

STANDARDIZATION OF A *SIDDHA* POLY HERBAL FORMULATION- *SOOTHAGAVAAYU CHOORANAM*

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Abstract- Most of the formulation in the *siddha* system is more effective. There are various formulations in *siddha* to treat PCOS. One among them is the poly herbal Preparation *Soothagavaayu chooranam* (*kuppaimeni–Acalypha indica*, *Pottrilaikarippaan- Eclipta prostrata*, *Siruserupadai – Glinus lotoides*, *Nilavaagai – Cassia angustifolia*) but it has not been validated. The drugs required for making *Soothagavaayu chooranam* were collected and authenticated by experts and prepared as per *siddha* literature *Anuboga vaithiya biramma ragasiyam* (part 8).The Main Purpose of this study is to standardized the drug *Soothagavaayu chooranam* by physicochemical, phytochemical And biochemical were evaluated as per PLIM guidelines. The obtained results were with in normal limits in this study, which is elaborately described in this paper.

Keywords: *Soothagavaayu chooranam*, Polyherbal formulation, Standardization, Physicochemical- Phytochemical Analysis.

1.INTRODUCTION

Herbals are mostly used in the *Siddha* system for its high therapeutic and potential values.Here is a contribution to the same in the treatment of PCOS (*SOOTHAGAVAAYU*), which is now been the major cause for female infertility^[1]

Soothagavaayu Chooranam, is a polyherbal formulation, has shown potential in treating *Soothagavaayu* its related complications. 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 perceive physicochemical screening –organoleptic nature, loss on drying, Total ash, Acid insoluble Ash, water soluble 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 *SOVC* According to PLIM guideline^[2]

2.MATERIALS AND METHOD

The herbal formulation of *Soothagavaayu chooranam* was taken as a trial drug has been taken from the *Siddha* literature“*Anuboga Vaithiya Biramma Ragasiyam part 8*”, Page No-227^[3] indicated as *Soothagavaayu*. Ingredients of *Soothagavaayu chooranam* were tabulated in Table-1.

Table-1: Ingredients of *Soothagavaayu chooranam*

S.No	NAME OF THE DRUG	SCIENTIFIC NAME	QUANTITY
1.	<i>Kuppaimeni</i>	<i>Acalypha indica</i>	5 varagan (21 gm)
2.	<i>Pottrilaikarippaan</i>	<i>Eclipta prostrata</i>	5 varagan (21 gm)
3.	<i>Siruserupadai</i>	<i>Glinus lotoides</i>	5 varagan (21 gm)
4.	<i>Nilavaagaiver</i>	<i>Cassia angustifolia</i>	5 varagan (21 gm)

COLLECTION OF THE RAW DRUG

The raw drugs *Kuppaimeni* (*Acalypha indica*), *Pottrilaikarippaan* (*Eclipta prostrata*), *Siruserupadai* (*Glinus lotoides*) was collected from Salem district, Tamilnadu. *Nilavaagaiver* (*Cassia angustifolia*) were collected from Govintharaju mudaliyar Nattu Marunthu kadai, parry's corner chennai, Tamilnadu.

IDENTIFICATION AND AUTHENTICATION OF THE RAW DRUG

Gunapadam experts And botanist from the Govt *Siddha* Medical College's PG Gunapadam (pharmacology) Department In Arumbakkam, Chennai, identified and validated all of the substances. Each sample has been labelled as 1112-

1115/PGG/320220100508/GSMC-CH/2020-2023 The identified product samples were maintained in the PG Gunapadam laboratory for future references.

