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Standardization of *Siddha* Formulation *Soothagavaayu Kudineer Chooranam*

M. Sangeetha[1], B. Bharathi[2], V. Velpandian[3], M. D. Saravanadevi[4]

[1]PG Scholar, Postgraduate Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-106, Tamilnadu, India
[2]PG Scholar, Postgraduate Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-106, Tamilnadu, India
[3]Professor, Postgraduate Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-106, Tamilnadu, India.
[4]Professor and Head of the Department, Postgraduate Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-106, Tamilnadu, India

Corresponding Author: M. Sangeetha

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ABSTRACT

Soothagavaayu kudineer chooranam is one of the polyherbal formulation mentioned in siddha system of medicine. The main objective of the study was to evaluate the quality of formulation *Soothagavaayu kudineer chooranam* by conducting Physicochemical, Biochemical and Phytochemical evaluation through advanced analytical techniques. The organoleptic nature of the drug provides the purity and quality of the formulation. The results obtained from physicochemical evaluation shows that the total ash value of *Soothagavaayu kudineer* was 2±0.1%, in which the acid insoluble ash was 3.1±1.94%,loss on drying at 105ºC of the formulation *Soothagavaayu kudineer chooranam* was noted to be7.43±0.97 in which water soluble extract value and alcohol soluble extract value was 33.9±1.34 and 0.085% respectively. Biochemical analysis of *Soothagavaayu kudineer chooranam* exhibit the appearance of carbonates and nitrates. High performance Thin Layer Chromatography analysis of *Soothagavaayu kudineer chooranam* reveals the appearance of two prominent peaks corresponds to presence of two versatile phytocomponents present within it. Rf value of the peaks ranges from 0.07 to 0.20. Phytochemical analysis of *Soothagavaayu kudineer chooranam* reveals the emergence of phytocomponents like Alkaloids, Flavonoids, Steroids, Triterpenoids, Coumarin, Phenol, Tannin, Protein, Saponins, Sugar, Betacyanin. In conclusion, the polyherbal formulation of *Soothagavaayu kudineer chooranam* possess significant phytocomponents and have beneficial effects towards treating various disorders.

Keywords: Standardization, Decoction, Physicochemical, *Soothagavaayu kudineer chooranam*, Polyherbal.

1. INTRODUCTION

In favour of the use of medicinal plants, they are the only resource available in nature which have comparatively few side effects. Synthetic drug in general has potent pharmacodynamic effects, but many also have strong and possibly dangerous harmful side effects. Nowadays Siddha treatment towards common people were well established and seek more attention about knowing herbal remedies and treatment procedures. Siddha system of medicine was indicated for the management of various ailments. There are 32internal medicines available in Siddha literature. In that decoction is considered to be one of the most effective dosage forms in Siddha system of medicine. Its life span was only 3hours and have to be used in fresh state due to loss of its therapeutic action [(1)].

Decoctions are made by pouring water to dry herbal/ plant parts or fresh ones and then dehydrated so that the water content is greatly reduced to 1/16th or 1/8th or 1/4th or 1/2th of its initial volume. Decoctions are water based extracts of herbal drugs which are easily absorbed into the body and enter into the blood stream quickly which gives faster action than other forms of medications. In order to prepare decoctions without difficulty in sourcing raw material premixed coarse powder of the kudineer formulations are available as kudineer chooranam.[(2)]*Soothagavaayu kudineer*, a polyherbal Siddha medication, has shown great potential in treating *soothagavaayu* and its related symptoms, lower backache and chest pain. But scientific evidences for *Soothagavaayu* have not been reported. So there is a need to develop a standardization technique by using preliminary guidelines. Therefore, the current investigation was done to detect physicochemical screening – organoleptic nature, loss on drying, Total ash, Acid insoluble Ash, Alcohol soluble extractive, water soluble extractive, High performance Thin Layer Chromatography (HPTLC), Heavy metal analysis, Sterility testing, Specific pathogen, Pesticide residue, Aflatoxin, Biochemical and phytochemical analysis of siddha formulation *SVKC* according to PLIM guidelines.

