STANDARDIZATION OF *SAMAN CHOORANAM*, A SIDDHA HERBO-MINERAL FORMULATION

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ABSTRACT:

Standardization of a Siddha Herbo-Mineral formulation is essential for establishing its authenticity, quality and efficacy. The present study highlights the standardization of Saman Chooranam (a Siddha Herbo-Mineral formulation containing 11 ingredients) which can make contributions to the existing literature. Standardization of formulation was done on the basis of its macroscopic and microscopic characters, physicochemical parameters, phytochemical screening, pH, particle size and High-performance thin layer chromatographic analysis. Phytochemical screening has shown the presence of alkaloids, flavonoids, steroids, Triterpenoids and phenols. Saman Chooranam has been used for various liver diseases, Anemia and peptic ulcer. From the investigations of the study, it can be concluded that the pharmacognostical and phytochemical evaluation of Saman Chooranam can be used as reference standard for the quality control/quality assurance purpose.

Keywords: Siddha medicine, *Saman Chooranam*, Standardization, Phytochemical screening.

1. INTRODUCTION:

In recent years, there has been a great demand for plant derived products in the developed countries.[1] These products are increasingly being sold as medicinal products, nutraceuticals and cosmeceuticals. Today many of the developing countries are using traditional or ancient system of medicines for curing the diseases. According to World Health Organization (WHO), 35% of the out-patients and 22% of the in-patients in rural China are treated with traditional Chinese medicines.

Plants are the good and natural sources of herbal medicines as they are free from toxicity or very less toxic. [1] The various indigenous systems which use plants for the ailment of diseases are Ayurveda, Unani, Siddha. Nowadays herbal medicines are widely used in different countries for the treatment of diseases and also the treatment through herbal medicines is accepted worldwide as they occur in nature, good for health and less toxic. [2] Now a days life style disorders like hypertension, diabetes, fatty liver, obesity etc., have become very common. The liver plays an important role in the innate immune response thereby providing the first line of defence against microbes and toxins. The liver is one of the most vital organs in human body responsible for metabolism and therefore it is more vulnerable to injury which can produce different diseases like hepatitis, cirrhosis, or hepatocellular carcinoma. Different environmental pollutants and drugs or chemicals are the major cause of these diseases. [3]

At the same time, hematological abnormalities are commonly occurred in chronic liver diseases. Many pathological reasons to causes of anemia. In chronic liver diseases, anemia is developed in 75% of patients. Folic acid and Vit B12 deficiency is the causes for anemia in chronic liver patients. Pancytopenia and hypocellular bone marrow conditions are may develop anemia in chronic hepatitis patients [4].

Siddha medicine is one of the traditional medicines in AYUSH system of medicines and majorly used in southern states of India. According to Siddha literatures, Liver diseases are mentioned in *Kalleeral Noigal*. In siddha literature mentioned anemia, jaundice, ascites is the main complication of *kalleral noigal.* According to Siddha literature common causes of Liver diseases, Anemia and Peptic ulcer are due to intake of more oily foods, no physical activities, unhealthy diet, irregular food habits which are increased in Pitham humors, drinking alcohol, liquor, and lifestyle modifications. These factors affected pitham humor and it vitiated. Affected pitham humor disturbed other vali and Iya humor results create liver diseases. Three humors vitiated and affect the udal Thadhukkal (physical constituents). Saaram and Senneer is one of the udal thaathukal. Saaram represents nourishing juice similar to plasma and Senneer represents the blood. Both are affected they leads to depletion of tissue system and obstruction in the natural functional pathway of piththuneer (Bile). And it also affects in two types of vaatham such as keezhnokkukal and

maelnokku kaal. All of these lead to create liver diseases. [5]

In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental methods of analysis.[6]

2. MATERIALS AND METHODS:

Preparation of Saman Chooranam:

The ingredients of preparation were procured from the Authenticated country drug store, Chennai, Tamilnadu and *Saman Chooranam* [7] was prepared in the Department of Gunapadam, Govt. Siddha Medical College, Chennai. Their identities were confirmed by correlating their morphological and microscopical characters with those given in the literature. The drugs were cleaned, dried and powdered. The drugs were weighed and mixed well.

