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STANDARDIZATION OF SILVISHA USIDHAM – A CLASSICAL SIDDHA FORMULATION FOR ALOPECIA AREATA

R. D. Indumathi[1]*, M. Meenakshi Sundaram[2] and V. Banumathi[3]

*[1]P.G. Scholar, Department of Kuzhanthai Maruthuvam, National Institute of Siddha. Chennai-47.

[2]Head of the Department, Department of Kuzhanthai Maruthuvam, National Institute of Siddha. Chennai-47.

[3]The Director, National Institute of Siddha. Chennai-47.

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***Corresponding Author**
R. D. Indumathi
P.G. Scholar, Department of Kuzhanthai Maruthuvam, National Institute of Siddha. Chennai-47.

ABSTRACT

Siddha system is unique among the Indian system of medicine and its drug sources are obtained from plants, minerals, metals and animals. Silvisha Usidham is a herbal drug was mentioned in Siddha literature Anuboga vaithiya navaneetha thirattu by B.Hakim abdula saayu. This herbal drug can use for the various kind of disease like Silvidam, Puzhuvettu, Viranam, Kadi, Kuttam. Standardization is a one point of safety and efficacy of the drug. Every effective and potent medicine of Siddha needs to be standardized. The present study deals with analyzing the physic-chemical characterization, Phytochemical, Biochemical analysis, HPTLC, Heavy metal analysis, Specific pathogen test, Aflotoxin and Pesticide residue of Silvisha Usidham to establish standard quality parameters. This study showed Refractive index determining the identity and purity, higher Saponification value improves absorption rate to the intestine, lesser free fatty acid make them less rancidity and also having healthy benefits. The implication of the present findings such as Microbial contamination, Specific pathogen, Aflatoxin, Pesticide residues and heavy metal analysis may be taken into consideration of the experimental formulation may be safe.

KEYWORDS: Physicochemical, Siddha, Silvisha Usidham and Standardization.

INTRODUCTION

Siddha system of medicine is a unique system among Indian medical systems. Siddhars are the spiritual scientists often they spend their lifetime in the way of experimenting the Nature of the herbs, minerals and animals in the forms of preparing various medicine for various indications. As a result of the experiments, they found a way to formulated so many valuable medicines which include small herbal preparations to the potent medicines. It plays a major role in treating acute and chronic ailments.

According to Siddha system of medicine five elements (Earth, Water, Fire, Air, and Space) of nature combination with each other and form the basis of three humors of the body namely *Vaatha*, *Pitha* and *Kapha*. Any derangements in humors results in development of 4448 diseases.[1]

The therapeutic potency of any drug in Siddha were designed depending on the following five unique properties namely, Suvai (Taste), Gunam (Properties), Veeriyam (Potency), Pirivu (Class), Mahimai (Action).[1] All the five properties are based on the Panchabootham (Five elements) present in the drug. The ingredients of Silvisha Usidham are Sesame oil, Peyathi, Cardamom and long pepper.[2] Sesame oil has Sweet taste, Peyathi has Astringent taste, Cardamom has Acrid taste and long pepper has Sweet taste. The ingredients of Silvisha Usidham possess Sweet, Astringent and Acrid.[3] The Presence of these tastes helps Silvisha Usidham for its therapeutic effect. Literary collections include drug review, which consist both botanical aspect, *Gunapadam* aspect and pharmacological review were support this study. Every effective and potent medicine of Siddha, needs to be standardized. Therapeutic efficacy of a medicine was related to its chemical constituents. Standardization of drug means confirmation of its identity and determination of its quality and purity and biological observations. Standardization of medicine starts from collection of raw material upto their clinical application and efficacy.

MATERIAL AND METHODS

Gingelly oil (Sesame *indicum*), Peyathi (Ficus *hispida*), Elarisithool (Elettaria *cardamomum*), Arisithippili (Piper *longum*) are used as an ingredient for the preparation of Silvisha Usidham.[2]

Collection of Raw Drug

The drugs were purchased from authorized country raw drug store in Chennai. Classical parameters were taken for the evaluation.

Identification and Authentication of The Drug

All the drugs were identified and authenticated by the Botanist, Department of Gunapadam, National Institute of Siddha, Tambaram Sanatorium, Chennai.