PURIFICATION OF THE DRUG

Purification process were done as per classical *Siddha* literature (Sarakkugalin suthi sei muraigal) ^[4]

PREPARATION OF SOOTHAGAVAAYU CHOORANAM

Each 5 *varagan* (21gm) of *kuppaimeni* (*Acalypha indica*), *Potrilai karippan* (*Eclipta prostata*), *Siruserupadai* (*Glinus lotoides*), *Nilavaagaiver* (*Cassia angustifolia*) were taken. Then dried and pounded into fine powder. Sieved the powder in a thin cotton cloth, then stored in a clean glass air-tight container and named as *Soothagavaayu Chooranam – SOVC*

INDICATION

As per *siddha* literature, evidence will be given 800 to 1000 gm with honey for *Soothagavaayu* .

ANALYSIS OF QUALITATIVE INVESTIGATION

The analysis of the trial drug *SOVC* was evaluated as per the PLIM (Pharmacopoeial Laboratory for Indian Medicines) guidelines. Physico-chemical, Phytochemical analysis, Biochemical analysis, Heavy metal analysis , TLC and HPTLC analysis, Sterility method, Specific pathogen test, Pesticide residue analysis, and Aflatoxins assay were done at Noble Research Institute, Perambur, Chennai.

PROPERTIES FOR ORGANOLEPTIC

The organoleptic characters of the sample drug were evaluated. 1gm of the *SOVC* was taken and the State, Nature, Appearance, Odour and other morphological characters were viewed by naked eye under natural light and results are noted.

PHYSICO-CHEMICAL ANALYSIS ^[5]

- Physicochemical Analysis was done
- Finding loss on drying Was done
- Finding total ash was done
- Determination of acid-insoluble ash was done
- Determination of water soluble ash was done
- Water soluble extraction was done
- Alcohol soluble extraction was done
- pH determination also done ^[6]
- Solubility test was done ^[7]
- Particle size determination was done^[8]

PHYTO-CHEMICAL ANALYSIS ^[9]

- Alkaloids analysis – was performed with Wagner’s Test

Carbohydrates - Benedict’s test

Tannins-Gelatin Test

Saponin--Foam’s Test

Phenols – Ferric chloride Test

Flavonoids- Reagent Test

Diterpens- Copper acetate Test

Quinones – Test For quinones

Gum and Musilage- Test For Gum and mucilage

TLC ^[10]

- HPTLC was done ^[11]
- Chromatogram development was done Biochemical analysis was done ^[12]
- Heavy Metal Analysis by AAS Standard was done ^[13]

TEST FOR STERILITY WAS DONE BY POUR PLATE METHOD ^[14]

SPECIFIC PATHOGEN TEST ^[15]

Table 2: A description of a particular media and their abbreviation

ORGANISM	ABBREVIATION	MEDIUM
<i>E-coli</i>	<i>EC</i>	<i>EMB Agar</i>
<i>Salmonella</i>	<i>SA</i>	<i>Deoxycholate agar</i>
<i>Staphylococcus Aureus</i>	<i>ST</i>	<i>Mannitol salt agar</i>

<i>Pseudomonas Aeruginosa</i>	<i>PS</i>	<i>Cetrimide Agar</i>
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- Pesticide Residue was done ^[16]
- Test for aflatoxins was done ^[17]

3.RESULT

Results of Organoleptic Characters

Organoleptic characters showed that *SOVC* is a fine powder, Pale brownish colour, odourless, and bitter taste. Test drug *SOVC* shown in fig -1 The results are tabulated in table-3



Fig-1.Soothagavaayu chooranam (SOVC)

Table 3: Organoleptic Characters of *Soothagavaayu chooranam*

S.NO	DESCRIPTION	RESULTS
1	State	Solid (Fine powder)
2	Nature	Fine
3	Odour	Strong Aromatic
4	Touch	Soft
5	Flow Property	Non free flowing
6	Appearance	Pale Brownish
7	Taste	Bitter

Particle Size Determination

Microscopic observation of the particle dimensions examination indicates that the mean intermediate particle dimension of the sample was sized up to be 60.72 μm which ensures the solubility, processing belongings, bioavailability, product uniformity, strength and the medicinal result of the *SOVC*.