Table 01: explains information about theIngredients of ***Saman Chooranam***

Table 01: Ingredients of Saman Chooranam

Si.No	**Ingredients**	**English Name**	**Scientific Name**	**Quantity**
1	*Indhuppu*	Rock salt	Impure Sodium chloride	5gms
2	*Perungayam*	Asafoetida	*Ferula asafoetida*	5gms
3	*Evatcharam*	Wood salt	ImpurePotassium Carbonate	5gms
4	*Kalluppu*	Common salt	Sodium chloride	5gms
5	*Kadukkai*	Yellow myrobalan	*Terminalia chebula*	5gms
6	*Chukku*	Dried ginger	*Zingiber officinale*	5gms
7	*Vaividangam*	Embelia ribes	*Embelia robusta*	5gms
8	*Sathicharam*	Mixture of fullers earth and Lime stone	Sodium, Potassium and calcium compound	5gms
9	*Kodiveli*	Leadwort	*Plumbago zeylanica*	5gms
10	*Thippili*	Long pepper	*Piper longum*	5gms
11	*Kurosani Omam*	Henbane seeds	*Hyoscyamus niger*	5gms

Drugs identification and authentication: [8]

All the above said ingredients were recognized and certified by Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai – 106 and experts of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai. A specimen sample coded as S17052201C. All the raw materials had been labeled and preserved for future reference.

Macroscopic Evaluation:

The powder was observed for its colour, odour, taste and appearance.

Microscopic Evaluation:

A small quantity of Chooranam was taken and a few drops of Dilute NaOH [9] was added and heated for one to two minutes. To the cleared Chooranam phloroglucinol and concentrated Hydrochloric acid in the ratio of 1:1 was added and kept as such for 3 to 4 minutes. Then 2 to 3 drops of dilute Hydrochloric acid was added and finally mounted in glycerin. The lignified tissues showed a pink colour. To detect the presence of starch grains, the Chooranam was mounted in 1 to 2 drops of dilute iodine and mounted in glycerine. Starch grains appeared as blue to purple colour.

Physicochemical Investigations:

In physicochemical investigation, loss on drying, total ash, acid insoluble ash, alcohol and water extractive values, pH and Particle Size Determination [10,11] were determined as per standard procedures.

All the parameters were performed according to the official methods prescribed in Indian Pharmacopoeia and [12] WHO on quality control methods for medicinal plant materials. [13]

Preliminary Phytochemical Analysis:

Preliminary phytochemical screening was carried out by using standard procedures. [14,15]

Heavy /toxic metals and microbial load analysis:

The formulation was also tested for the presence of heavy/ toxic metals and for the microbial load as per WHO guidelines. [16,17]

PESTICIDE RESIDUE [17], [18]

Test sample was extracted with 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.

AFLATOXIN ASSAY BY Thin Layer Chromatography (TLC) [19]

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 and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365nm.

High Performance Thin Layer Chromatography Analysis [20]

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.

3. RESULT AND DISCUSSIONS:

As a part of standardization procedure, the formulation was tested for relevant physical and chemical parameters and also subjected to microbial screening through quality control measures.

Macroscopic Evaluation:

The formulation was light brown in colour having a characteristic odour and pungent taste (Table 2).

Table no: 2: Macroscopic evaluation of formulation

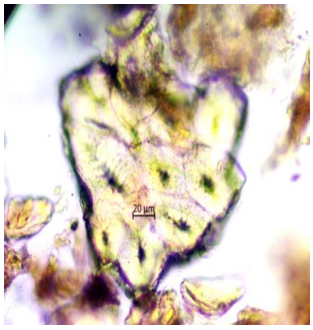
S. No	Parameter	Result
1.	State	Solid
2.	Nature	Very Fine
3.	Odour	Characteristic
4.	Touch	Soft

5.	Flow Property	Free Flowing
6.	Appearance	Pale Brownish
7.	Taste	Salty with pungent
8.	Character	Astringent

Microscopic Evaluation:

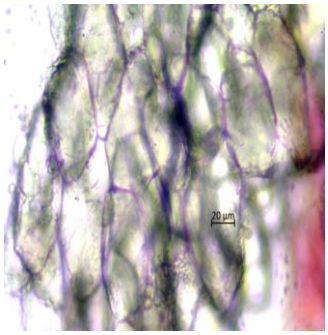
The powdered formulation has shown the presence of sclereidal mesocarp cells and stone cells from endocarp for *Embelia robusta*, beaded walled cells and perisperm cells from *Piper longum*. Surface view of yellow coloured collapsed layer and testa from *Hyoscyamus niger*, pitted vessels and cells with brownish content from *Plumbago zeylanica*, surface view of epicarp and branched sclereids from *Terminalia chebula*, oleoresin cells and sac shaped starch grains from *Zingiber officinale.*

***Embelia robusta*:**



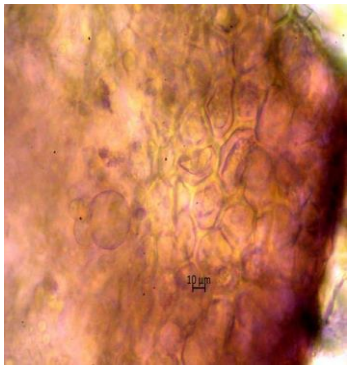
Sclereidal layer from mesocarp **Stony endocarp**

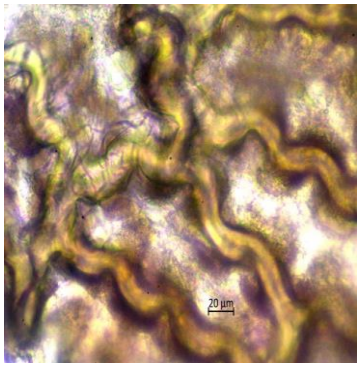
Piper longum:



Beaded walled cells **Perisperm cells**

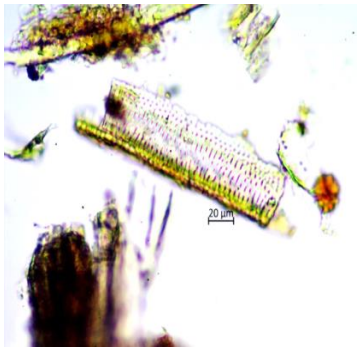
Hyoscyamus niger:

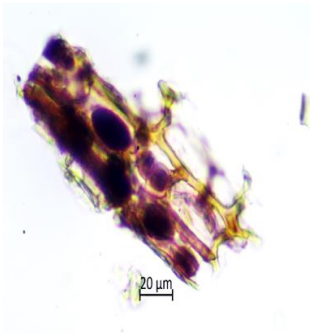




Cells from the epicarp **Surface view of testa**

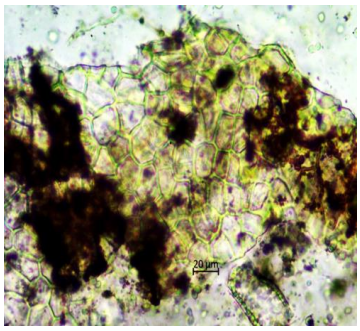
Plumbago zeylanica:

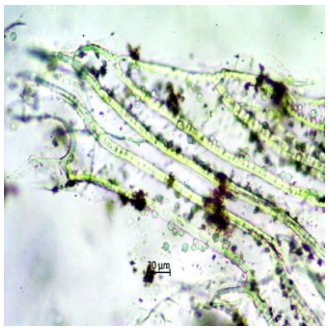




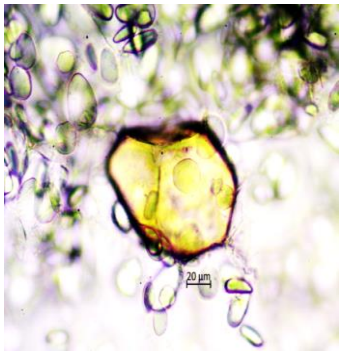
Pitted vessel **Cells with brownish content**