Classical Method of Silvisha Usidham Purification and Preparation of The Ingredients

All the drugs mentioned here were purified as per the Siddha literature.[3]

Ficus *hispida*: Remove petiole and vein of the leaves

Elettaria *cardamomum*: Fry to the extent that the contents become golden color

Piper *longum***:** Fruit of Piper *longum* was soaked in lemon juice and it was dried in sunlight until the juice gets evaporated. Then it was fried well.

Table 1: Formulation Composition of Silvisha Usidham.

S.NO	INGREDIENTS	WEIGHT IN GRAMS
1	Gingelly oil (Sesame *indicum*)	40 palam (1400gms)
2	Peyathi (Ficus *hispida*)	80 palam (2800gms)
3	Elarisithool (Elettaria *cardamomum*)	1 palam (35gms)
4	Arisithippili (Piper *longum*)	1 palam (35gms)

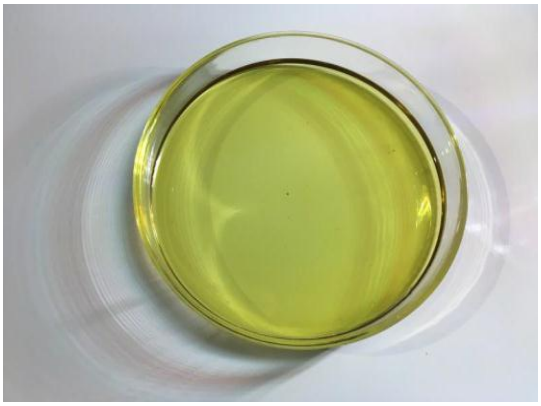
INGREDIENTS OF SILVISHA USIDHAM

Gingelly oil (Sesame *indicum*)

Peyathi (Ficus *hispida*)

Elarisithool (Elettaria cardamomum)

Arisithippili (Piper longum)



Silvisha Usidham

Classical Method of Preparation

The Purified ingredients were made into fine powder then mixed with peyathi juice together combined with gingelly oil and heated till the waxy consistency is obtained and kept in an air tight container.

Test Drug Preparation and Method of Physiochemical Analysis

1. Determination of specific gravity

Fill the dry sp. gravity bottle with prepared Silvisha Usidham in such a manner to prevent entrapment of air bubbles after removing the cap of side arm. Insert the stopper, immerse in water bath at 50^{O}C and hold for 30 min. Carefully wipe off any substance that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side and quickly weigh. Calculate the weight difference between the Silvisha Usidham and reference standard.

2. Determination of Iodine value

About 20 gm of test Silvisha Usidham was transferred into Iodine flask. To which 10 ml of chloroform was added and warmed slightly and cooled for 10 minutes. Followed by this about 25 ml of Wiji's solution was added in the same flask and shaken well. The flask was allowed to stand for 30mins and refrigerated for an hour. T About10 ml of KI solution was added to this and titrated against 0.1 N Sodium thiosulphate solutions until the appearance of yellow color. 1 ml of starch indicator was added and again titrated against the sodium thiosulphate solution from the burette. Disappearance of blue colour indicates end point. Repeat the above procedure without taking Silvisha Usidham and note the corresponding reading for blank titration.

3. Determination of saponification value

About 2 gm of test Silvisha Usidham was transferred into the round bottomed flask. To this about 20 ml of 0.5 N alcoholic KOH solutions was added to the round bottomed flask. Repeat the same procedure without taking the sample for blank titration. Reflux both Silvisha Usidham and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the Silvisha Usidham using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.

4. Determination of Viscosity value

Viscosity determination were been carried out using Ostwald viscometers. Measurement of viscosity involves the determination of the time required for a given volume of liquid to flow through a capillary. The liquid is added to the viscometer, pulled into the upper reservoir by suction, and then allowed to drain by gravity back into the lower reservoir. The time that it takes for the liquid to pass between two etched marks, one above and one bellow the upper reservoir, is measured.

5. Determination of Refractive Index

Determination of RL was carried out using Refractometer.

6. Determination of Weight per ml

Weight per ml was determined using the comparative weight calibration method, in which the weight of 1ml of the base of the formulation was calculated and then weight of 1 ml of Silvisha Usidham were been calculated. The difference between weight variations of the base with respect to Silvisha Usidham calculated as an index of weight per ml.

7. Determination of pH

Sample being liquid in nature the direct litmus evaluation method was adopted to check the pH of the sample.

