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STANDARDIZATION OF THE SIDDHA HERBAL–MINERAL FORMULATION DRUG *"SAMUTHARA CHOORANAM"*

Kavinaya A. B.*[1] and Mohamed Musthafa M.[2]

[1]PG Scholar, PG Dept of Sirappu Maruthuvam, Govt Siddha Medical College, Chennai, Tamilnadu, India.
[2]Professor and Head of the Department of Sirappu Maruthuvam, Govt Siddha Medical College, Chennai, Tamilnadu, India.

***Corresponding Author: Kavinaya A. B.**
PG Scholar, PG Dept of Sirappu Maruthuvam, Govt Siddha Medical College, Chennai, Tamilnadu, India.

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ABSTRACT

Standardization of Siddha formulaion drugs is very essential to order to justify their acceptability in the modern system of medicine. The herbal-mineral formulation drug "Samuthara chooranam" from the siddha text have vital importance in standardization which will encompass the entire field of study from the cultivation of medicinal plants to its clinical application. Herein standardization parameters like Organoleptic characters, Physicochemical analysis, Heavy metal analysis, TLC and HPTLC analysis, Phytochemical analysis and Sterility test are carried out as per Ayush guidelines. The outcome of this study clearly proves the quality, purity, safety and potency of the drug which will help the medicine to survive and succeed in future researches on both clinically and economically.

KEYWORDS: Samuthara chooranam, TLC and HPTLC.

INTRODUCTION

The Siddha system of medicine is one of the long-lived traditional systems which has a unique art of healing founded by great spiritual scientist called 'Siddhars'. Here thousands of raw drugs were used in the treatment of many diseases. According to WHO, 60% of the world's population depends on traditional medicine and 80% of the population in developing countries depends almost entirely on traditional medicine. As a result of increasing demand for herbal medicines, the need for standardization of herbal products is very essential for the global acceptance. Standardization confirms the identity of the product, determine its quality and purity and detection of the nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observations.

I have chosen the herbal-mineral formulation drug "Samuthara chooranam" from the Siddha text of 'Pranarashamirtha Sindhu' for treating all types of Vatha and Gunmam diseases. Hence my aim of this study is to evaluate the Qualitative and Quantitative analysis of "Samuthara chooranam" which may also help the medicine for the widespread acceptance of globally, scientifically and economically. Organoleptic characters, Physicochemical analysis, Heavy metal analysis, TLC and HPTLC analysis, Phytochemical analysis and Sterility test were carried out as the standardization parameters(as per AYUSH guidelines)

MATERIALS AND METHODS

Selection of Drugs

Samuthara chooranam consists of Kadukkai (Terminalia chebula), Omam (Trachyspermum ammi), Inji (zingiber officinale), Thippili (Piper longum), Perungayam (Ferula asafoetida), Vaividangam (Embelia ribes), Induppu (Rock salt), Kaluppu (Sodium chloride impura), Yavacharam (Potassium carbonate) were purchased from the raw drug shop R.N.RAJAN & CO, Paris. After getting proper authentication from the Head of the Department of Medicinal Botany and Pharmacology (Gunapadam), GSMC, Chennai-106 the medicines were prepared.

Method of Purification

Herbal Drugs

Purified and dried under the classical text.

Mineral Drugs

Induppu

Soaked in rice vinegar for 3 days, dried in sunlight till all water content gets evaporated and then the salt was powdered in a stone mortar.

Yavacharam

Dissolved in Goat's urine, filtered, dried in sunlight and then the salt was powdered in a stone mortar.

Kaluppu
Soaked in rice vinegar for a few seconds. Remove the humidity of kaluppu by placing it in dry cotton cloth and dried in sunlight. At last, the salt was powdered in a stone mortar.

Method of Preparation
Above purified drugs were grinded separately and mixed well together. Then filter them as a fine powder and weighed. Atlast the powder was stored in the air tight container.

Administration: Two grams (twice daily) for 48 days with ghee as the adjuvant.

1. Organoleptic Characters
State
Solid.

Appearance
Greenish brown in color.

Nature
Mild coarse in nature.

Taste
Astringent with mild Pungent.

Odor
Strong characteristic.

