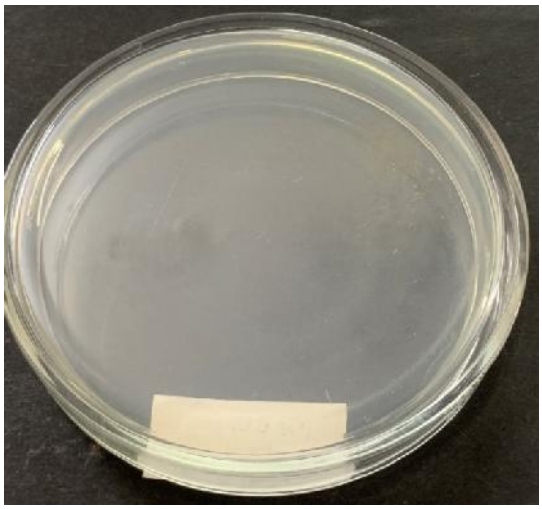
Methodology for pesticide:

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 for aflatoxin:

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

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.

Test for Sterility

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45oC 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 37o 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
Total bacterial count	Absent
Total fungal count	Absent

Methodology for specific pathogen

Methodology

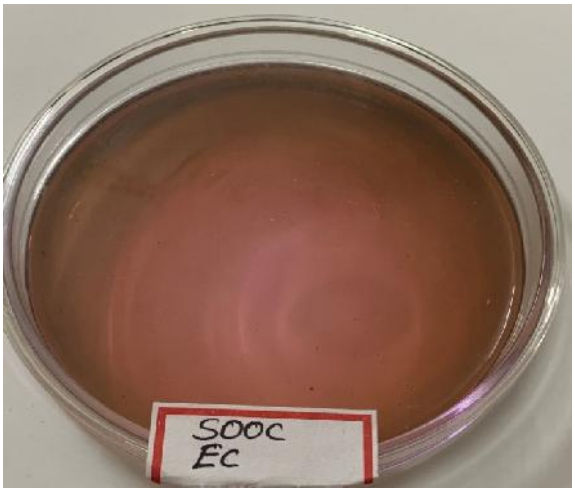
Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37oC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation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

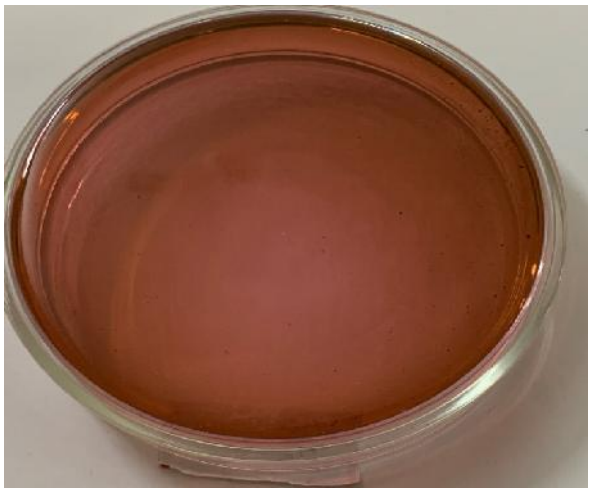
Result

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

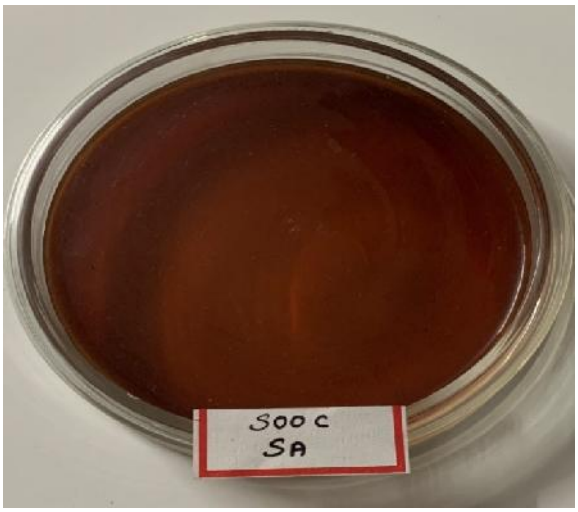
Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

