PHARMACOGNOSTICAL,QUALITATIVE SCREENING OF SIDDHA-HERBOMINERAL DRUG "SAGALAVAAYU CHOORANAM" FOR RHEUMATOID ARTHIRITIS

[1]Dr.M.Jagadeesh babu, [2]Dr.R.Sathya, [3]Dr.M.D.Saravana devi M.D(s)

[1,2]PG scholar, [3]Guide, Professor, Head of the Department
PG Gunapadam, Gsmc,chennai.

***Abstract*- Rheumatoid arthritis is most auto immune disorder followed by inflammation of joints. The studies stated the prevalence rate of rheumatoid arthritis is estimated at 0.5–1.0% worldwide. Symptoms of rheumatoid arthritis are closely related to *mudaku vatham* in the *Siddha* system of medicine. The siddha medical system is an ancient medical system that was documented by siddhars in southern India. In the *Siddha* system of medicine, which has various formulas based on herbal, mineral, and animal origins, they pay more attention to improving the quality of life. *Siddhars* formulated many effective preparations for rheumatoid arthritis without any side effects. Sagalavaayu cooranam is a polyherbal-mineral combination that includes *sukku (Zingeria officinale), milagu (Piper nigrum), thippili (Piper longum), seeragam (Cuminum cyminum), kadukaai (Terminalia chebula), nellikaai (Phylanthus emblica), thaandrikaai (Terminalia)*, and some other herbal ingredients, with fuller's earth indicated as mudaku vatham (rheumatoid arthritis). With advanced technologies and research methods, The current study provides a detailed screening report of sagalavaayu chooranam, including organoleptic properties, aflatoxin assay by TLC, pysio-chemical parameters like loss on drying, total ash, acid insoluble acid, water soluble and alcohol soluble extract, pH, and particle size. The presence of Carbonates, Chlorides, Sulfates, and Phosphates in the test drug is revealed by biochemical analysis. The test drug is free of microbial contamination, and heavy metals and pesticide residues were below the quantification limit. The sudy was validated according to PLIM guidelines. Following pharmacognostical and qualitative testing, sagalavaayu chooranam confirmed the regulatory requirement and also possessed significant phytocomponents, which contributed to the beneficial effect on rheumatoid arthritis.**

***KEYWORD*: Rheumatoid arthritis-siddha medicine-mudaku vatham-sagalavaayu chooranam-herbo mineral drug-pharmacognostical and quantitative screening -PLIM guidelines.**

INTRODUCTION: The *Siddha* system of medicine is one of the traditional systems of medicine practised in the southern part of India, mainly in Tamil Nadu. *"Unavae marunthu, marunthae unavu"* is our ancestors' golden truth for living a prosperous life. It was founded by *Siddhars, who lived a long time ago and treated diseases with the most primitive forms of medicine and diagnostic methods. The chief principal philosopher, Sage Agathiyar, contributed many methodologies and techniques to the Siddha system of medicine.* There are 4448 diseases explained by the *Siddhars*. ***Mudaku Vatham*** is one of the 80 *Vadha* diseases. It is symptomatically similar to rheumatoid arthritis. Rheumatoid arthritis is an auto-immune condition in which the immune system attacks and damages the lining of the joints, resulting in pain and inflammation. The most common auto-immune disorder is rheumatoid arthritis, which is followed by joint inflammation. In the Siddha system of medicine, there are various formulas based on herbal, metal, mineral, and animal origins. They pay more attention to improving the quality of life. So the Siddha system of medicine has many effective medicines for Rheumatoid athritis without any side effects. siddha medicine Sagalavaayu chooranam is a polyherbal formulation drug like *sukku (Zingeria officinale), milagu (Piper nigrum), thippili (Piper longum), seeragam (Cuminum cyminum), kadukaai (Terminalia chebula), nellikaai (Phylanthus emblica), thaandrikaai (Terminalia bellerica), vaaividangam*(*Embelia ribes*), *perungayam*(*Ferula asafoetida*), *kadugurohini(veratriviridi rhizome), koshtam*(*costus speciosus*),sadamanjil(*Nardostachys jatamansi*),*elam*(*Elettaria cardamomum*), *athimathuram(Glycyrrhiza glabra), thaalisapathiri(Abies Webbiana),kirambu(Syzygium aromaticum), santhanam(Santalum album), sirunaagapooo(Mesua ferrea), sugar(Saccharum Offinerum),pooneeru(fuller's earth).* In the classical literature "*Dhanwanthiri vaithiyam 1000*," the trial drug ***sagalavaayu*** chooranam was indicated as vatham, pitham, santhu vaatham, ***mudaku vatham***, muga vatham, isupu vatham, and sagala vaayu. Standardizing herbal formulations is a crucial step in determining the potency of medicines based on the concentration of their active ingredients and other physicochemical and phytochemical characteristics. In order to support their acceptance in the contemporary medical system, the quality evaluation of herbal formulations is of utmost significance. The absence of strict quality control profiles for herbal resources and their formulations is one of the biggest issues the herbal sector is dealing with. The need to ensuring quality control of medicinal plant products by employing modern techniques and appropriate standards has been stressed by the WHO in a number of resolutions. As it directly affects how effectively herbal medications work, the phytochemical profile is particularly important. A complex analytical technique with numerous marker-based standardisations is HPTLC. It offers both qualitative and quantitative data on a drug, allowing for an evaluation of the drug's quality. Sagalavaayu Chooranam

used physicochemical and phytochemical factors to explain the standardisation parameters in this study. As per PLIM guidelines, the study validation was completed.