2. Physicochemical Evaluation
Percentage Loss on Drying
10gm of test drug was accurately weighed in evaporating dish. The sample was dried at 105oC for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/Wt of the sample X 100

Determination of Total Ash
3 g of 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.

Total Ash = Weight of Ash/Wt of the Crude drug taken X 100

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.

Acid insoluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

Determination of Water Soluble Ash
The ash obtained by total ash test will be boiled with 25 ml of water for 5 mins. The insoluble matter is collected in crucible and will be washed with hot water and ignite for 15mins at a temperature not exceeding 450ºC. Weight of the insoluble matter will be subtracted from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

Water Soluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

Determination of Alcohol Soluble Extractive
About 5 g of test sample will be macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing 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.

Alcohol sol extract = Weight of Extract/ Wt of the Sample taken X 100

Determination of Water Soluble Extractive
About 5 g of the test sample will be macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing 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.

Water soluble extract = Weight of Extract/ Wt of the Sample taken X 100

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

3. 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 KN 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 sample.

Sample Digestion
Test sample 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.

4. TLC and HPTLC Analysis
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 different solvent system Toulene: Ethyl Acetate: Acetic Acid (1.5:1:0.5) 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. 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 fingerprint of phytochemicals which is suitable for confirming the identity and 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 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 extract and Rf values were tabulated.

5. Phytochemical Analysis
Extraction
Sample Extraction were carried out with water and the resulting extract was utilized for the 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 had 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
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 Benedict'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.

6. 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
About 1ml of the 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. Grown colonies of organism was then counted and calculated for CFU.

RESULTS AND DISCUSSIONS

Organoleptic Characters
Table 1: Organoleptic character of SC.

S. No	Characters	Results
1.	State	Solid
2.	Appearance	Greenish Brown
3.	Nature	Mild Coarse powder
4.	Taste	Astringent with mild Pungent
5.	Odor	Strong Characteristic

Physicochemical Evaluation
Table 2: Physicochemical evaluation of SC.

S. No	Parameter	Mean (n=3) SD
1.	***Loss on Drying at 105 °C (%)***	24.67 ± 1.06
2.	***Total Ash (%)***	1.544 ± 0.31
3.	***Acid insoluble Ash (%)***	0.33 ± 0.04
4.	***Water Soluble Ash (%)***	4.46 ± 1.10
5.	***Alcohol Soluble Extractive (%)***	21.22± 0.53
6.	***Water soluble Extractive (%)***	9.98 ± 1.15
7.	***PH***	4.5

Heavy Metal Analysis
Table 3: Heavy Metal Analysis of SC.

S. No	Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
1.	Mercury	253.7 nm	BDL	1 ppm
2.	Arsenic	193.7 nm	0.268 ppm	3 ppm

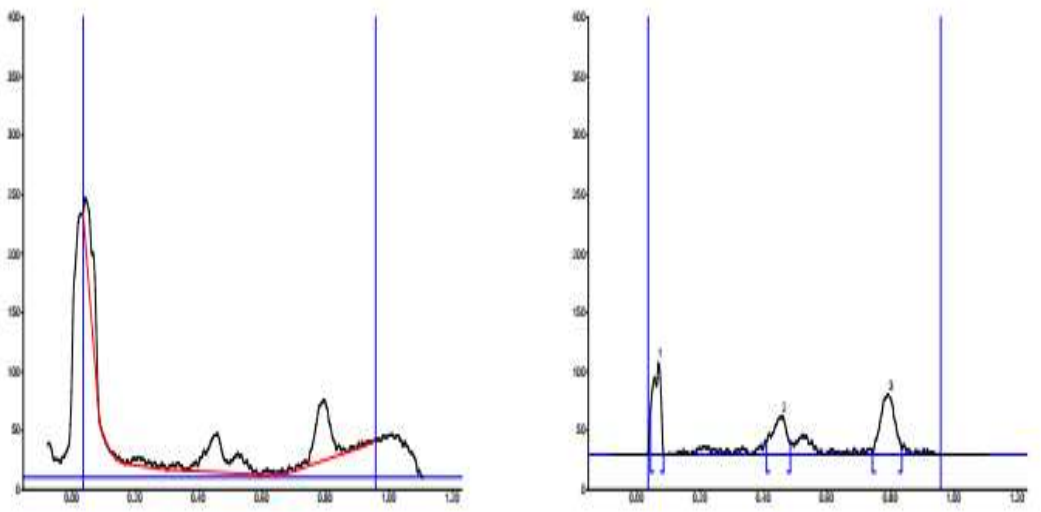
BDL- Below Detection Limit

Report and Inference
- Results of the present investigation(Table.3) has clearly shows that the sample SC has no traces of Mercury and further shows the presence of Arsenic at 0.268 ppm level and hence it was considered that the heavy metals mercury was absent in the sample SC.
- The reported heavy metal arsenic seems very low (0.268 ppm) when compare to the allowed recommended limit of 3ppm.