Profile for Solubility

Table 4: Solubility Profile of *Soothagavaayu chooranam*

S.NO	SOLVENT USED	SOLUBILITY/ DISPERSIBILITY
1	Chloroform	In soluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	In soluble
5	Hexane	In soluble
6	DMSO	Soluble

pH determination

The pH of *SOVC* is 5.5 and it is acidic. In oral administration the acidic nature of the drug enhances rapid absorption in the stomach. So *SOVC* is suitable for oral Administration.

Table 5: Results of Physicochemical Analysis of *Soothagavaayu chooranam*

S.NO	PARAMETERS	PERCENTAGE
1	Loss on drying	9.04%
2	Total ash value	16.37%
3	Acid insoluble ash	6.67%
4	Water soluble ash	3.70%
5	Water soluble extraction	19.15%
6	Alcohol soluble extraction	7.053%

Qualitative Phytochemical Screening of *SOVC*

The Qualitative Phytochemical Analysis results indicate that the medication *SOVC* shows the presence of Alkaloids, Carbohydrates, Tannin, Saponin, Phenols, Flavonoids, Diterpenes, Quinones, Gum and Musilage. The result were tabulated in the table,

Table 6: Result of Qualitative Phytochemical Screening

S.NO	TEST	OBSERVATION
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Tannin	+
4.	Saponin	+
5.	Phenols	+
6.	Flavonoids	+
7.	Diterpenes	+
8.	Quinones	+
9.	Gum and Mucilage	+

+ Indicates positive

**Fig :2 Qualitative Phytochemical Analysis of *SOVC*****HPTLC Analysis of *SOVC***

The sample drug's HPTLC fingerprinting analysis reported the existence of two significant peaks, indicating the available of two different Phyto components. The peaks Rf values range from 0.02 to 0.24