MATERIALS AND METHODS

selection of medications

The traditional Siddha book "Dhanwanthiri vaithiyam 1000," published by Thamarai Noolagam, Page No. 135 is where the trial medication "sagalavaayu chooranam" was consumed.

Table.no:01 INGREDIENTS OF THE MEDICATION

S.No	*DRUG NAME*	BOTANICAL NAME	QUANTITY
1.	*SUKKU*	*Zingeria officinale*	1 *palam*(35gm)
2.	*MILAGU*	*Piper nigrum*	1 *palam*(35gm)
3.	*THIPPILI*	*Piper longum*	1 *palam*(35gm)
4.	*SEERAGAM*	*Cuminum cyminum*	1 *palam*(35gm)
5.	*KADUKAAI*	*Terminalia chebula*	1 *palam*(35gm)
6.	*NELLIKAAI*	*Phylanthus emblica*	1 *palam*(35gm)
7.	*THAANDRIKAAI*	*Terminalia bellerica*	1 *palam*(35gm)
8.	*VAAIVIDANGAM*	*Embelia ribes*	1 *palam*(35gm)
9.	*PERUNGAYAM*	*Ferula asafoetida*	1 *palam*(35gm)
10.	*KADUGUROHINI*	*veratriviridi rhizome*	1 *palam*(35gm)
11.	*KOSHTAM*	*costus speciosus*	1 *palam*(35gm)
12.	*SADAMANJIL*	*Nardostachys jatamansi*	1 *palam*(35gm)
13.	*ELAM*	*Elettaria cardamomum*	1 *palam*(35gm)
14.	*ATHIMATHURAM*	*Glycyrrhiza glabra*	1 *palam*(35gm)
15.	*THAALISAPATHIRI*	*Abies Webbiana*	1 *palam*(35gm)
16.	*KIRAMBU*	*Syzygium aromaticum*	1 *palam*(35gm)
17.	*SANTHANAM*	*Santalum album*	1 *palam*(35gm)
18.	*SIRUNAAGAPOOO*	*Mesua ferrea*	1 *palam*(35gm)
19.	*POONEERU*	*fuller's earth*	1 *palam*(35gm)
20.	*SUGAR*	*Saccharum Offinerum*	19 *palam*(665gm)

Collection of raw drug:

The raw drugs *sukku (Zingeria officinale), milagu (Piper nigrum), thippili (Piper longum), seeragam (Cuminum cyminum), kadukaai (Terminalia chebula), nellikaai (Phylanthus emblica), thaandrikaai (Terminalia bellerica), vaaividangam(Embelia ribes), perungayam(Ferula asafoetida), kadugurohini(veratriviridi rhizome), koshtam(costus speciosus),sadamanjil(Nardostachys jatamansi),elam(Elettaria cardamomum), athimathuram(Glycyrrhiza glabra), thaalisapathiri(Abies Webbiana),kirambu(Syzygium aromaticum), santhanam(Santalum album), sirunaagapooo(Mesua ferrea), sugar(Saccharum Offinerum),pooneeru(fuller's earth)* were purchased from the authenticated raw medicine retailer RN RAJAN and CO, Parrys, Chennai, Tamil Nadu

Identification and verification of the drug:
In the Government Siddha Medical College in Arumbakkam, Chennai, Gunapadam professionals recognised and verified all drugs. Each sample has been labelled as …….... For future use as references, the identified product samples were kept in the PG Gunapadam laboratory.

Drug preparation and purification:
According to traditional Siddha literature Sarakkugalin suthi sei muraigal, the purifying procedure was followed. The ingredients listed above were taken and ground into a fine powder in the specified amount. *Sagalavaayu* Chooranam (SVC) was made by sieving the powder with a thin cotton cloth and keeping it in a clean, airtight container.