TLC and HPTLC Analysis

TLC Analysis at 254 nm

TLC Analysis at 366 nm



HPTLC finger printing of sample SC.

Table 4: HPTLC Analysis of SC.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	39.0	0.07	78.7	48.29	0.08	0.4	1461.7	33.99
2	0.41	11.3	0.46	33.0	20.28	0.48	10.2	1089.4	25.33
3	0.74	2.4	0.80	51.2	31.43	0.84	5.0	1749.9	40.69

Report and Inference

HPTLC finger printing analysis of the sample SC(Table.4) 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 0.04 to 0.74. Further the peak 1 occupies the major percentage of area of 48.29 % which denotes the abundant existence of such compound. Followed by this peak 3 and 2 occupies the percentage area of 31.43 and 20.28 %.

Phytochemical Analysis

Table 5: Phytochemical analysis of SC.

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 Presence and - indicates Absence of the Phytocomponents

Tests Done For

Alkaloids

Flavanoids

Glycosides

Steroids

Triterpenoids

Coumarins

Phenol

Tanin

Protein

Saponin

Sugar

Anthocyanin

Report and Inference

Table. 5 shows the Samuthara chooranam indicates the presence of rich Flavanoids, Glycosides, Phenol, Tannin, Saponin and Sugar.

Sterility Test by Pour Plate Method

Observation

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

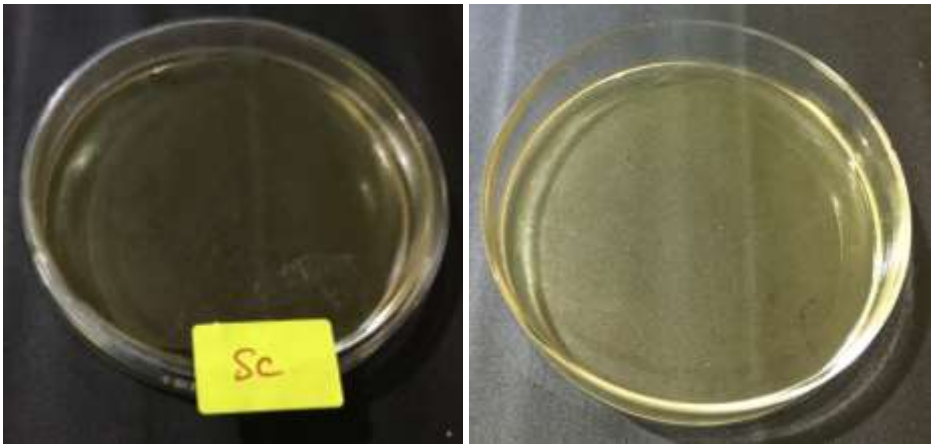


Table 6: Sterility test by Pour Plate Method for SC.

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	

Report and Inference

No growth / colonies were observed (Table.6) in any of the plates inoculates with the test sample.

CONCLUSION

Heavy Metal Analytical study clearly shows that the metal mercury was absent and the metal arsenic seems very low trace when compared to the allowed recommended limit in the sample Samuthara chooranam. Thus the drug "Samuthara chooranam" is very safe in recommending for the clinical trial.

Phytochemical study indicates the presence of rich Flavanoids, Glycosides, Steroids, Phenol, Tanin, Saponins and Sugar in "Samuthara chooranam" which may help to reduce the inflammation of joints. It shows the identity of the drug.

Sterility test indicates that there no growth/colonies were observed in the plates inoculates with the test sample SC. It shows the purity of the drug "Samuthara chooranam".

This experimental study clearly demonstrates the Qualitative and Quantitative analysis of "Samuthara chooranam" which will help to conduct further clinical studies and standard researches.

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