8. Acid Value

Accurately 5 g of Silvisha Usidham was weighed and transferred into a 250 mL conical flask. To this, a 50 mL of neutralized alcohol solution was added. This mixture was heated for 10 min by heating mantle. Afterwards, the solution was taken out after 10 min and 1 or 2 drops of phenolphthalein indicator was added. This solution was titrated against KOH solution from the burette. The appearance of pink color indicated the end point. The volume of consumed

KOH solution was determined and the titration of Silvisha Usidham was carried out in triplicate and the mean of the successive readings was used to calculate the acid-value of the respective sample by following expression.

Acid value = Titter Value X 0.00561X 1000 / Wt of test sample (g)

9. Peroxide value

5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Table 2: Physico-chemical analysis.

S.No	Parameter	Silvisha Usidham
1	Specific Gravity	0.9760
2	Viscosity at 50^{o}C (Pa s)	6.905
3	Refractive index	1.24
4	Iodoine value (mg I2/g)	104.77
5	Saponification Value (mg of KOH to saponify 1gm of fat)	149.55
6	pH	4
7	Weight per ml	0.029 g/ml
8	Acid Value mg KOH/g	0.766
9	Peroxidase Value mEq/kg	0.528

Table 3: Organoleptic character.

S.NO	Parameters	Results
1	Colour	Pale Yellowish
2	Odour	Slightly Pungent
3	Taste	Slightly acrid
4	State of matter	Liquid
5	Consistency	Free flowing greasy

Preliminary Phytochemical Screening[4]

The preliminary Phytochemical screening test was carried out for extracts of Silvisha Usidham as per the standard procedure.

1. 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.

2. 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 color.

3. 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.

4. 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.

5. 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% ammomia solution is added to it. Pink colour indicates presence of glycosides.

6. 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 color indicates the presence of Flavonoids.

7. 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.

8. Test for steroids

Salkowshi's Test: 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.

9. 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.

10. 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.

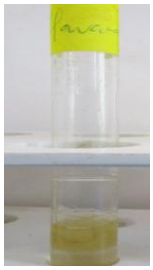
11. Test for Carbohydrates

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.

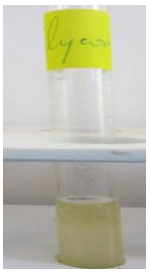
12. Test for Proteins: (Biuret Test)

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.

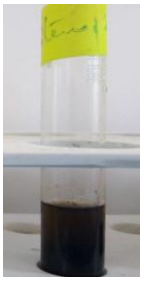
Test for Alkaloids



Test for Flavonoids



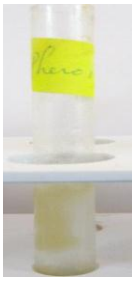
Test for Glycosides



Test for Steroids

Test for Triterpenoids

Test for Coumarins



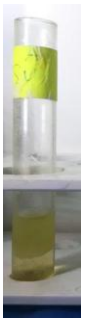
Test for Phenols

Test for Tanins

Test for Proteins

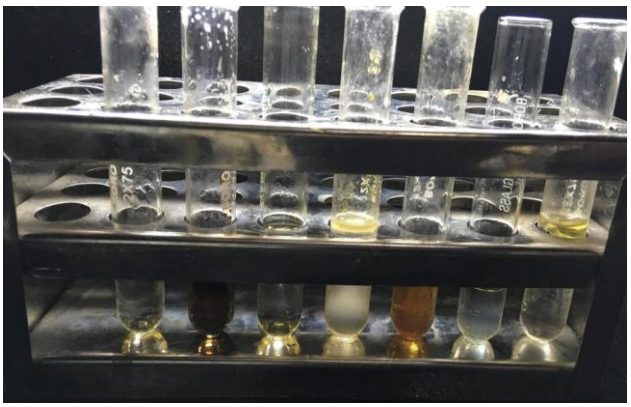
Test for Saponins

Test for Carbohydrates

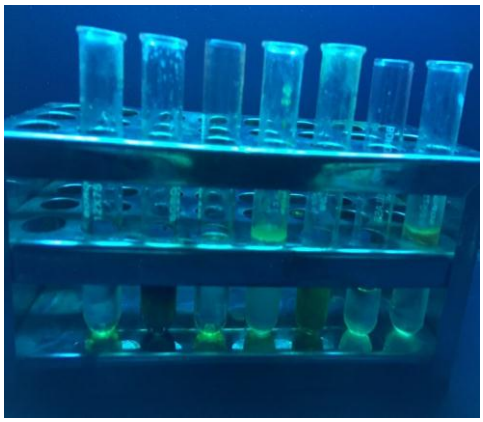


Test for Anto/ Beta cyanins

Fluorescence analysis in dried powder



Visible light



Short UV- light (254 nm) **Long UV- light (365 nm)**

Table 4: Fluorescence Analysis in Dried Powder.