DRUG PROFILE
Route of Administration : Enteral route
Dose : *verukadi alavu (1354mg)*
Adjuvant: Warm water
Indication: *vatham ,pitham, santhu vaatham, mudaku vatham, muga vatham, isupu vatham, sagala vaayu*

Organoleptic properties :
The state, nature, odor, feel, flow property, physical appearance, and taste were noted from the prepared drug SVC.
Physicochemical analysis :
The following physicochemical analysis was done and their results were noted.
Loss on drying
An accurately weighed 1g of SVC formulation was taken in a tarred glass bottle. The crude drug was heated at 105ºC for 6 hours in an oven at a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

Total Ash
Weighing accurately 2g of SVC formulation was added in the crucible at a temperature 600ºC in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.
Acid insoluble ash
Ash above obtained from SVC, was boiled for 5 min with 25 ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.
Water soluble ash
Total ash of SVC, 1g was boiled for 5 min with 25 ml water and insoluble matter collected on an ash-less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450ºC in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.
Water soluble extractive
5gm of air dried drug, fine powdered SVC was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 100ºC and weighted. The percentage of water soluble extract was calculated with reference to the air dried drugs.
Acid soluble extractive
1 gm of air dried drug fine powdered SVC was macerated with 20 ml alcohol in a closed flask for 24 hours. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100ºC and weighted. The percentage of alcohol soluble extract was calculated with reference to air dried drug.
Solubility :
pH determination :
Phytochemical analysis :
The following tests were carried out for the phytochemical analysis. Preliminarily the sample was dissolved with dilute Hydrochloric acid for several tests.
Test for Alkaloids :
Mayer's test
Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow color precipitate indicates the presence of alkaloids.
Dragendroff's test
Filtrates were treated with Dragendroff's reagent (Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.
Wagner's test
Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
Test for Carbohydrates :
Molisch's test

To 2 ml of plant sample extract, two drops of alcoholic solution of α-naphthol are added. The mixture is shaken well and a few drops of concentrated sulphuric acid are added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

Benedict's test

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Saponin :

Foam test

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Test for Phenols :

Ferric chloride test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Test for Tannin :

Gelatin test

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

Test for Flavonoids

Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless in addition to dilute acid, indicates the presence of flavonoids.

Lead acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Test for Diterpenes :

Copper acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicating the presence of Quinones.

Gum and Mucilage

To 1 ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examined for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

Biochemical analysis :

5 gm of SVC was dissolved with 50 ml of distilled water. Boiled well for 10 minutes and cooled. Filtered the extract and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Analysis of Specific acid radicals :

Test for Carbonates :

1 ml of the test solution was added with 1 ml of concentration (conc.) HCl. Formation of brisk effervescence indicates the presence of carbonates.

Test for chlorides

2 ml of test solution was added with about 1 ml of silver nitrate solution. Appearance of White precipitate indicates the presence of chlorides.

Test for sulfates

1 ml of the test sample was added to dilute H_2SO_4 till effervescence ceases followed by this about 1 ml of barium chloride solution was added. Appearance of white precipitate indicates the presence of sulfates.

Test for sulfides

1 ml of the test sample about 2 ml of HCl was added with slight warming the mixture. Formation of colorless gas with the smell of rotten egg indicates the presence of sulfides.

Test for phosphates

2 ml of test solution treated with 2 ml of Ammonium molybdate solution followed by addition of 2 ml of concentrated nitric acid. Formation of yellow precipitate Indicates the presence of phosphates.

Test for Fluoride and Oxalate

2 ml of the test solution about 2 ml of dil acetic acid and 2 ml of Calcium chloride solution was added. Formation of white precipitate Indicates the presence of Fluoride/ Oxalate.

Test for Borates

2ml of the test solution was added with sulphuric acid and 95% alcohol followed by exposure to flame. Appearance of green flame Indicates the presence of Borates.

Test for Nitrates

0.5 ml of test solution heated with copper turning followed by addition of sulphuric acid. Appearance of reddish brown gas Indicates the presence of Nitrates.

Analysis of Specific basic radicals :

Test for Lead

1 ml of the test solution added with 2 ml of potassium chromate solution. Formation of yellow precipitate indicates the presence of lead.

Test for Arsenic

1 ml of the test solution added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution. Formation of brownish red precipitate indicates the presence of Arsenic.

Test for Mercury

1 ml of the test solution added with 2 ml of 10% (2N) sodium hydroxide (NaOH) solution. Formation of yellow precipitate indicates the presence of mercury.

Test for Copper

1 ml of the test solution added with 1 ml of Ammonium hydroxide (NH_4OH) solution. Formation of blue precipitate indicates the presence of copper.

Test for Ferric

1 ml of test solution, about 2 ml of potassium ferrocyanide was added. Formation of blue precipitate indicates the presence of ferric.

Test for Ferrous

1 ml of test solution, about 1 ml of potassium ferricyanide solution was added. Formation of blue precipitate indicates the presence of ferrous.

Test for Zinc

1 ml of the test solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears. Formation of white precipitate indicates the presence of Zinc.