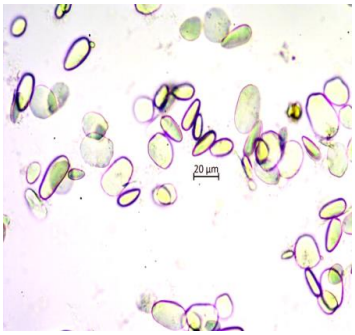
Terminalia chebula:





Surface view of the epicarp **Branched sclereids*Zingiber officinale:***





Oleoresin cells **Sac shaped starch grains**

Physicochemical Investigations:

Loss on drying gives an idea of moisture present in the drug. Ash value of a drug provides an idea of the earthy matter or the inorganic components and other impurities present along with the drug. Extractive values are useful for the determination of exhausted or adulterated drugs. The water soluble extractive was high in the formulation. The results of physico-chemical constants of the drug powder are presented in Table 3.

The physicochemical parameters of *Saman Chooranam* were determined and the results given in Table no: 3

S.No	Parameter	Result (Mean (n=3) SD)
1.	Loss on Drying at 105 °C (%)	6.133 ± 2.19
2.	Total Ash (%)	4.967 ± 0.152
3.	Acid insoluble Ash (%)	0.047 ± 0.014
4.	Water soluble Extractive (%)	20.03 ± 0.923
5.	Alcohol Soluble Extractive (%)	9.667 ± 0.83
6.	pH	6.2
7.	Particle size	82.81 ± 18.42 µm

Preliminary Phytochemical Analysis:

Preliminary phytochemical analysis has revealed the presence of alkaloids, flavonoids, steroids, Triterpenoids and phemols. (Table

4)**Table no: 4 Phytochemical Analysis of *Saman Chooranam***

S.NO	TEST	OBSERVATION
1.	Alkaloids	+
2.	Flavanoids	+
3.	Steroids	+
4.	Triterpenoids	+
5.	Coumarin	+
6.	Phenol	+
7.	Tannin	+
8.	Sugar	+
9.	Betacyanin	+

Heavy /toxic metals and microbial load analysis:

Heavy metals have been associated with various adverse effects including status epilepticus, fatal infant encephalopathy, hepatotoxicity, congenital paralysis and deafness. Many case studies have reported serious adverse conditions due to heavy metals in siddha and other herbal drugs. The formulation was found free from the Lead, Arsenic, Cadmium and Mercury. Results are tabulated in table 5.

Various microorganisms can contaminate herbal drugs and cause serious health hazards. Table.5 represents the count of total bacteria, total yeast and mould. Escherichia coli, Pseudomonas aeruginosa, Salmonella species, Staphylococcus aureus were found to be absent in the formulation. The results revealed the absence of these microorganisms thereby confirming the nontoxic nature of the formulation

Table 5: Heavy/ Toxic metals in the formulation

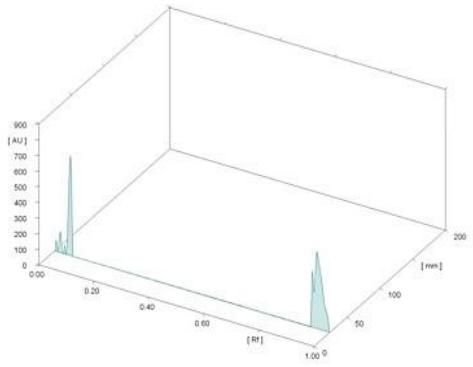
Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	2.28 PPM	**10 ppm**
Arsenic	193.7 nm	BDL	**3 ppm**
Cadmium	228.8 nm	BDL	**0.3 ppm**
Mercury	**253.7 nm**	**BDL**	**1 ppm**

Table 6: Microbial load of the formulation

Microbes	Count
Total Bacterial Count	NMT 10^5CFU/g
Total Fungal Count	NMT 10^3CFU/g
E-coli	Absent
Salmonella	Absent
Staphylococcus Aureus	Absent
Pseudomonas Aeruginosa	Absent

