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STANDARDIZATION AND PHYSIO-CHEMICAL ANALYSIS OF VARMA POLYHERBAL FORMULATION SITRAMUTTI KIYAZHAM- I

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ABSTRACT

Introduction: Siddha system of medicine is a traditional system of medicine. Siddha is themother medicine of ancient Tamils/Dravidians of peninsular South India. The word siddha means established truth. Varmam is the branch of siddha system of medicine. Varma maruthuvam is based on the energy flow that flows throughout the body. Most of the varma medicines are effective but they have not been standardized yet. Standardization of Siddha Varma Polyherbal formulation becomes essential to establish the monograph of the particular formulation along with this, it encompasses the Genuity, purity and safety of the preparations intended for usage in patients. "SITRAMUTTI KIYAZHAM- I (SK-I)", a polyherbal formulation in varma medicine, is traditionally used to varma kaayam (pain), thodu varmangal, padu varmangal, thatu varmangal. **Aim and objective:** To standardize "SITRAMUTTI KIYAZHAM- I (SK-I)", a siddha varma polyherbal formulation using physiochemical analysis and other analytical techniques such as HPTLC and TLC, Heavy metal analysis. **Materials and Methods:** Varma polyherbal formulation "Sitramutti kiyazham –I (SK-I)", drug mentioned in the textbook of "Formulary of Varma medicine (Varma marunthu sei muraigal 5th edition)" which is used to treat varma kaayam (pain). A scientificapproach was undertaken to standardize the varma polyherbal formulation SK-I by conducting a through physiochemical evaluation, HPTLC and TLC thereby ensuring the establishment of its quality and efficacy parameters. **Results:** The results obtained from physicochemical evaluation shows that the Total ash value of Sitramutti kiyazham –I (SK-I) was "0.4±0.226%", in which the acid insoluble ash was "0±0", loss on drying at 105ºC of the formulation SK-I was noted to be "4.867±0.450" in which water soluble extract value and

alcohol soluble extract value was “19.48±2.66”, and “2.81±0.055” respectively. The pH value of the drug 7.15 which is indicates it is slightly alkaline (basic). HPTLC finger printing analysis of the sample reveals the presence of eight prominent peaks corresponds to the presence of eight components present with in it. Heavy metal analysis by AAS result shows no traces of heavy metals such as Mercury, Arsenic, Lead and Cadmium. Microscopic observation of the particle size analysis reveals that the average particle size of SK-I was found to be 112.9±32.5 µm. **Conclusion:** The preliminary physiochemical analysis of the siddha drug SK-I provides a foundational fingerprint for future clinical studies, there by facilitating the establishment of its safety, efficacy and quality parameters.

KEYWORDS: Sitramutti kiyazham - I, SK-I, Varma polyherbal formulation, Physiochemical analysis, HPTLC.

INTRODUCTION

Varmam is based on energetics – Life energy. Life energy is concentrated at somevital points in the human body where bones, tendons, nerves, blood vessels come together and over the vital organs like brain, heart, lungs, liver, spleen etc., These points are called as Varmam points. The total number of Varmam points in human body are more than a thousandamong which 108 are important points (Padu varmam 12, Thodu varmam 96).Many points are bilateral points lying in both the halves of the body, others which lie in the axis are unpaired points. Siddha Varmam is non - invasive and time saving. Varmam is the Hall Markof Siddha Medicine based on vital energy circulation in our body. Any disturbance in the flowof this energy leads to disease. Varmam Points are the places where this vital energy resides and activates both body and mind. By the proper stimulation of Varmam Points, restoration of normal function of body is possible. Varmam treatment includes two main elements varmam physical manipulation and varmam medicines (Kiyazham, Ennai, Chooranam, Nei/Gritham.etc). Herbal drugs have been used widely because of its availability, cost effectiveness and safer than other drugs. Varma drugs requires standardization for global acceptance. Thus, the author preferred to choose Varma drug SK-I for standardization. It was aimed to validate the qualitative and quantitative screening for SK-I scientifically to prevent adulterations. Thus, Organoleptic properties, Physio-chemical parameters, HPTLC finger printing aspects according to PLIM guidelines, Heavy metal analysis by AAS were carried out in this drug.