2. MATERIALS AND METHODS

Selection of the drug:

The trial drug *Soothagavaayu kudineer chooranam* was taken from *Anuboga Vaithiya Navaneedham* part -9 for the treatment and management of *Soothagavaayu* and its related symptoms.[(3)] *Soothagavaayu kudineer chooranam* comprises of the following ingredients.

Table 1: Ingredients of *Soothagavaayukudineer chooranam*

S.no	Tamil name	Botanical name
1	Erukkuverpattai	Calotropis procera
2	Kandankathiriver	Solanam xanthocarpum
3	Kodiveli	Plumbago indica
4	Mukkavelaiver	Tephrosia purpurea
5	Saranaiver	Trianthemadecandra
6	Arisithipilli	Piper longum
7	Sevviyam	Piper nigrum
8	Chukku	Zingiber officinale

Collection of the Raw Materials:

The raw drugs *Chukku* [Zingiber officinale], *Sevviyam* [Piper nigrum], *Arisithipili* [Piper longum], *Kodiveli* [Plumbago indica] were bought from Authenticated country store in Chennai, Tamilnadu. *Erukkuverpattai* [Calotropis procera], *Mukkavelaiver* [Tephrosia purpurea], *Saranaiver* [Trianthemadecandra] was collected from Tirunelveli district, Tamilnadu. *Kandankathiriver* [Solanam xanthocarpum] was collected from Tenkasi district, Tamilnadu.

Identification and Authentication of The Drug:

All drugs were recognized and authenticated by Botanist in Government Siddha Medical College, Arumbakkam, Chennai. Each sample has been labelled as 10038-10045/ PGG/321912109/GSMC-CH/2019-2022. The identified product samples were maintained in the PG Gunapadam laboratory for future references.

Purification of the Drug:

Purification process were made according to the procedures mentioned in the classical Siddha literature

Preparation of *Soothagavaayu Kudineer Chooranam:*

The above given ingredients were taken in an equal quantity, then pounded into coarse powder. The obtained decoction powder was then stored in clean air-tight container and named as *SVKC*

Fig:1

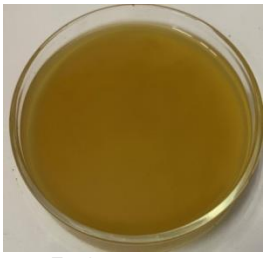

Fig:2

All the above investigations were performed at Noble Research Solution, Perambur at Chennai

PHYSICO-CHEMICAL EVALUATION [4,5]

Percentage Loss on Drying

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

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC 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.

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.

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 tarred 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.

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 tarred 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.

PARTICLE SIZE DETERMINATION [6]:

Methodology

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

THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS [7]

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 was used to spot the sample for TLC applied sample volume 10-microliter by using pipette at distance of1 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.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) ANALYSIS [8]:

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.

HEAVY METAL ANALYSIS BY ATOMIC ABSORPTION SPECTROMETRY [9]

Standard: Hg, As, Pb and Cd – Sigma

Methodology

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

Standard preparation

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

STERILITY TEST BY POUR PLATE METHOD [10]

Methodology

Test sample was inoculated in sterile Petri dish to which about 15 ml of molten agar 45ºC were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37º C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

TEST FOR SPECIFIC PATHOGEN [11]

Methodology for Specific Pathogen

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

Table 2: Detail of Specific Medium and their abbreviation

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

PESTICIDE RESIDUE [12,13]

Extraction

Test sample were 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 millilitres of toluene and heat again until the acetone is

completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter

AFLATOXIN [14]

Procedure

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, 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

BIOCHEMICAL ANALYSIS OF ACIDIC AND BASIC RADICALS [15]

1. Test for Carbonates:

1 ml of the test solution was taken and about 1 ml of concentration (conc.) HCL was added, Formation of brisk effervescence indicates the presence of carbonate.