Culture by E-coli(EC) specific medium

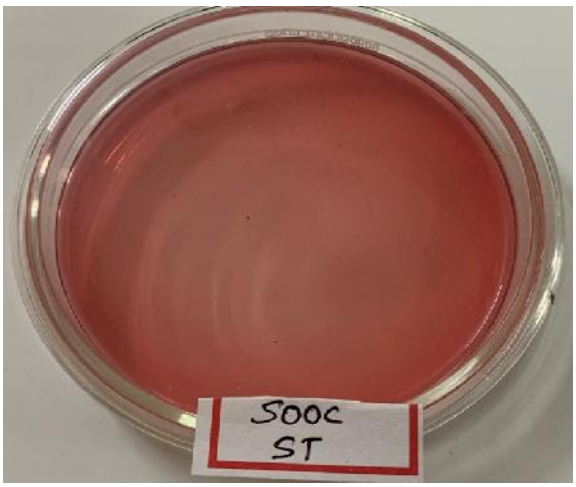


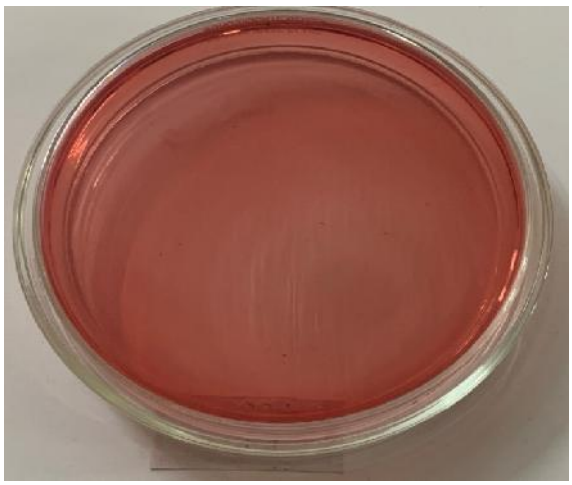


Culture plate with Salmonella (SA) specific medium

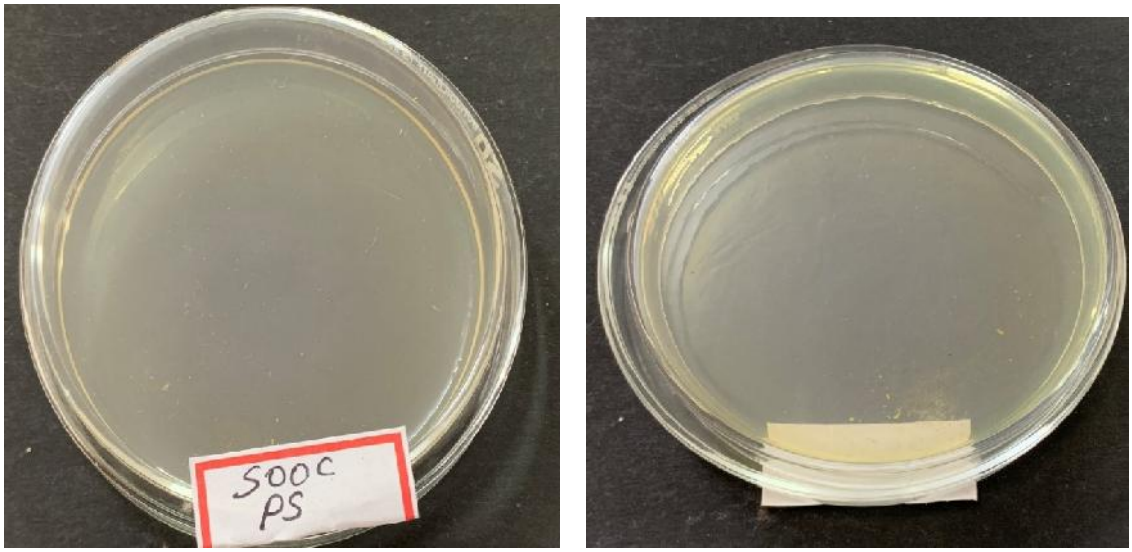


Culture plate with staphylococcus aureus (ST) specific medium





Culture plate with pseudomonas aeruginosa (PS) specific medium



Discussion

Polyherbal preparation or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The therapeutic role of number of herbal drugs in disease management is still being enthusiastically researched due to their less side effect and affordable properties. It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities. It is a largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants Siddha system of traditional medicine has numerous formulations which are utilized for the treatment of disease in mankind since several years. Till now there are several formulations which need to be standardized and evaluated for its potency. To know any information about any medicinal plant, there is a necessary to go throughall the available texts of siddha and also the previo us reviews from recent research. Phytochemical investigations and biological reviews on the plants will lead to the valuable information which can help the scientists to know more advanced knowledge about these plant species. The result of the phytochemical analysis indicates that the formulation SOOC shows the alkaloid, coumarins, saponins, tannins, glycosides, flavanoids, steroids, Triterpenoids, aanthocyanin, Carbohydrate, proteins. The result of HPTLC analysis shows phytoconstituents present in each sample and has no traces of heavy metal cadmium, whereas the sample shows the presence of Lead at 5.28 ppm, Arsenic has BDL and Mercury 0.99 ppm. The sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.It was observed form the results of In-vitro anti-microbial assay that the formulation SOOC possesses significant antimicrobial activity against E.coli, Solmonella. Staphylococcus aureus, Pseudomonas aeruginosa. Siddha also has standardized protocols for purification and detoxification of certain phytocomponents used in specific formulations. As this will greatly reduce the toxicity and also enhance the therapeutic efficacy of the formulation. The chances of occurrence of an adverse event are very minimal in Siddha when compare to any other therapies in the world this is mainly because the 90 % of ingredients used in the preparing formulations are compatible with the biological system of the humans and animals. In the present study specific pathogenic bacteria and Aflotaxin, heavy metals, pesticide, Sterility are absent in SOOC formulation. It is as per WHO norms. So it proves that SOOC is free from microbial contamination. The findings of this study also highlighted the

safety of the Soothaga Chooranam . The information obtained from preliminary phytochemical screening will be use full in finding out the reality of the drugs.

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