S.No	Experiment	Visible light	Short UV – Light 254 nm	Long UV – Light 365 nm
1	Sample + Conc. Hcl	Mild yellowish	Mild yellow Floresent	Yellowish
2	Sample + Conc. Sulphuric Acid	Greenish brown	Greenish brown	Crimson red
3	Sample + Conc. Nitric acid	Lime Yellow	Mild Florescent yellow	Crimson brown
4	Sample + Sodium hydroxide in water	Creamy white	Pale yellowish white	Pale yellowish
5	Sample + Ferric chloride	Pale yellowish white	Florescent green	Reddish brown
6	Sample + glacial acetic acid	Turbid white	Milky white	Pale yellowish
7	Sample + Water	White	Milky white	Pale yellowish

Table 5: Phytochemical analysis.[4]

S.no	Phytochemical	Test name	Observation
1.	Alkaloids	Mayer's test	+
2.	Flavanoids	Dil.ammonia + h_2so_4	-
3.	Glycosides	**Borntrager's test**	-
4.	Steroids	Salkowski's test	+
5.	Triterpenoids	**Liebermann–burchard test**	+
6.	Coumarin	10% naoh	+
7.	Phenol	**Lead acetate test**	-
8.	Tannin	Ferric chloride test	+
9.	Protein	**Biuret test**	-
10.	Saponins	Frothing test	-
11.	Sugar	**Benedict's test**	-
12.	Anthocyanin	**Aanthocyanin:**	-

Note: +-> Indicates Presence and - -> Indicates Absence of the Phytocomponents.

BIOCHEMICAL ANALYSIS

Preparation of Extract

5gm of Silvisha Usidham was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

Table 6: Biochemical analysis.

S.no	Experiment	Observation	Inference
1.	Physical Appearance of extract	Pale yellowish in colour	
2.	Test for Silicate A 500mg of the sample was shaken well with distilled water.	Sparingly soluble	Presence of Silicate
3.	Action of Heat: A 500mg of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved.	Absence of Carbonate
4.	Flame Test: A 500mg of the sample was made into a paste with Con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame	Absence of copper
5.	Ash Test: A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No yellow color flame	Absence of sodium

S.no	Experiment	Observation	Inference
	I. Test For Acid Radicals		
1.	**Test For Sulphate:** 2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	Cloudy appearance present	**Presence of Sulphate**
2.	**Test For Chloride:** 2ml of the above prepared extract was added with 2ml of dil-HCl until the effervescence ceases off.	No Cloudy appearance was formed	Absence of Chloride
3.	**Test For Phosphate:** 2ml of the extract was treated with 2ml of dil.ammonium molybdate solution and 2ml of Con.HNo3	No Cloudy yellow appearance present	Absence of Phosphate
4.	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulphate solution.	Cloudy appearance present.	**Presence of carbonate**
5.	**Test For Nitrate:** 1gm of the extract was heated with copper turning and concentrated H2So4 and viewed the test tube vertically down.	No Brown gas was evolved	Absence of nitrate
6.	**Test For Sulphide:** 1gm of the extract was treated with 2ml of Con. HCL	No rotten egg smelling gas was evolved	Absence of Sulphide
7.	**Test For Fluoride & Oxalate:** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil. calcium chloride	No cloudy appearance.	Absence of fluoride and oxalate