Test for Silver

1 ml of the test solution was added with 1 ml of conc. HCL followed by the appearance of curdy white precipitate. Boil the precipitate with water. It does not dissolve. Add NH_4OH solution in it and add 1 ml dilute HNO_3. Formation of curdy white precipitate indicates the presence of silver.

Test for Magnesium

1 ml of the test solution added with 2 ml of sodium hydroxide (NaOH) drop wise until indication appears. Formation of white precipitate indicates the presence of Magnesium.

Particle size determination :

It was carried out by dissolving the sample with distilled water in 1/100th dilution. Mounted to the slide and viewed under an optical microscope. The average size of the particles were calculated.

Identification - HPTLC :

Pre-coated HPTLC graded plates and auto sampler were used to achieve precision, sensitive, significant separation both qualitatively and quantitatively.

Instrument - CAMAG TLC SCANNER III

TLC Plate - Aluminium Coated Silica Gel – Merck

Mobile Phase - Chloroform: n-Butanol: Methanol: Water: Acetic Acid (4:1:1:0.5:0.5)

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

Heavy metal analysis :

The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determine 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 samples were digested with 1 mol/L of HNO3.

Standard preparation : As & Hg- 100 ppm sample in 1 mol/L HCl

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

Sterility test :

Pour plate method

SVC was mixed with sterile distilled water and the mixture was used for the sterility evaluation. About 1 ml of the SVC extract was inoculated in sterile petri dish to which about 15 ml of molten agar 45ºC was added. Agar and SVC were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it for about 10 minutes. Plates were then inverted and incubated at 37ºC for 24 - 48 hours and further extended for 72 hours for fungal growth observation. Grown colonies of organisms were then counted and calculated for CFU.

Table no: 02 - **Microbial load**

Test	Specification
Total bacterial count	NMT 10^5CFU/g
Total fungal count	NMT 10^3CFU/g

Test for specific pathogens :

Test sample was directly inoculated into 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 pathogens identified by their characteristic color with respect to pattern of colony formation in each differential media.

Table No 03 Specific Medium and their abbreviation

Organism	Abbreviation	Medium
Escherchia coli	*EC*	*EMB Agar*
Salmonella spp.	*SA*	*Deoxycholate agar*
Staphylococcus Aureus	*ST*	*Mannitol salt agar*
Pseudomonas Aeruginosa	*PS*	*Cetrimide Agar*

Pesticide residue :

Test sample was extracted with acetone and followed by homogenization for a brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of the test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent had 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 a membrane filter.

Aflatoxins :

Standard - Aflatoxin B1
Aflatoxin B2
Aflatoxin G1
Aflatoxin G2

Solvent - Standard samples was dissolved in a mixture of chloroform and acetonitrile (9.8 : 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Standard aflatoxin was applied on the surface to pre-coated TLC plates 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

RESULTS

Organoleptic properties

Fig.no:01 - Prepared SVC

SVC is soft and brown in appearance, smells extremely aromatic, and tastes astringent and little salty

Table.no:04 - Organoleptic properties of SVC

S.No	Parameter	Result
1.	State	Solid
2.	Nature	Fine
3.	Odour	Strongly aromatic
4.	Touch	Soft
5.	Flow property	Non-free flowing
6.	Appearance	Brown in color
7.	Taste	astringent and slightly salty

Table.no:05 Physicochemical analysis :

The observed values of the physic-chemical properties are given below

S.No	Parameter	Result
1.	Loss on drying	8.86%
2.	Total Ash	1.55%
3.	Acid insoluble ash	0.93%
4.	Water soluble ash	1.96%
5.	Water soluble extractive	19.98%
6.	Acid soluble extractive	11.20%
7.	pH	7.5

Solubility :

Table.no:06 - Solubility test of SVC

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

Phytochemical analysis :

Table.no:07 - Phytochemical analysis of SVC

S.No.	Phytochemicals	Test Name	H_2O Extract
1	Alkaloids	Mayer's Test Dragendroff's Test Wagner Test	-ve -ve +ve

2	Carbohydrates	Molisch's Test Benedict Test	+ve +ve
3	Saponin	Foam Test	+ve
4	Phenols	Ferric Chloride Test	+ve
5	Tannins	Gelatin Test	+ve
6	Flavonoids	Alkaline Reagent Test Lead acetate	+ve +ve
7	Diterpenes	Copper Acetate Test	-ve
8	Quinones	Test for Quinones	+ve
9	Gum & Mucilage	Test for Gum & Mucilage	+ve

Fig.no:02 - Phytochemical screening of SVC

Biochemical analysis :