2. Test for chlorides:

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

3. Test for sulfates:

1 ml of the test sample was taken and add diluted H2SO4 till effervescence ceases, followed by this, about 1 ml of barium chloride solution was added. Appearance of white precipitate indicates the presence of sulfates.

4. Test for sulphides:

1 ml of the test sample was taken and about 2 ml of HCL was added with slight warming the mixture, Formation of colourless gas with the smell of rotten egg indicates the presence of sulfides.

5. Test for phosphates:

2 ml of test solution was taken and treated with 2 ml of ammonium molybdate solution followed by addition of 2ml of concentrated nitric acid, Formation of yellow precipitate Indicates the presence of phosphates

6. Test for Fluoride and Oxalate:

2 ml of the test solution was taken and about 2 ml of dilute acetic acid and 2ml of calcium chloride solution was added, Formation of white precipitate Indicates the presence of Fluoride/ Oxalate.

7. 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 denotes the presence of Borates

8. 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

ANALYTICAL INVESTIGATION ON TEST FOR BASIC RADICALS

1. Test for Lead:

1 ml of the test solution Mixed with 2 ml of potassium chromate solution, observation of yellow precipitate indicates the presence of lead.

2. Test for Arsenic:

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

3. Test for Mercury:

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

4. Test for Copper:

1 ml of the test solution added with 1 ml of Ammonium hydroxide (NH4OH) solution, outcome of blue precipitate indicates the presence of copper.

5. Test for Ferric:

To 1 ml of test solution, about 2 ml of potassium ferrocyanide was added,

outcome of blue precipitate indicates the presence of ferric.

6. Test for Ferrous:

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

7. Test for Zinc:

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

8. Test for Silver:

1 ml of the test solution was added with 1 ml of conc. HCL followed by appearance of curdy white precipitate. Boil the precipitate with water. It does not dissolve. Add NH4OH solution in it and add 1 ml dilute HNO3, outcome of curdy white precipitate indicates the presence of silver.

9. Test for Magnesium:

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

PHYTOCHEMICAL EVALUTION [16]

Test drug *SVKC* was subjected to Preliminary phytochemical screening of the following components.

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 colour 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 chloroform is added and shaken, chloroform 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. Sulfuric acid. Appearance of yellow colour indicates the presence of Flavonoids.

Test for phenols:

Lead acetate test:

To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

a) 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.

b)Betacyanin:

To the test sample, 2ml of HCl was added and heated for 5 mins at 100ºC.

Formation of pink colour indicates the presence of betacyanin

Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

3. RESULT

PHYSICO-CHEMICAL EVALUATION OF *SVKC*

Organoleptic characters:

The drug *SVCK* was coarsely powdered and the results were mentioned in Table: 3

Table 3 : Organoleptic characters of *SVKC*

Description	*Soothgavaayu chooranam*	*Soothagavaayu kudineer*
State	Solid (Coarse powder)	Liquid
Nature	Fibrous and leafy coarse Material	Non Viscous
Odour	Characteristic	Aromatic
Touch	Hard Texture	Non greasy
Flow Property	Non free flowing	Free Flowing
Appearance	Pale Brownish	Yellowish Brown

Solubility Profile:

The drug *SVKC* for solubility profile was given in Table 4

Table 4: Solubility profile of*SVKC*

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

The results for physicochemical analysis were tabulated in Table 5

Table 5 : Results of physicochemical evaluation of *SVKC*

S.No	Parameter	Mean (n=3) SD
1.	*Loss on Drying at 105 °C (%)*	7.433 ± 0.9713
2.	*Total Ash (%)*	2 ± 0.1
3.	*Acid insoluble Ash (%)*	3.167 ± 1.94
4.	*Water soluble Extractive (%)*	33.9 ± 1.345
5.	*Alcohol Soluble Extractive (%)*	0.54 ± 0.08544

PARTICLE SIZE DETERMINATION:

Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be 10.4±4.41µm further the sample has particle with the size range of lowest 5 µm to highest 15 µm

