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STANDARDIZATION OF THE SIDDHA HERBAL DRUG "SAGALA VAAIVU KUTTHALUKU KIYAZHAM" (SVKK)

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ABSTRACT

Standardization of Siddha formulation drug is very essential to justify their acceptability in the modern system of medicine. The herbal drug sagalavaaivukutthalukukiyazham from Siddha text have vital importance in standardization which will encompass the entire field of study from the cultivational of medicinal plants to its clinical application. Here the 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 result of this study clearly proves the quality, purity, safety and potency of the drug which will helps the medicine to survive and succeed in future researches on both clinically and economically.

KEYWORDS: Siddha drug, Sagalavaaivukutthalukukiyazham, TLC and HPTLC.

INTRODUCTION

The Siddha system is one the long lived traditional system 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, the need for 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 observation.

I have chosen the herbal drug "Sagalavaaivukutthalukukiyazham" from the Siddha text of "Thanvandrivaithiyam 1000" for treating all type of vatha diseases. Hence my aim of this study is to evaluate the qualitative and quantitative analysis of SVKK which may also help the medicine for the widespread acceptance of globally, scientifically and economically.

MATERIAL AND METHODS

Sagala Vaaivu Kutthaluku Kiyazham consist of Chukku (zingiberofficinale), Velulli (Allium sativam), Karuvapattai (Cinnamomumverum), Kadugurohini(Picrorhizakurroa), Kazharchikai (Caesalpinia crista), Vasambu (Acorus calamus), Kadugu (Brassica nigra), Kodiveliverpattai (Plumbago zylanica).

Source of Raw Drug and Purification

The raw trial drugs are purchased from a famous traditional raw drug R.N. Rajan shop in Chennai. The raw drugs are authenticated by medicinal botanist in government Siddha medical college Chennai. Then raw drugs are purified separately in gunapadam department laboratory of government Siddha medical college Chennai as per classical text book.

1. Oraganoleptic characters

Sample Description

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. METAL ANALYSIS BY

Standard: Hg and As- 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 SVKK was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury and arsenic concentrations in the test sample SVKK.

Sample Digestion

Test sample SVKK digested with 1mol/L HCl for determination of arsenic and mercury.

Standard reparation

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

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.

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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 quantization 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.

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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 colour.

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% ammonia 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 were been added followed by addition of few drops of conc. Sulphuric 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.

Test for 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 Benedic'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.

Reference: Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol: Wright Scientechnica; 1975:36-45

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 45^0C 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 37^0C for 24-48 hours. Grown colonies of organism was then counted and calculated for CFU.

RESULTS AND DISCUSSIONS

Organoleptic Characters

Table 1: Organoleptic Characters of SVKK.

State	Solid- Crude raw Material	Decoction- Water Extraction-
Appearance	Dark Brownish	Brownish
Nature	Fibre/ Woody	Liquid with micro solutes
Odour	Moderate Aromatic Characteristic	Strong Aromatic Characteristic

Physicochemical Evaluation

Table 2: Physicochemical Evaluation of SVKK.

Final Test report SVKK

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 ℃ (%)	6.93 ± 1.94
2.	Total Ash (%)	14.29 ± 1.74
3.	Acid insoluble Ash (%)	17.73 ± 0.80
4.	Water Soluble Ash (%)	18.2 ± 0.72
5.	Alcohol Soluble Extractive (%)	27.47 ± 0.91
6.	Water soluble Extractive (%)	17.78 ± 5.49
7.	PH	4

Heavy Metal Analysis

Table 3: Heavy Metal Analysis of SVKK Test Report of the Sample SVKK.

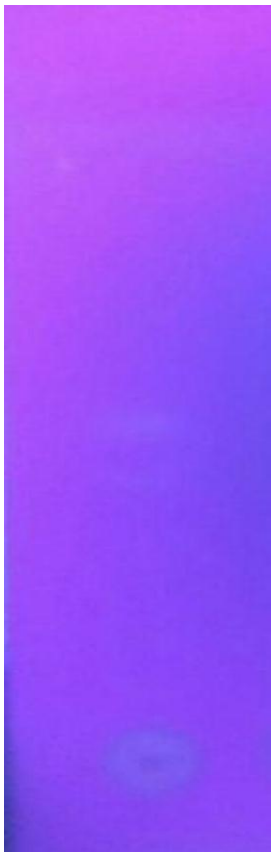
S .No	Name of the Heavy	Absorption Max Λ max	Result Analysis	Maximum Limit
1.	Mercury	253.7nm	BDL	1ppm
2.	Arsenic	193.7nm	BDL	3ppm
BDL- Below Detection Limit				

RESULT

Results of the present investigation has clearly shows that the sample SVKK has no traces of Mercury and Arsenic and hence considered that these heavy metals are absent in the sample SVKK provided for analysis.