	solution and heated**.**		
8.	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution were placed.	No characteristic changes were noted.	Absence of nitrite
9.	**Test For Borate:** 2 Pinches (50mg) of the extract was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	No Appearance of bluish green color.	Absence of borate
I. Test For Basic Radicals			
1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil.potassium iodine solution.	No Yellow precipitate was obtained	Absence of lead
2.	**Test For Copper:** One pinch (25mg) of extract was made into paste with Con. HCl in a watch glass and introduced into the non-luminuous part of the flame.	No blue colour appeared	Absence of copper
3.	**Test For Aluminium** To the 2ml of extract dil.sodium hydroxide was added in 5 drops to excess.	No yellow Colour appeared characteristic changes chara	Absence of Aluminium.
4.	**Test For Iron:** a. To the 2ml of extract, added 2ml of dil.ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNO3 were added	Mild Red colour appeared	**Presence of Iron**
5.	**Test For Zinc:** To 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. ammonium chloride was added.	No White precipitate was formed	Absence of Zinc
6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution	No Cloudy appearance and white precipitate was formed	Absence of calcium
7.	**Test For Magnesium:** To 2ml of extract dil. sodium hydroxide solution was added in 5 drops to excess.	No White precipitate was obtained	Absence of magnesium
8.	**Test For Ammonium:** To 2ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution were added.	Brown colour appeared	**Presence of ammonium**
9.	**Test For Potassium:** A pinch (25mg) of extract was treated with 2ml of dil. sodium nitrite solution and then treated with 2ml of dil. cobalt	No Yellow precipitate was obtained	Absence of potassium

	nitrate in 30% dil. glacial acetic acid.		
10.	**Test For Sodium**: 2 pinches (50mg) of the extract was made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved. of yellow colour flame	Absence of sodium
11.	**Test For Mercury**: 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No Yellow precipitate was obtained	Absence of Mercury
12.	**Test For Arsenic** 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No Brownish red precipitate was obtained	Absence of arsenic

III. Miscellaneous			
1.	**Test For Starch** 2ml of extract was treated with weak dil.Iodine solution	Blue colour developed	**Presence of starch**
2.	**Test For Reducing Sugar** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes were noted.	No Brick red colour is developed	Absence of reducing sugar
3.	**Test For The Alkaloids** a) 2ml of the extract was treated with 2ml of dil.potassium lodide solution. b) 2ml of the extract was treated with 2ml of dil.picric acid. c) 2ml of the extract was treated with 2ml of dil.phosphotungstic acid.	Yellow colour developed	**Presence of Alkaloid**
4	**Test For Tannic Acid** 2ml of extract was treated with 2ml of dil. ferric chloride solution	No Blue-black precipitate was obtained	Absence of Tannic acid
5	**Test For Unsaturated Compound** To the 2ml of extract, 2ml of dil. Potassium permanganate solution was added.	Potassium permanganate was not decolourised	Absence of unsaturated compound
6	**Test For Amino Acid** 2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent was added**.**	No Violet colour appeared	Absence of amino acid
7	**Test For Type of Compound:** 2ml of the extract was treated with 2 ml of dil. ferric chloride solution.	No green and red colour developed No Violet colour developed No Blue colour developed.	Absence of quinolepinephrinepyrocatecho antipyrine Aliphatic amino acid and meconic acid. Apomorphine salicylate and Resorcinol were absent Morphine, Phenol cresol and hydrouinone were Absent.

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 SU 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 sample SU.

Sample Digestion

Test sample SU 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.

Table 7: Test Report of the Sample SU.

Name of the heavy metal	Absorption max A max	Result analysis	Maximum limit
Mercury	253.7nm	BDL	1ppm
Lead	217.0nm	0.010ppm	10ppm
Arsenic	193.7nm	BDL	3ppm
Cadmium	228.8nm	BDL	0.3ppm

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 Toluene: 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.

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 quantitation of nano grams level of samples. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of Phytochemical which is suitable for confirming the identity and purity of medicinal plant raw materials.