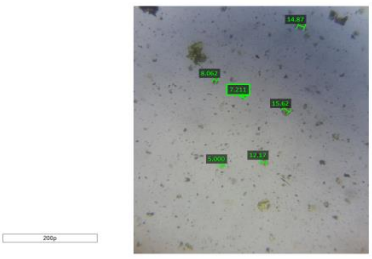

Fig 3: Microscopic Observation of Particle Size for the sample *SVKC*

HPTLC Analysis Of *SVKC*

HPTLC finger printing analysis of the sample reveals the presence of two prominent peaks corresponds to presence of two versatile phytocomponents present within it. Rf value of the peaks ranges from 0.07 to 0.20.

Fig : 4 TLC Chromatogram of *SVKC*

TLC Visualization of *SVKC* at 366 nm

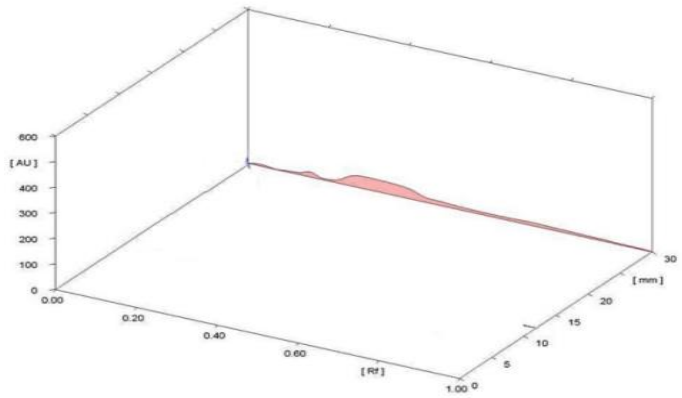

Figure 5: HPTLC Chromatogram of *Soothagavaayu kudineer Chooranam* 3D - Chromatogram

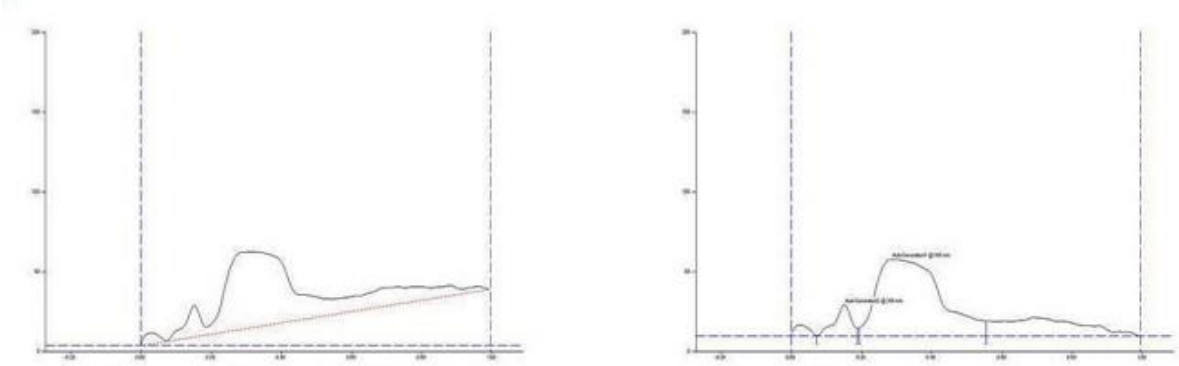

Fig: 6 HPTLC finger printing of Sample *SVKC*

Peak Table

Table: 6 Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area %
1	0.07	0.3	0.15	19.9	29.23	0.19	5.1	239.0	9.47
2	0.20	5.3	0.29	48.1	70.77	0.56	9.7	2284.2	90.53

HEAVY METAL ANALYSIS BY ATOMIC ABSORPTION SPECTROMETRY

The result for Heavy metal analysis of the trial drug the was tabulated in table 7

Table 7: Heavy metal analysis

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
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

RESULT FOR SPECIFIC PATHOGEN

The observation of the trial sample and the results were mentioned in table 8.