Table.no:08 - Test for acid radicals results

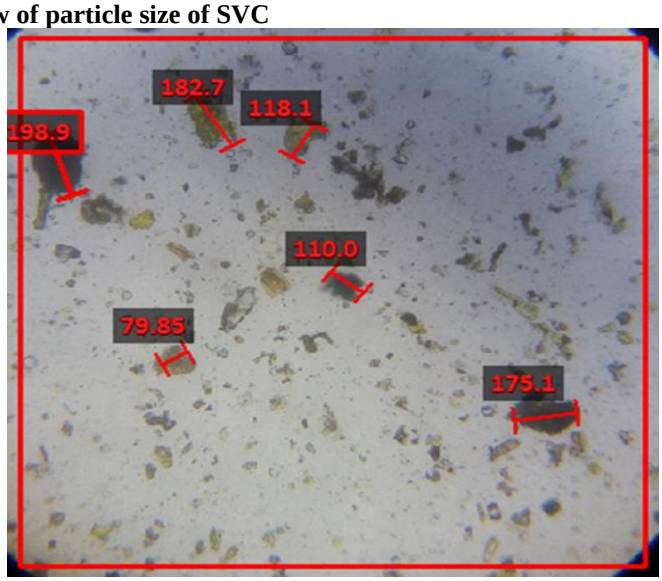
S.No	Specific radical	Observation	Test report
1.	Test for carbonates	Presence of brisk effervescence	Presence
2.	Test for chlorides	Absence of White precipitate	Absence
3.	Test for sulfates	Presence of white precipitate	Presence
4.	Test for sulphides	Absence of rotten egg smell	Absence
5.	Test for phosphates	presence of yellow precipitate	presence
6.	Test for Fluoride & Oxalate	Absence of white precipitate	Absence
7.	Test for Borates	Absence of green flame	Absence
8.	Test for Nitrates	Absence of reddish brown color	Absence

Table.no:09 - Results of Test for basic radicals

S.No	Specific radical	Observation	Test report
1.	Test for Lead	Presence of yellow precipitate	Presence
2.	Test for Arsenic	Absence of brownish red precipitate	Absence
3.	Test for Mercury	Presence of yellow precipitate	Presence
4.	Test for Copper	Absence of blue precipitate	Absence
5.	Test for Ferric	Absence of blue precipitate	Absence
6.	Test for Ferrous	Absence of blue precipitate	Absence
7.	Test for Zinc	Absence of white precipitate	Absence
8.	Test for Silver	Absence of curdy white precipitate	Absence
9.	Test for Magnesium	Absence of white precipitate	Absence

Particle size determination :

Fig.no:03 - Microscopic view of particle size of SVC



Identification - HPTLC :

HPTLC finger printing analysis of the sample reveals the presence of four prominent peaks corresponds to the presence of four versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.01 to 0.53.