MATERIALS AND METHODS

SELECTION OF THE TRIAL DRUG: In this research paper purified and prepared "SK-I" was taken as a trial drug for Lumbar spondylosis from the varma literature "Formulary of Varma medicine (Varma marunthu sei muraigal 5th edition pg. no-57)"

Table No 1: Ingredients with Botanical name of Sitramutti kiyazham –I (SK-I).

INGREDIENTS	BOTANICAL NAME	FAMILY	PARTSUSED	RATIO
Sitramutti	*Sida cordifolia*	Malvaceae	Root	Equal ratio
Isangu	*Cleodendrum inerme*	Lamiaceae	Root	Equal ratio
kandankathiri	*Solanum surattense*	Solanaceae	Root	Equal ratio
Senjitti	*Tragia involucrata*	Euphorbiaceae	Root	Equal ratio
Vilvam	*Aegle marmelos*	Rutaceae	Root	Equal ratio
Nathaisoori	*Spermacoce hispida*	Rubiaceae	Root	Equal ratio
Chinni	*Acalypha fruiticosa*	Euphorbiaceae	Root	Equal ratio
Adathodai	*Adathoda vasica*	Acanthaceae	Root	Equal ratio
Sundai	*Solanum torvum*	Solanaceae	Root	Equal ratio
Arathai	*Alpinia galangala*	Zingiberaceae	Rhizome	Equal ratio
Amukkura	*Withania somnifera*	Solanaceae	Root tubers	Equal ratio
Athimathuram	*Glycyrrhiza glabra*	Leguminosae	Root tubers	Equal ratio
Meerai	*Commiphora myrrha*	Burseraceae	Resin	Equal ratio

COLLECTION OF THE DRUG MATERIALS: The raw drugs was collected from MSS Aasaan and sons store Nagarcoil, Kanyakumari district, Tamilnadu.

IDENTIFICATION AND AUTHENTICATION OF THE DRUGS: All the raw materials were identified and authenticated by the Botanical and Pharmacological experts at Government Siddha Medical College, Arumbakkam and Chennai.

METHODS OF PURIFICATION: In SK-I all the drugs were dried Root except *Alpinia galangala* (dried Rhizome), *Withania somnifera* (dried Root tubers), *Glycyrrhiza glabra* (dried Root tubers) *and Commiphora myrrha* (Resin). The root, root tubers and resin were cleaned with white cloth.

PREPARATION OF THE DRUG: Equal amount of each of purified *Sida cordifolia, Cleodendrum inerme, Solanum surattense, Tragia involucrata, Aegle marmelos, Spermacoce hispida, Acalypha fruiticosa, Adathoda vasica, Solanum torvum, Alpinia galanga, Withania somnifera, Glycyrrhiza glabra, Commiphora myrrha* are taken and ground into coarse powder using a store motor. To this 1.3 L of water is added and heated until it gets reduced to 1/8th part to form Sitramutti kiyazham - I.

ADMINISTARTION OF THE DRUG

Form of the medicine: Kiyazham (decoction)Route of administration: Oral (Internal)

Dose: 60ml twice a day depending on the severityShelf life: 3 hours.

INDICATION: *Varma kaayam, Thatuvarmangal, paduvarmangal, thodu varmangal,aathara varamam, ull varamam, sayavarmam, nadukku varmam.*

STANDARDIZATION OF THE DRUG

1. Organoleptic characters of SK-I

State, nature, odour, touch, flow property, appearance of the drug was noted.

2. Physiochemical properties of SK-I

A. Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105°c for 5 hours and then weighed.

B. Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400^{o}C until it turns white in colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

C. 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.

D. Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o}C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

E. Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four

hours, shaking frequently during six hours and allowing it to stand and for eighteen hours.

Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o}C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

F. pH determination

Required quantity of test sample was admixed with distilled water and the subjected to screening using pH meter.

3. Heavy metal analysis by AAS

Methodology: Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model 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 samplewere digested with 1mol/L of HNO3.