Table 8: Results for specific pathogen

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

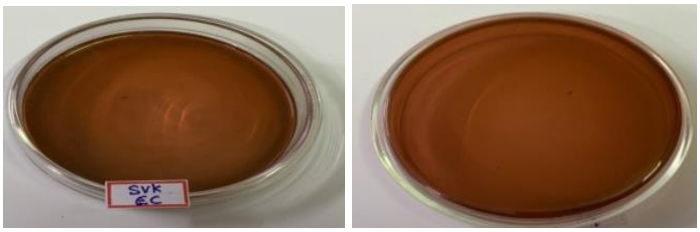

Fig: 7 Culture plate with E-coli (EC) specific medium

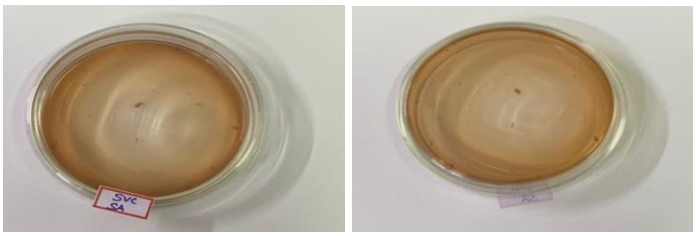

Fig:8Culture plate with Salmonella (SA) specific medium

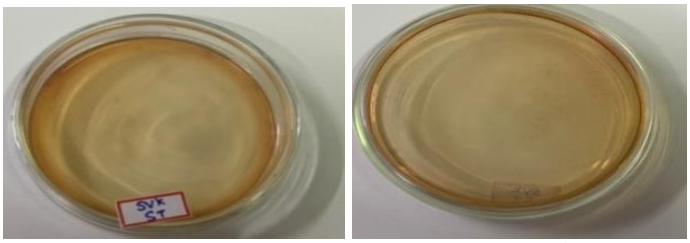

Fig:9 Culture plate with Staphylococcus Aureus (ST) specific medium

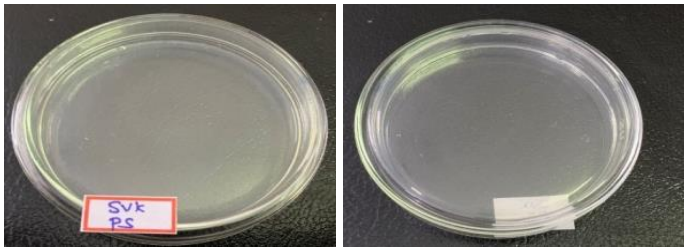

Fig: 10 Culture plate with Pseudomonas Aeruginosa (PS) specific medium

RESULT FOR MICROBIAL CONTAMINATION

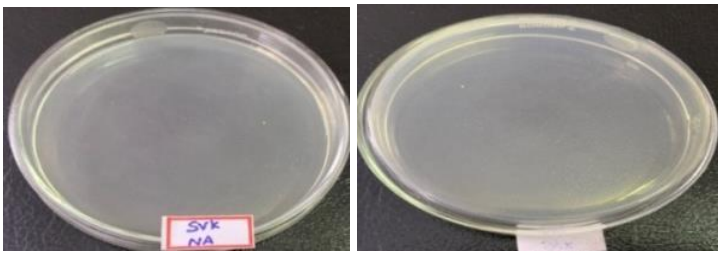

Fig : 11 Microbial contamination by pour plate method

Test for microbial contamination of the given sample was done and the results were given in Table 9.