TLC and HPTLC

TLC Analysis at 254 nm **TLC Analysis at 366 nm**



HPTLC Finger Printing Of SVKK

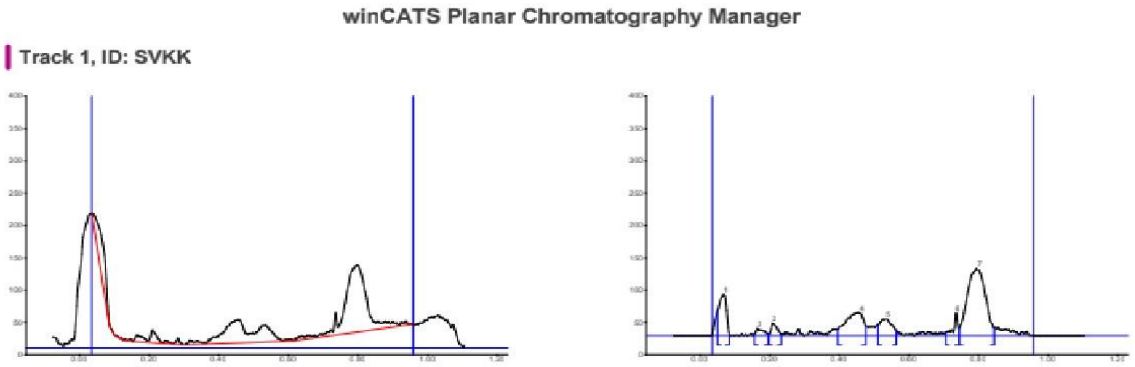


Table 4: HPTLC Analysis of SVKK.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	38.8	0.07	63.6	21.59	0.08	1.4	1037.3	13.27
2	0.16	2.5	0.17	11.0	3.73	0.20	2.9	178.7	2.29
3	0.20	2.5	0.21	19.9	6.75	0.23	2.4	249.7	3.19
4	0.40	10.2	0.46	35.7	12.12	0.48	14.7	1336.9	17.10
5	0.51	15.3	0.54	25.6	8.70	0.56	8.3	677.6	8.67
6	0.71	4.9	0.74	35.0	11.87	0.74	10.6	295.9	3.79
7	0.75	11.7	0.80	103.8	35.23	0.85	12.5	4040.6	51.69

HPTLC finger printing analysis of the sample SVKK reveals the presence of 7 prominent peaks Corresponds to presence of seven versatile components present within it. Rf value of the peaks ranges from 0.05 to 0.75. Further the peak 7 occupies the major percentage of area of 35.23% which denotes the abundant existence of such compound. Followed by this peak 1 and 4 occupies the percentage area of 21.59 and 12.12%.

Phytochemical Analysis

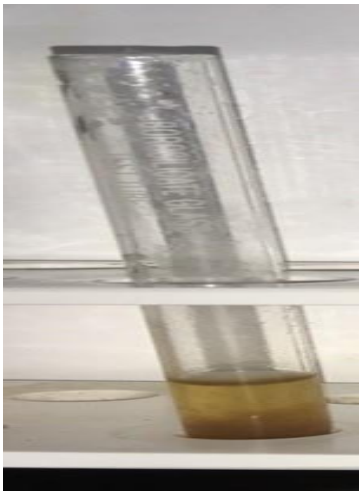
Table 5: Phytochemical Analysis of SVKK.

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.