Standard reparation: As & Hg- 100 ppm sample in 1mol/L HCl, Cd & Pb- 100 ppmsample in 1mol/L HNO3.

4. Particle size determined by Microscopic method

Particle size determination was carried out by the optical microscopic method. In which the sample was dissolved in sterile distilled water (app 1/100th dilution). Diluted samples were mounted on the slide and fixed with the stage of an appropriate location. Light microscopic images were drawn with a scale micrometer to arrive at the average particlesize. Minimum of 30 observations were made to ascertain the mean average particle sizeof the sample.

5. Thin Layer Chromatography (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.

6. 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 phototherapeutics.

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 sample and their respective Rf values were tabulated.

RESULTS

PHYSIOCHEMICAL ANALYSIS

Table 2: Organoleptic character of SK-I.

State	Solid	Liquid
Nature	Woody Fibrous	Non Viscous
Odour	Characteristic	Mild odour
Touch	Hard	Non greasy
Flow Property	Non Free flowing	Free Flowing
Appearance	Dark Brownish	Whitish

Table 3: Solubility Profile of SK-I.

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

Table 4: Physiochemical evaluation of SK-I.

S. No	Parameter	Mean (n=3) SD
1.	***Loss on Drying at 105 °C (%)***	4.867 ± 0.450
2.	***Total Ash (%)***	0.4 ± 0.226
3.	***Acid insoluble Ash (%)***	0 ± 0
4.	***Water soluble Extractive (%)***	19.48 ± 2.66
5.	***Alcohol Soluble Extractive (%)***	2.81 ± 0.055
6.	***pH***	7.15

HEAVY METAL ANALYSIS BY AAS

Table 5: Heavy metal analysis results of SK-I.

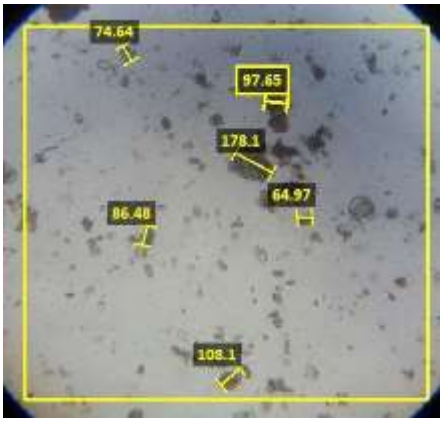
Name of the HeavyMetal	Absorption MaxΛ max	Result Analysis	MaximumLimit
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

BDL- Below Detection Limit

Results of the present investigation have clearly shows that the samplehas no traces of heavy metal such as Mercury, Arsenic, Lead and Cadmium.

Particle Size Determination by Microscopic Method

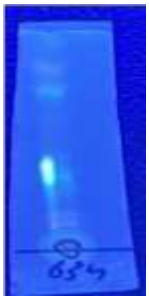
Microscopic Observation of Particle Size for the sample SK-I



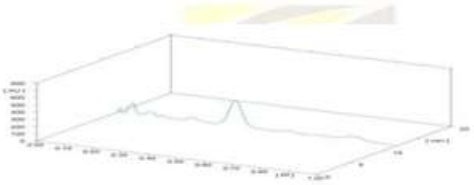
REPORT: Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be 112.9 ± 32.5 µm.