HPTLC REPORT

TLC Analysis at 254 nm

TLC Analysis at 366 nm

HPTLC finger printing of Sample SU

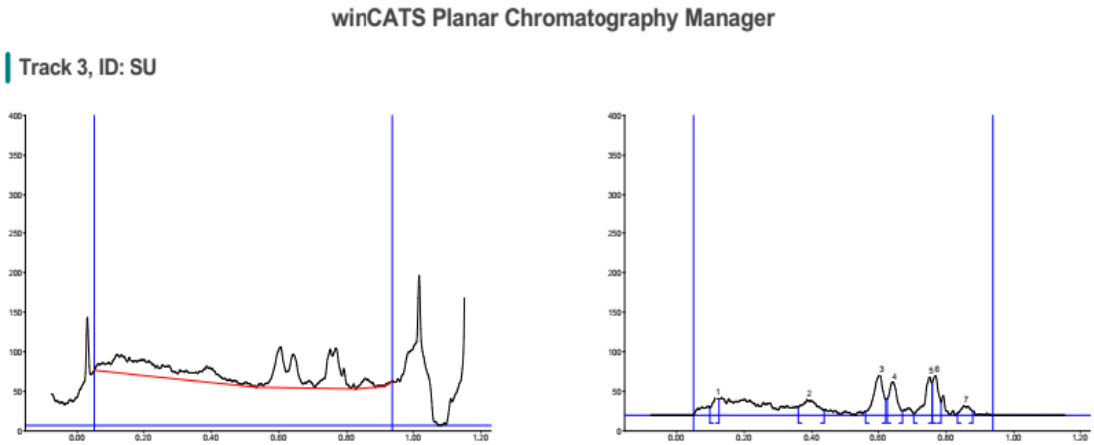


Table 8: HPTLC finger printing analysis of the Silvisha Usidham.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area %
1.	0.10	9.7	0.12	22.8	9.10	0.13	20.3	333.6	7.45
2.	0.36	9.9	0.39	20.2	8.06	0.44	6.4	694.8	15.51
3.	0.56	5.3	0.61	51.6	20.61	0.62	20.3	1074.4	23.98
4.	0.63	20.9	0.64	42.6	17.05	0.67	5.3	749.1	16.72
5.	0.71	1.8	0.75	49.6	19.84	0.76	41.1	743.0	16.58
6.	0.67	42.6	0.77	51.1	20.44	0.79	16.4	638.8	14.26
7.	0.84	2.8	0.86	12.3	4.90	0.88	5.4	247.2	5.52

REPORT

Sample SU reveals the presence of seven prominent peaks corresponds to presence of seven versatile phytocomponents present within it. Rf value of the peaks ranges from 0.10 to 0.84. Further the peak 3 occupies the major percentage of area of 23.98% which denotes the abundant existence of such compound. Followed by this peak 4 and 5 occupies the percentage area of 16.72 and 16.58%.

Test for Specific Pathogen

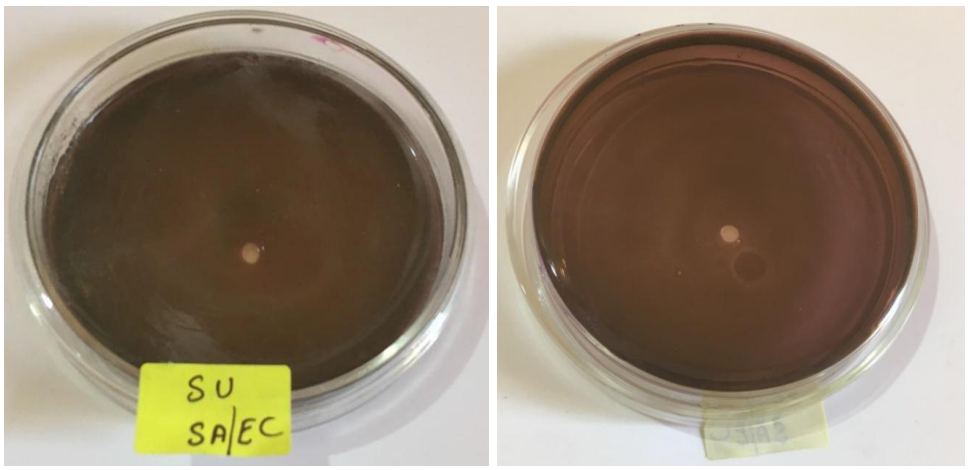
Methodology

About 0.5 gms of test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by spread plate method. The plates were incubated at 37oC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation in each differential media.