Fig 3: TLC Chromatogram of SOVC

TLC Visualization of SOVC at 366nm

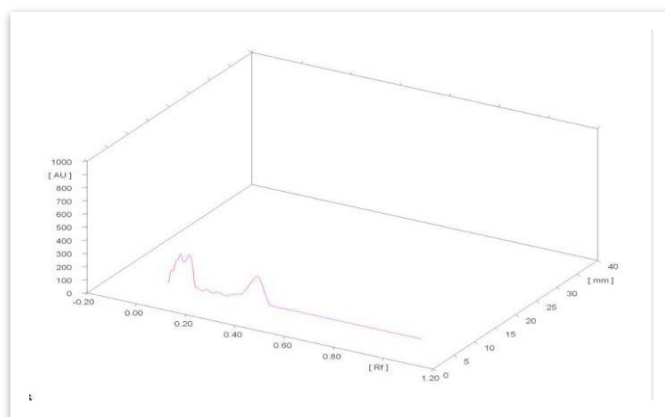


Fig 4: HPTLC Chromatogram 3D- Chromatogram

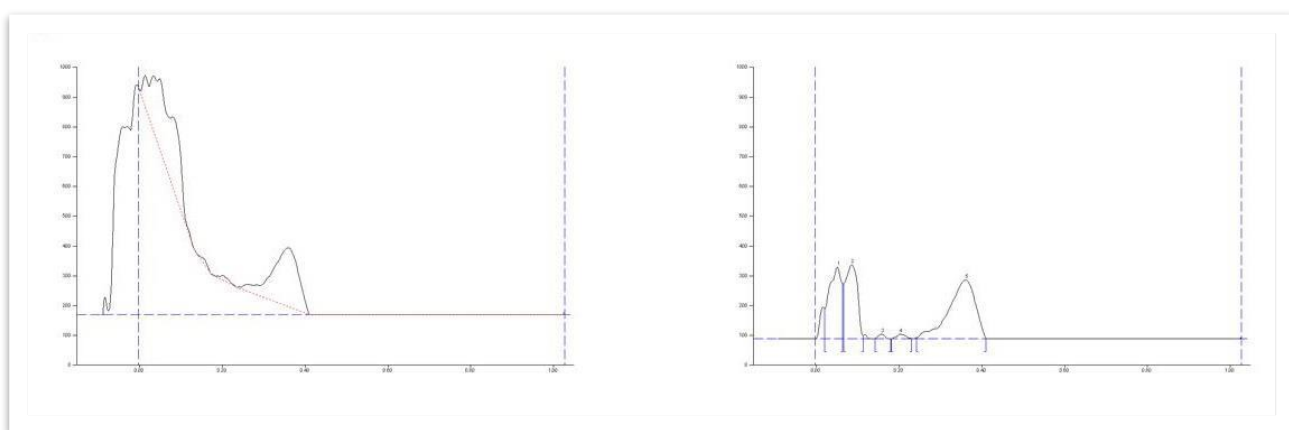


Fig 5: HPTLC fingerprinting

Table 7: HPTLC peak table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	102.0	0.05	240.8	33.43	0.06	185.4	4253.3	26.15
2	0.07	186.0	0.09	248.4	34.48	0.11	10.8	4302.2	26.45
3	0.14	0.7	0.16	16.7	2.32	0.18	0.4	146.1	0.90
4	0.18	0.0	0.20	16.9	2.34	0.23	1.9	202.7	1.25
5	0.24	5.3	0.36	197.5	27.42	0.41	2.5	7361.4	45.26

AAS's Heavy Metal Analysis

The present investigation revealed that the SOVC included no traces of heavy metals such as lead, arsenic, mercury, cadmium. The observed results of heavy metals analysis were tabulated in Table-8.

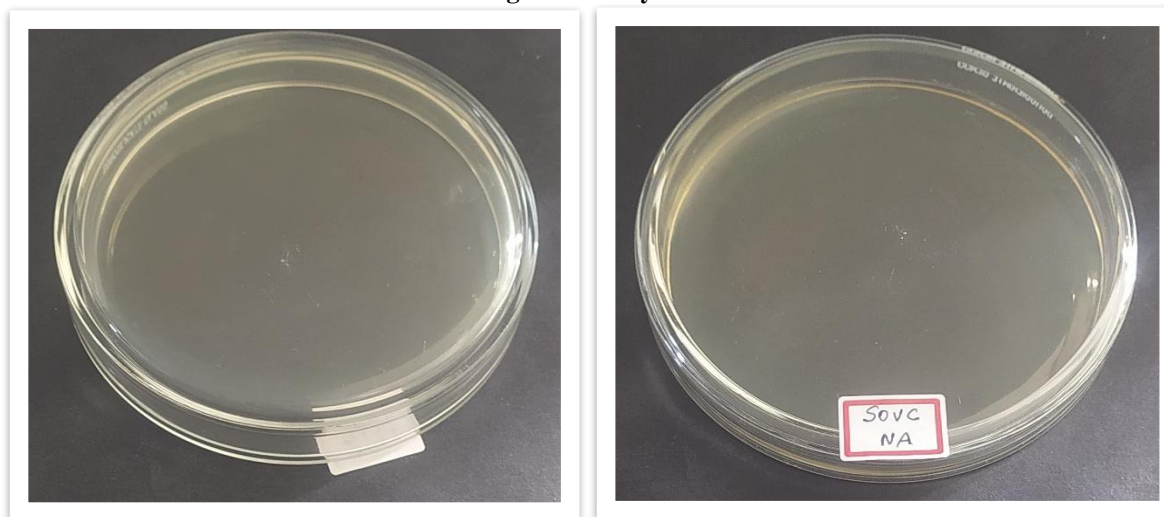
Table 8: Results of Heavy Metal Analysis of SOVC

Name of the Heavy Metal	Absorption Max A max	Result Analysis	Maximum Limit
Lead	217.0 nm.	3.86 PPM	10 ppm
Arsenic	193.7 nm	0.15 PPM	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Arsenic	253.7 nm	0.31 PPM	1 ppm