HPTLC REPORT

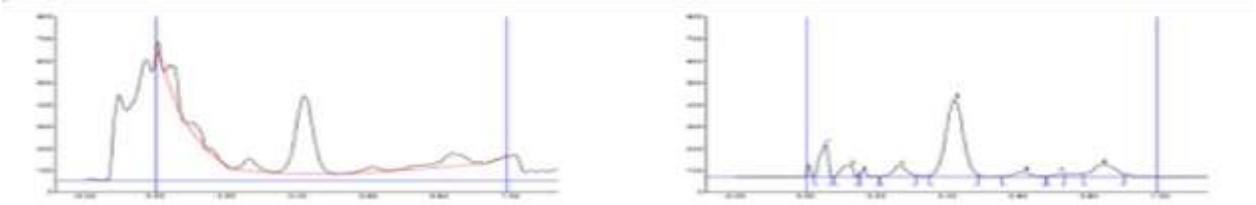
TLC Visualization of SK-I at 366nm

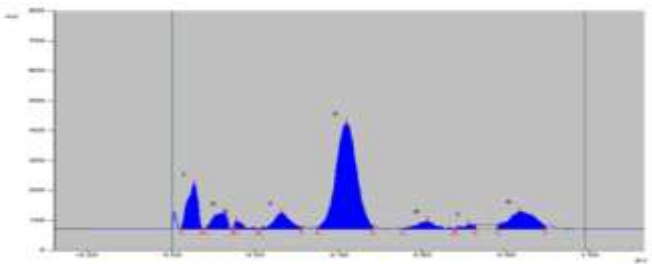


3D - CHROMATOGRAM



HPTLC finger printing of SK-I





Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	4.1	0.06	152.3	20.55	0.07	4.5	1462.6	11.59
2	0.08	2.4	0.13	53.5	7.21	0.15	13.7	748.5	5.93
3	0.15	21.1	0.16	25.6	3.46	0.21	0.0	233.7	1.85
4	0.21	0.0	0.27	54.7	7.38	0.31	7.9	867.6	6.87
5	0.35	5.3	0.42	354.4	47.83	0.49	11.4	7100.7	56.25
6	0.56	1.3	0.62	25.8	3.48	0.68	1.7	494.9	3.92
7	0.68	3.1	0.72	16.1	2.17	0.73	15.4	196.5	1.56
8	0.78	14.3	0.84	58.7	7.92	0.90	11.0	1519.4	12.04

REPORT: HPTLC finger printing analysis of the sample reveals the presence of eight prominent peaks corresponds to the presence of eight components present with in it. Rf value of the peaks ranges from 0.03 to 0.78.

DISCUSSION

The drug SK-I was coarsely powder and dark brownish colour. Fresh preparation of its extract shows non-Greasy, dark brownish with mild odour characteristic. Oral bio availability depends on several factors including aqueous solubility, drug permeability etc. The drug SK-I soluble in specific solvent like ethanol, water and DMSO nearby proves its efficiency of solubility increasing in bio available at in the stomach indirectly. The result derived from the physiochemical evaluation divulge that loss on drying at 105°c(%) value of SK-I was 4.867 ± 0.450 which indicates the moderate moisture content could increase the stability and shelflife of the drug which is suitable for medicine preparation. Total Ash value (%) 0.4 ± 0.226which notes the presence of inorganic components. Water soluble extractive and alcohol soluble extractive value (%) was “19.48±2.66” and “2.81±0.055” respectively which indicates that the drug contains potential for better dissolution and absorption. The pH value of the drug was 7.15 which indicates that the drug is slightly alkaline and that the drug is wellsuited for most biological applications. Results of the heavy metal investigation have clearly shows that the sample has no traces of heavy metal such as Mercury, Arsenic, Lead and Cadmium**.** Microscopic observation of the particle size analysis reveals that the average particle size of SK-I was found to be 112.9±32.5 µm. Rf value of the peaks ranges from 0.03 to 0.78. This

study constitutes a preliminary exploration of SK-I physiochemical properties providing an essential foundation for future research which can build upon these insights to elucidate the complex characteristics of this varma polyherbal formulation. By expanding on this study discoveries, subsequent research can deeper into the therapeutic potential of SK-I, ultimately contributing to a more comprehensive understanding of its properties and applications in traditional medicines.

CONCLUSION

This study successfully standardized Sitramutti kiyazham-I (SK-I) in accordance with PLIM guidelines and established protocols. The comprehensive standardization of this Siddha herbal formulation involved a range of parameters, including Organoleptic characters, Physicochemical parameters, TLC visualization at 366nm and HPTLC fingerprinting analysis. Results of the Heavy metal analysis have clearly shows that the sample has no traces of heavy metal such as Mercury, Arsenic, Lead and Cadmium. The results of this standardization effort will serve as a valuable tool for authenticating and evaluating the safety and quality of SK-I.

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