Table 9: Results for microbial contamination

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 FOR PESTICIDE RESIDUES:

The results showed that there were no traces of pesticides residues in the given sample and the result was tabulated in table 10:

Table 10: Results for pesticide residues

Pesticide Residue	Sample SVK	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

RESULT FOR AFLATOXIN

The results shown that there were no spots were being observed in the test sample loaded on TLC plates when compare to the standard which denotes that the sample was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

Table 11: Results for Aflatoxin

Aflatoxin	Sample *SVKC*	AYUSH Specification Limit
B1	Not Detected	0.5 ppm
B2	Not Detected	0.1 ppm
G1	Not Detected	0.5 ppm
G2	Not Detected	0.1 ppm

RESULTS OF BIOCHEMICAL ANALYSIS OF *SVKC*

Test for Acid Radicals and Basic Radicals was done and the results for Biochemical analysis of the sample were tabulated in table 12&13

Table 12: Test forAcid Radicals

Specific Radical	Test Report
Test for carbonates	Positive
Test for chlorides	Negative
Test for sulfates	Negative
Test for sulphides	Negative
Test for phosphates	Negative
Test for Fluoride and Oxalate	Negative
Test for Borates	Negative
Test for Nitrates	Positive

Table 13: Test for Basic Radicals

Specific Radical	Test Report
Test for Lead	Negative
Test for Arsenic	Negative
Test for Mercury	Negative
Test for Copper	Negative
Test for Ferric	Negative
Test for Ferrous	Negative
Test for Zinc	Negative
Test for Silver	Negative
Test for Magnesium	Negative

QUALITATIVE PHYTOCHEMICAL EVALUATION OF *SVKC*

The Results for the Analysis of phytochemical present in the given sample was mentioned and the outcome were tabulated in table 14

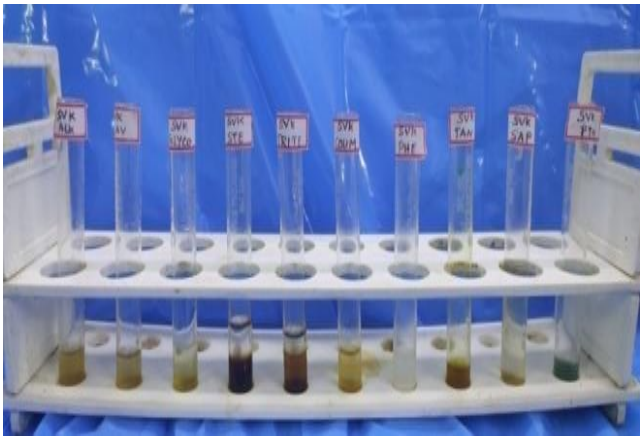

Fig –12 Phytochemical Analysis of *Soothagavaayu kudineer chooranam*

Table 14: Qualitative Phytochemical Analysis of *Soothagavaayu kudineer*

S.NO	TEST	OBSERVATION
1	Alkaloids	Positive
2	Flavanoids	Positive
3	Glycosides	Negative
4	Steroids	Positive
5	Triterpenoids	Positive
6	Coumarin	Positive
7	Phenol	Positive
8	Tanin	Positive
9	Protein	Positive
10	Saponins	Positive
11	Sugar	Positive
12	Anthocyanin	Negative
13	Betacyanin	Positive