Fig.no:04 TLC Visualization of SAVC at 366 nm

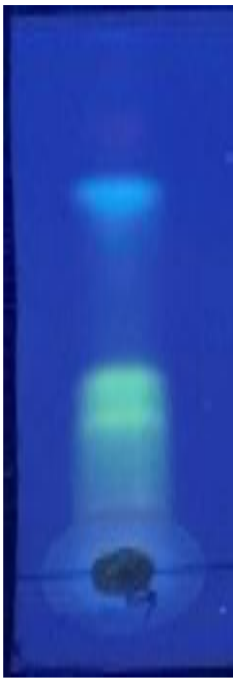


Fig.no:05 - 3D chromatogram

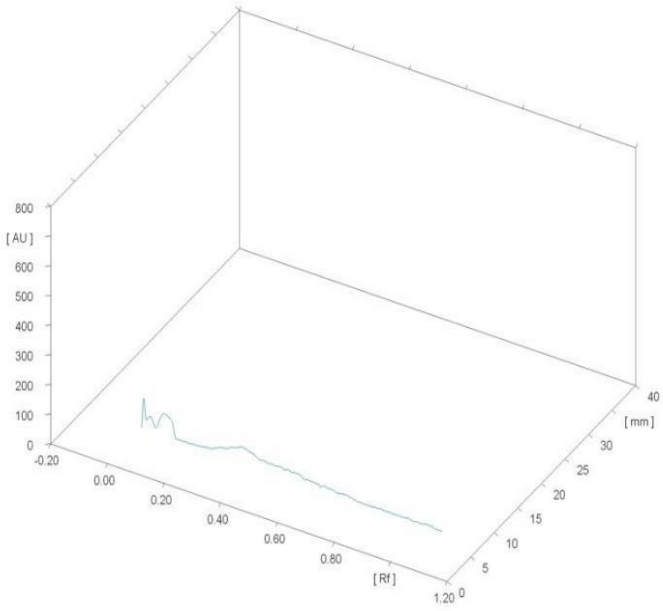


Fig.no:06 - HPTLC fingerprinting of SVC

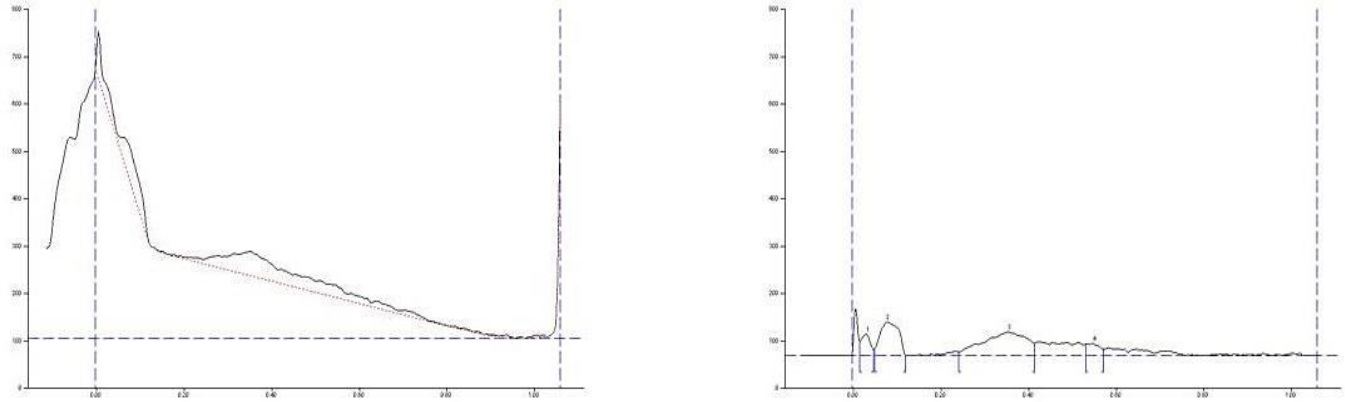


Table.no:10 - Peak table of SVC

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.01	29.8	0.03	45.2	23.36	0.05	13.0	535.4	9.94
2	0.05	14.2	0.08	71.5	36.92	0.12	0.1	1661.7	30.86
3	0.24	7.7	0.35	50.6	26.14	0.42	26.5	2750.7	51.08
4	0.53	24.5	0.55	26.3	13.59	0.57	13.7	437.4	8.12

Heavy metal analysis :

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metal such as Arsenic and Cadmium, whereas the sample shows the presence of Lead and Mercury 0.92 and 0.57 PPM level as listed in the table.

Table.no:11 - Heavy metal analysis report

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	**217.0 nm**	**0.92 ppm**	**10 ppm**
Arsenic	**193.7 nm**	**BDL**	**3 ppm**
Cadmium	**228.8 nm**	**BDL**	**0.3 ppm**
Mercury	**253.7 nm**	**0.57 ppm**	**1 ppm**

BDL- Below Detection Limit ppm - Parts per million

Sterlity test :

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

Fig.no:07 - Results of sterlity test - pour plate method.

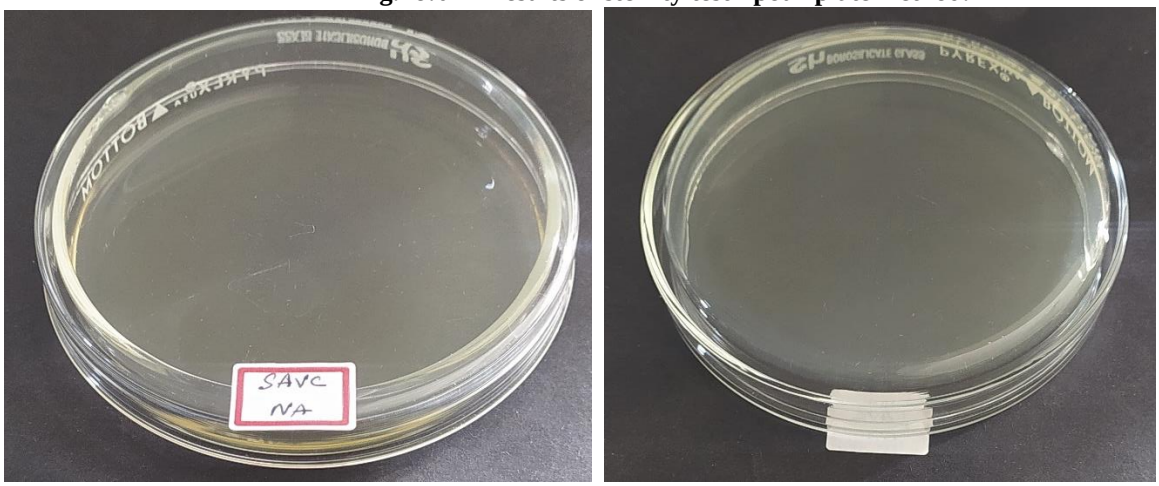


Table.no:12 - Results of sterility test -pour plate method

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**	

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

Test for specific pathogen :

The results of tests for specific pathogens showed the absence of *E.coli, Salmonella spp, Staphylococcus aureus, Pseudomonas aeruginosa* in SVC.