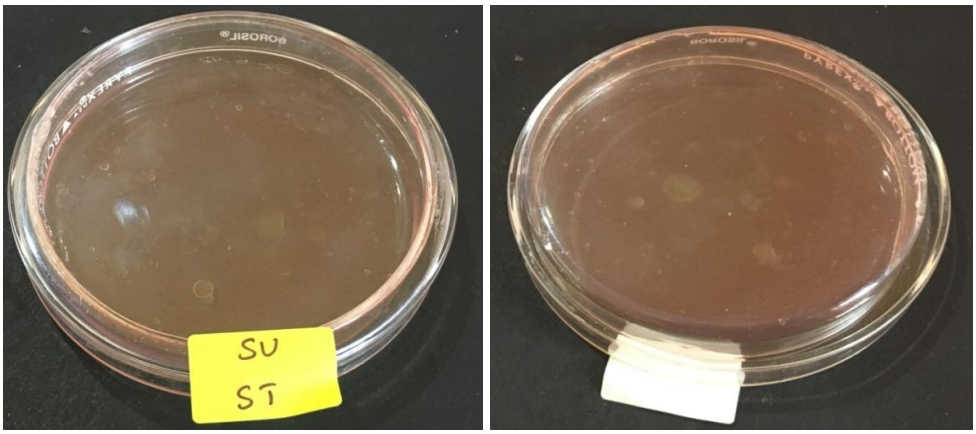
Table 9: Test for Specific Pathogen.

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

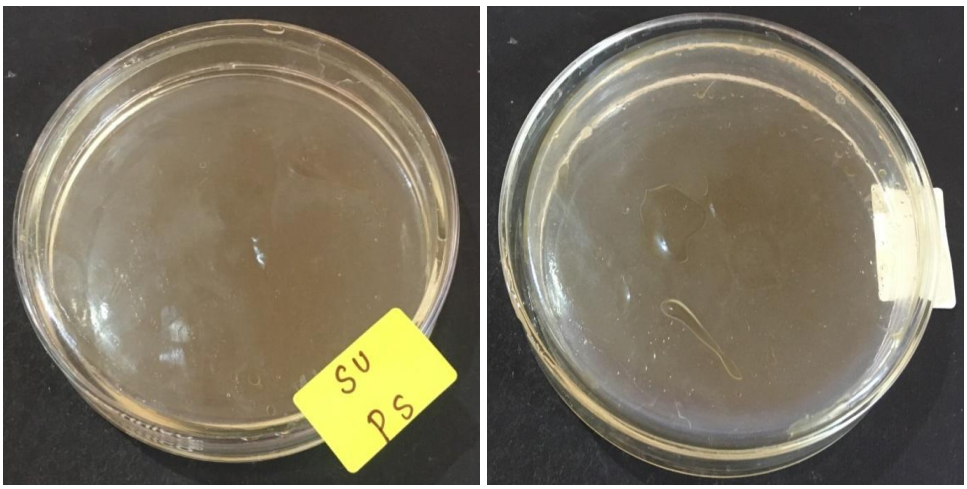
Culture plate with E-coli and Salmonella specific medium



Culture plate with Staphylococcus Aureus specific medium



Culture plate with Pseudomonas Aeruginosa specific medium



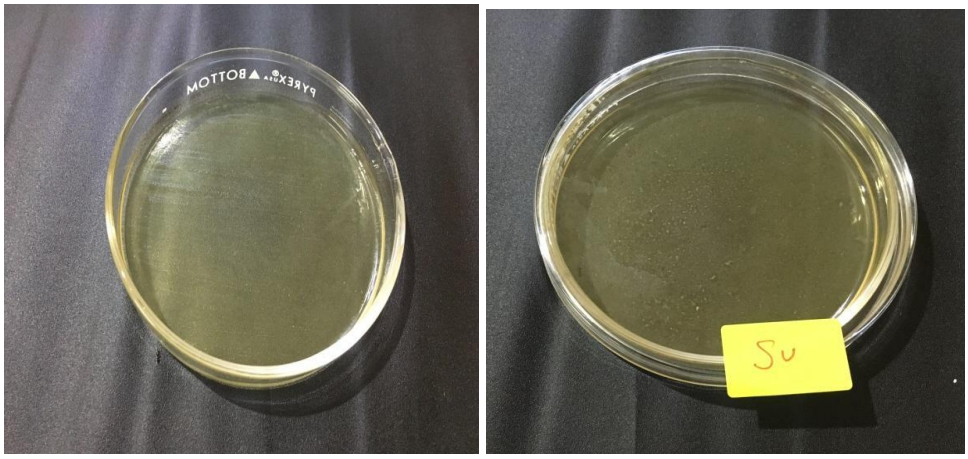
STERILITY TEST BY POUR PLATE METHOD

Methodology

About 1ml of the 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. Grown colonies of organism was then counted and calculated for CFU.