Fig: 3 TLC Visualization of SC at 366 nm

Fig: 4 3D – Chromatogram



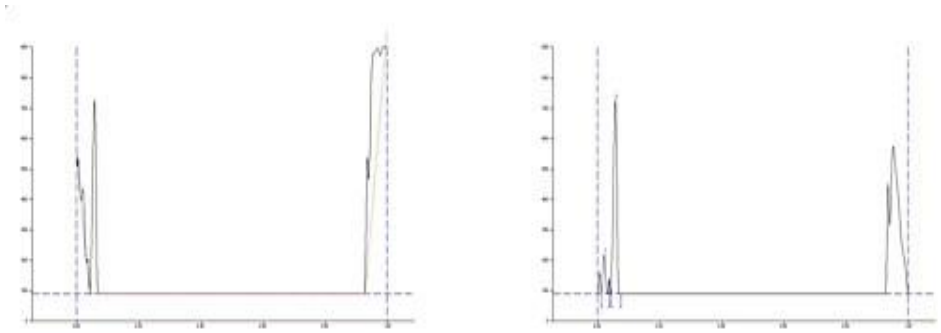


Fig: 5 & 6 HPTLC finger printing of Sample SC (*Saman Chooranam*)

Peak Table No: 7

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	19.2	0.02	124.8	20.58	0.03	30.4	1433.7	14.52
2	0.03	30.8	0.05	158.6	26.16	0.06	0.7	1547.0	15.67
3	0.08	5.3	0.14	171.4	28.28	0.17	1.1	3805.8	38.55
4	0.30	9.5	0.35	67.1	11.06	0.39	19.9	2341.1	23.71
5	0.48	3.1	0.51	12.5	2.06	0.52	7.3	224.5	2.27
6	0.96	8.5	0.97	71.9	11.86	0.99	1.8	520.6	5.27

Pesticide Residue and Aflatoxin Assay:

The result 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.

Table No: 8 Test Result Analysis of the Sample SC [*Saman Chooranam*]

Pesticide Residue	Sample SC	AYUSH Limit (mg/kg)
I. Organo Chlorine Pesticides		
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**
III. Pyrethroid		
Cypermethrin	**BQL**	**1mg/kg**

BQL- Below Quantification Limit

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 was

free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. So the drug is not harmful. [18]

Table No: 9 Test Result of Aflatoxin

Aflatoxin	Sample SC	AYUSH Limit	Specification
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. CONCLUSION:

The above results and interpretations of *Saman chooranam* shows that physicochemical results LOD 6.133 ± 2.19 %, total ash 4.967 ± 0.152 (%), acid insoluble ash 0.047 ± 0.014 (%), water soluble extractive 20.03 ± 0.923 (%), alcohol soluble extractive 9.667 ± 0.83 (%). solubility nature shows soluble in ethanol, water& DSMO, Phyto chemicals reported are Alkaloids, Carbohydrates, Phenols, Tannins, Flavonoids, triterpenoids, coumarin, steroids, betacyanin. *Saman chooranam* has no traces of heavy metal Cadmium, Mercury, Arsenic and the presence of Lead at 2.28 PPM level which is BDL. There were no traces of pesticide residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the drug *Saman chooranam.* No spots there were being identified in the sample *Saman chooranam* when compared to the standard which indicates that the drug was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. In specific pathogen and sterility test there was no growth after incubation period which indicates the drug is free from microorganism. HPTLC finger printing analysis of the *Saman Chooranam* reveals the presence of six prominent peaks corresponds to the presence of six versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.03 to 0.96. *Saman chooranam*, a Siddha herbomineral formulation is exposed to many studies to validate its effectiveness and safety via a defined standardization process. The review article of *Saman chooranam* confirms that the potentcy of drug which indicated for hepatic disorders. It is advised to bring the formulation for further investigation via pharmacological studies and clinical trials for global acceptance.

CONFLICT OF INTEREST:

The authors have declared no conflict of interest.

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