Table.no:13 - Test for specific pathogen in SVC

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

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

Fig.no:08 Culture plate with E-coli (EC) specific medium

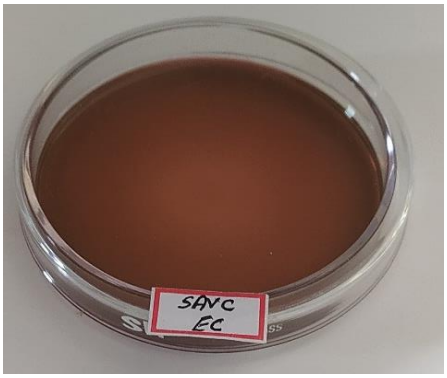
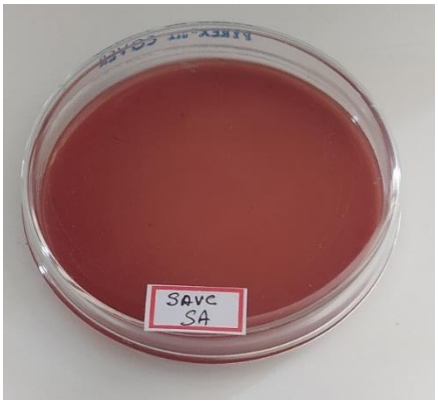


Fig.no:09 Culture plate with Salmonella (SA) specific medium



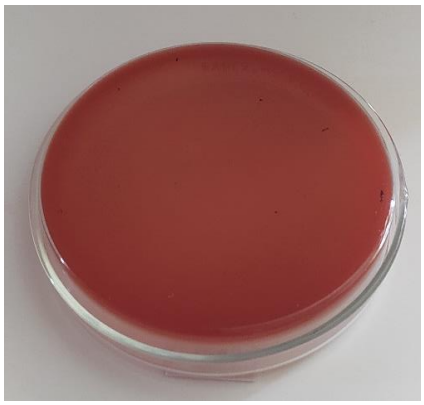


Fig.no:10 Culture plate with Staphylococcus Aureus (ST) specific medium

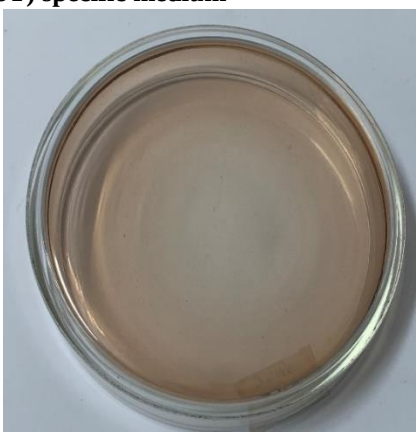
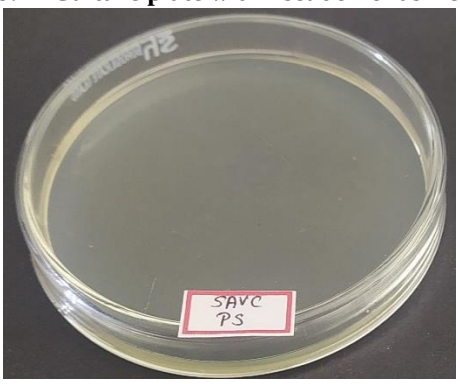
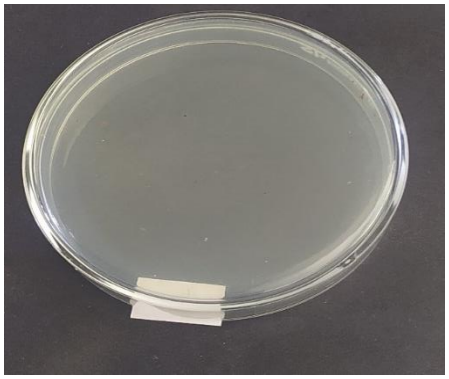


Fig.no:11 Culture plate with Pseudomonas Aeruginosa (PS) specific medium





Pesticide residue :

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

Table.no:14 Test Result Analysis of the Sample SVC

Pesticide Residue	Sample SVC	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)

Aflatoxins :

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.

Table.no:15 Aflatoxin test result

Aflatoxin	Sample SVC	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
B2	Not Detected - Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected - Absent	0.1 ppm (0.1mg/kg)