Table 10: Sterility Test By 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	



PESTICIDE RESIDUE[5,6]

Extraction

About 10 g weight equivalent to test substance were extracted with 100 ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene R and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Table 11: Test Result Analysis of the Sample SU.

Pesticide Residue **I.Organo chlorine Pesticides**	**Sample SU**	**AYUSH Limit (mg/kg)**
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	0.2mg/kg
Dichlorovos	BQL	1mg/kg
III.Pyrethroid		
Cypermethrin	0.2mg/kg	1mg/kg

BQL- Below quantification Limit

AFALOTOXIN[7]

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.

Test solution: Concentration 1 µg per ml.

Procedure

Standard aflatoxin was applied on to the surface to pre coated TLC plate 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 saturated 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.

Table 12: Aflotoxin Report.

AFLOTOXIN	SAMPLE SU	AYUSH Specification
B1	Not Detected-Absent	0.5ppm
B2	Not Detected-Absent	0.1ppm
G1	Not Detected-Absent	0.5ppm
G2	Not Detected-Absent	0.1ppm

RESULT AND DISCUSSIONS

The observation of organoleptic, physicochemical and Phytochemical analysis of Silvisha Usidham is mentioned in (Table 2, 3 and 5), whereas biochemical analysis mentioned in (Table 6) respectively. The present study dealt with analyzing the physiochemical, phytochemical characterization, Heavy metal analysis, HPTLC, Microbial contamination and Pesticide residue of Silvisha Usidham to establish standard quality parameters.

Refractive index is used in determining the identity and purity and the results showed the given sample was having more purity.

The saponification value indicates the average molecular weight or chain length of all fatty acids present. It improves the absorption rate to the intestine there by increase nutritional

value and therapeutic values. In the present study higher saponification value in Silvisha Usidham shows that it contains shorter.

The acid value indicates the presence of free fatty acids in the Silvisha Usidham. The free fatty acid is the responsible of rancidity compound, flavor and stability. Lesser free fatty acid makes them less rancidity. It suggests that Silvisha Usidham contains less free fatty Acids and chances of rancidity are less.

The Iodine value indicates the degree of unsaturation of fat which in turn denotes the less rancidity of fats and also having healthy benefits. In this study, Silvisha Usidham contains more Iodine value which suggests the presence of higher unsaturated fatty acid bonds and the chance of rancidity will be less. Unsaponification matter indicates the non-fatty matter which contains more non fatty active volatile components.

The biochemical analysis of Silvisha Usidham shows the presence of Iron, Sulphate, Carbonate, Ammonium and Starch. The Phytochemical analysis shows the presence of Alkaloid, Steroids, Triterpenoid, Coumarin and Tannin. The presence of these chemicals helps Silvisha Usidham for its therapeutic effect.

For the evaluation of microbial contamination, total bacterial and fungal content were less than 10 cfu/ml. In specific pathogen analysis E.coli, Salmonella spp, S.aurens, Pseudomonas aeruginosa were totally absent. All the Pesticide residue and Aflatoxin showed absent. Heavy metal analysis indicated that the level of lead was below the detectable limit.

HPTLC study was done to obtain the fingerprints of the Silvisha Usidham and it was also done to get standard markers. In the present study, HPTLC densitometric scan of petroleum ether extract of unsaponifiable matter of Silvisha Usidham at 366nm showed 7 peaks which covered the area of corresponding RF values. Maximum spots were observed in our sample which indicates more active constituents in it. There are the standard markers of the components which can be used as referral standards.

In general, based on the results of this study it was found that Saponification value and Iodine value were higher which indicates higher active constituents were present in Silvisha Usidham and it can reduce the chance of rancidity thereby increase the quality. The implication of the present findings such as Microbial contamination, Specific pathogen,

Aflatoxin, Pesticide residues and heavy metal analysis may be taken into consideration of the experimental formulation may be safe.

CONCLUSION

From the above study Silvisha Usidham was prepared as per classical textual standard operative procedure and subjected to various studies to reveal its potency and effectiveness against the disease mentioned in Anuboga Vaithiya Navaneetha Thirattu. This present study suggests Silvisha Usidham has remarkable medicinal value in the treatment.

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