+ -> Indicates Positive and - -> Indicates Negative

4. DISCUSSION

The drug *SVKC* was coarsely powdered with hard texture and pale brownish colour. Fresh preparation of its extract shows non greasy, yellowish brown with aromatic odour. Oral bio-availability depends on several factors including Aqueous solubility, drug permeability etc., The drug *SVKC* soluble in specific solvent like Ethanol, Water and Dimethyl sulfoxide thereby it proves its efficiency of solubility increasing in bio-availability in the stomach indirectly. The loss on drying was found to be 7.433±0.9713%which indicates the moisture content of the drug. Total ash value was found to be 2±0.1% which notes the presence of inorganic components. Acid insoluble ash was 3.167±1.94which indicates that the drug contains minimum amount of siliceous matter. The water and alcohol soluble extractive values were found to be 33.9±1.345% and 0.54±0.08544% which proof that the secondary metabolites are extractable with above solvents and it shows the high polar secondary metabolites such as tannins, proteins, triterpenoids, flavonoids, coumarin, phenol etc.,[17] HPTLC finger printing analysis of the sample reveals the appearance of two prominent peaks corresponds to presence of two versatile phytocomponents present within it. Rf value of the peaks ranges from 0.07 to 0.20. Heavy metal analysis clearly shows that the sample has no traces of heavy metals such as Lead, Arsenic, Mercury and Cadmium. These results indicate that the trial drug is extremely safe as it contains heavy metals within specified limits which reveals the safety of the drug. Results obtained from the test for specific pathogen denotes that No growth /colonies were seen in any of the plates inoculated with the test sample *SVKC* which confirms the absence of E-coli, Salmonella, Staphylococcus Aureus and Pseudomonas aeruginosa in the sample. Analysis of Pesticide residue is an important parameter for quality control of drug and the results obtained further confirms that there were no traces of pesticides residues such as Organochlorine, Organophosphorus and Pyrethroids in the *SVKC*. The results obtained from the test for Aflatoxin shown that there were no spots were been identified in the *SVKC* loaded TLC plates when compare to the standard, which denoted that the sample was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 which proof that the trial drug is free from toxicity and does not perform any carcinogenic activity. The Biochemical analysis for Basic radicals of *SVKC* reveals the presence of Carbonates and Nitrates. Calcium is needed for the generation of muscle contraction nerve conduction and release of hormones. Presence of calcium in the test sample, it may be given for the treatment hormonal and metabolic disorders and also used for the management of low calcium levels such as Osteoporosis, Osteomalacia/ Rickets, Hypoparathyroidism and certain muscle disease (latent tetany).[18] Due to the presence of nitrate, it performs physiological functions in various systematic activities and possess beneficial activity towards healthy lifestyle. Phytochemical analysis indicates that the medication *SVKC* indicates the presence of Alkaloids which possess anti-inflammatory, anti-cancer, and used as a local anesthetic and pain relief.[19]The trial drug contains Flavonoids which exhibits anti- diabetic, anti-oxidative, anti-inflammatory and anti-carcinogenic Activity[20]. Thus presence of Flavonoids in the test sample it may protect cells from oxidative damage and also reduces blood glucose levels. Presence of Steroids the drug may deliver anti-

inflammatory and immune modulating activities.[21] Presence of Triterpenoids exhibit Anti oxidant and Anti-inflammatory activities. It is also useful in treating hyperglycemia, metabolic syndrome and obesity.[22] Coumarin has pharmacological properties like anti inflammatory, anti hyperglycaemic and antioxidant[23] and the presence of coumarin can be good in treating Inflammatory disorders, Diabetics and polycystic ovarian syndrome. Presence of Phenols exhibits anti-oxidant activity which is effective in protecting cells from oxidative damage.[24] Presence of Tannin helps to reduce inflammation of mucous membrane and inhibition of carcinogenesis.[25]. Presence of Saponins acts as an immunological adjuvant by increasing the immune response.[26] Presence of Sugar in the test sample acts as an energy source for obvious functions of the body thereby regulating hormonal dysfunction and functions of various tissues and organs. Betacyanin has potential therapeutic efficacy in the treatment of dyslipidemia, cancer and cardiovascular diseases.[27] Due to the presence of betacyanin in the trial is given for reducing abnormally elevated cholesterol or fats thereby treating obesity which is the major cause for polycystic ovarian syndrome. phytoconstituents can be used as a major tool for obtaining a quality control profile of drug. However the presence of these phytoconstituents hence proved that the trial drug will be effective in treating various disorders.

5. CONCLUSION

Results obtained from the above discussion; this was finally concluded that the Siddha formulation *SVKC* possess potent biologically active components which may helps in treating various disorders. Investigation of those specifications with the help of modern analytical tools helps in standardization of *SVKC*. Hence this present investigation had generated an evidence-based data with respect to purity, standards, physico-chemical, phytochemical and biochemical nature of the formulation *SVKC*.

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