DISCUSSION

Preclinical research focuses on a drug's safety and efficacy and comprises in vitro and in vivo trials. One of the most important procedures is to standardise the sample drug SVC, which ensures the medicine's quality. SVC was soft and brown in colour, had a strong aromatic odour, and tasted tannic and slightly salty. The drying loss of SVC was 8.86%, indicating that the moisture content of a medication should not exceed 0.01 g. The total ash value of SVC was 1.55%, the acid-insoluble ash value was 0.93%, and the water-soluble ash value was 1.96%, indicating that the medication contains less siliceous compounds. The water-soluble extractive value of 19.98% and the alcohol-soluble extractive value of 11.20% were useful to determine the phytoconstituents of the drug SVC. Phytochemical tests show that SVC contains alkaloids, carbohydrates, saponin, phenols, flavonoids, quinones, gum, and mucilage. Tests for acid radicals show that carbonate and sulphate are present. A test for basic radicals shows that lead and mercury are present. HPTLC fingerprinting analysis of SVC reveals the presence of eight prominent peaks, which correspond to the presence of four versatile phytocompounds present within it. The heavy metal toxicity of herbal products depends upon their bioavailability and the cation exchange of the soil in which they are grown. The bioaccumulation of these heavy metals causes oxidative stress and a broad spectrum of toxic effects in plants. The drug SVC contains arsenic and cadmium below the detection limit, whereas lead and mercury are at 0.92 and 0.57 ppm, respectively, within their mentioned limits. The sterility test showed that no growth or colonies of any microbial growth were observed, which ensured the microbial sterility of SVC. Tests for specific pathogens showing the absence of microbial growth in their medium explain that the drug was not infected by E. coli, *Salmonella spp., Staphylococcus aureus, or Pseudomonas aeruginosa.* Tests for pesticide residue in a drug showed that there were no traces of pesticide residues such as organochlorine, organophosphorus, organocarbamates, and pyrethroids—the most important parameters in plant-based herbal drugs, which in turn are contaminated by various agricultural practises. Aflatoxins are dangerous mycotoxins found in agricultural products, yet the herbomineral medicine SVC was shown to be free of Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2, demonstrating the treatment's safety.

CONCLUSION

The first and most critical phase in the medication development process is standardisation. It was done in accordance with the PLIM guidelines' analytical requirements for chooranam (fine powder). Based on the findings of the following tests, the medicine SVC is considered extremely safe for oral administration. While preclinical in-vitro research on the medication

Sagalavaayu Chooranam have been completed, additional in-vivo and clinical investigations are needed to demonstrate its therapeutic efficacy.

REFERENCES:

1. WHO international standard terminologies on Siddha medicine. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA3.0 IGO
2. Thiyagarajan R, Gunapadam (Thathu Jeeva Vaguppu) part II & III, Directorate Indian Medicine & Homoeopathy, Chennai
3. Ramachandran SP Dhanwanthiri vaithiyam 1000 [art 1.Chennai: Thamarai noolagam;1996.Page.no:135
4. Pharmacopeial Laboratory for Indian Medicine [PLIM] Guideline for Standardization and evaluation of Indian medicine which include drugs of Ayurveda, Unani and Siddha systems, Department of AYUSH, Ministry of Health and Family Welfare, Govt of India.
5. Aanaivaari Anandhan. Sarakku Suththi Sei Muraigal. Indian Medicine and Homeopathy Department, Chennai-106; 2008.
6. Indian Pharmacopoeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopoeia commission, 2014.
7. Xu Z. Particle and Size Distribution. *Fundamentals of Air Cleaning Technology and Its Application in Cleanrooms*. 2013;1-46. Published 2013 Aug 7. doi:10.1007/978-3-642-39374-7_1
8. Wagner H. Plant Drug Analysis. A thin Layer chromatography Atlas.2nd ed. Heidelberg: Springer-Verlag Belgium; 2002:305, 227.
9. Lukasz Komsta, Monika Waksmundzka-Hajnos, Joseph Sherma . Thin Layer Chromatography in Drug Analysis . CRC Press, Taylor and Francis.
10. Fazal Ur Rehman Shah, Nasir Ahmad, Khan Rass Masood, Jose R Prelta- Videa, Firoz ud Din Ahmad: Heavy metal toxicity in plants: July 2010.
11. Pour Plate Method: Procedure, Uses, (Dis) Advantages • Microbe Online [Internet]. Microbe Online. 2022 [cited 13 April 2022]
12. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—11th ed. CLSI document M02- A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
13. WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues. WHO Geneva. 2007.
14. Nema S Shaban , Khaled A Abdou. Nour El-HoudaY Hassan : Impact of toxic heavy metals and pesticide residues in herbal products ; Beni-Suef University Journal of Basic and Applied Sciences : Vol-5, Issue-1 , MArch2016, pg.no -102-106
15. Lohar. D.R. Protocol for testing of ASU medicines. Pharmacopoeial Laboratory for Indian Medicines. Ministry of AYUSH. 2007.
16. Luciana de CASTRO. Determining Aflatoxins B1, B2, G1 and G2 in Maize Using Florisil Clean Up with Thin Layer Chromatography and Visual and Densitometric Quantification. Ciênc. Tecnol. Aliment. vol.21 no.1 Campinas. 2001.
17. David G. Schmale III, Gary P.Munkvold , Mycotoxins in crops: A threat to Human and Domestic animal health, The American phytopathology society.