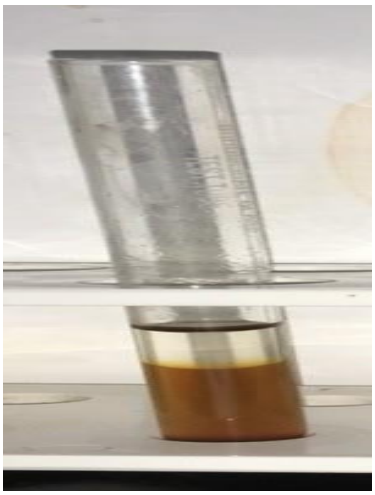
Test for Alkaloids

Test for Flavonoids



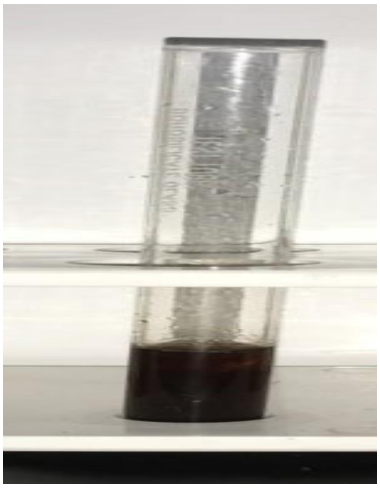
Test for Glycosides

Test for Steroids



Test for Triterpenoids

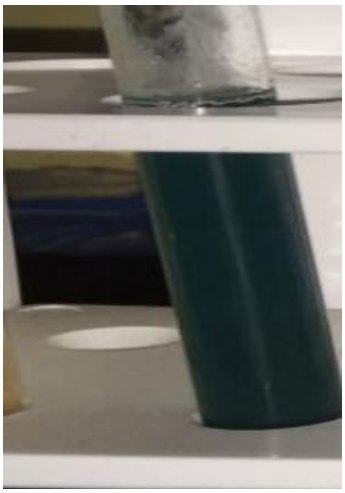
Test for Tanin



Test for Phenols

Test for coumarins

Test for Proteins



Test for Saponins

Test for Carbohydrates

Test for Anthocyanins / Beta cyanins

Sterility test by pour plate method for SVKK.

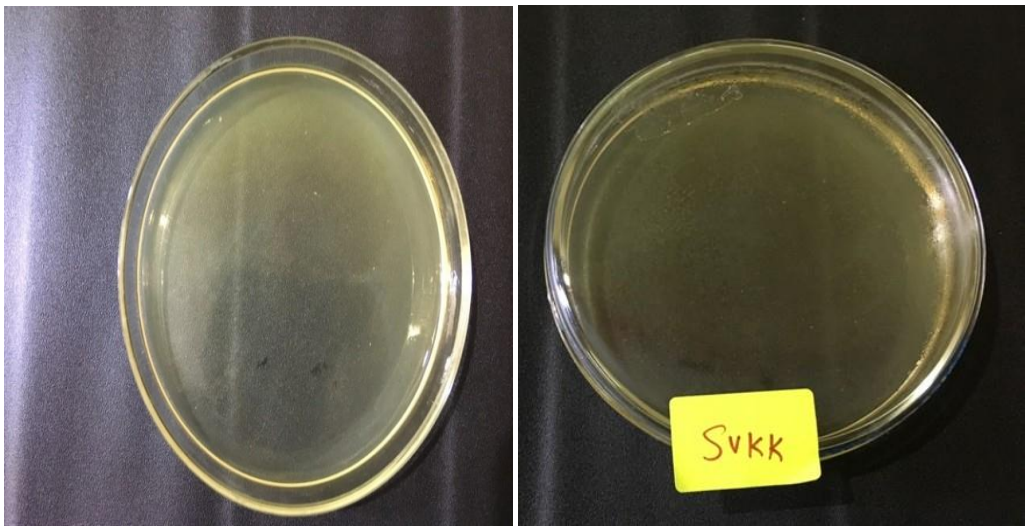


Table: 6 Sterility test by Pour Plate Method for SVKK.

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	

RESULT

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

CONCLUSION

Heavy Metal Analytical study clearly shows that the metals mercury and arsenic seems very low trace when compared to the allowed recommended limit in the sample SVKK. Thus the drug sagalavaaivukutthalukukiyazham is very safe in recommending for the clinical trial.

Phytochemical study indicates the presence of rich flavonoids, steroids, tri terpinoids, phenols, coumarin, tannin, saponins and sugar in SVKK which helps to reduce the inflammation and pain. It shows the identity drug.

Sterility test indicates that there is no growth/colonies were observed in the plates inocutaes with test sample SVKK. It shows the purity of the drug Sagalavaaivukutthalukukiyazham.

This experimental study clearly demonstrate the Qualitative and Quantitative analysis of SVKK. Which will help to conduct further clinical studies and standard researches.

ACKNOWLEDGEMENT

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