BDL – Below Detection Limit

Test for Sterility Using the Pour Plate Method

Fig : 6 Sterility Test



Observation

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

Result

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

Table 9: Result of Sterility test of SOVC

Test	Result	Specification	As per AYUSH/WHO

Total Bacterial Count	Absent	NMT 105CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 103CFU/g	As per AYUSH specification

Results for Specific Pathogen

The test results of specific pathogen for *E.coli*, *Salmonella*, *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in test drug SOVC reveal no traces of the four mentioned specific pathogens. The results were tabulated in Table-10—culture plates in Fig 7, Fig 8, Fig 9, Fig 10.

Table 10: Result of specific pathogen of SOVC

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

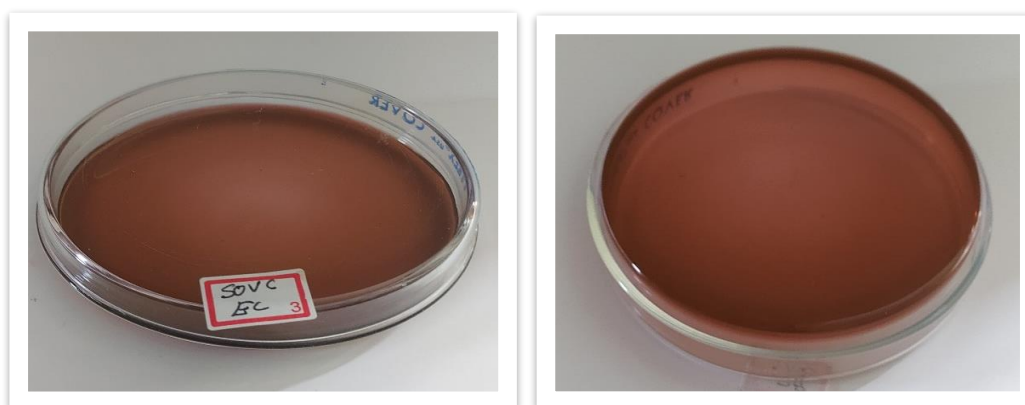


Fig 7. Culture plate with E-coli (EC) specific medium



Fig 8. Culture plate with Salmonella(SA) -specific medium

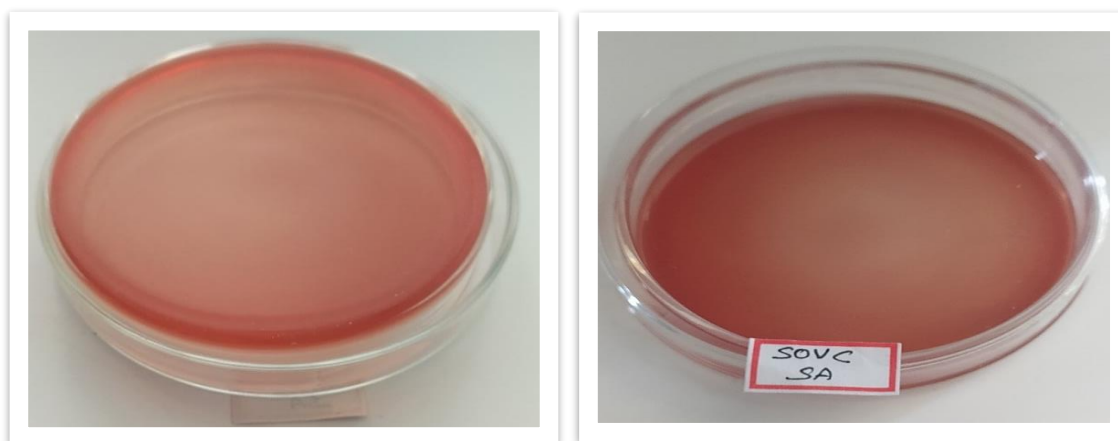


Fig 9. Culture plate with *Staphylococcus Aureus* (ST) specific medium

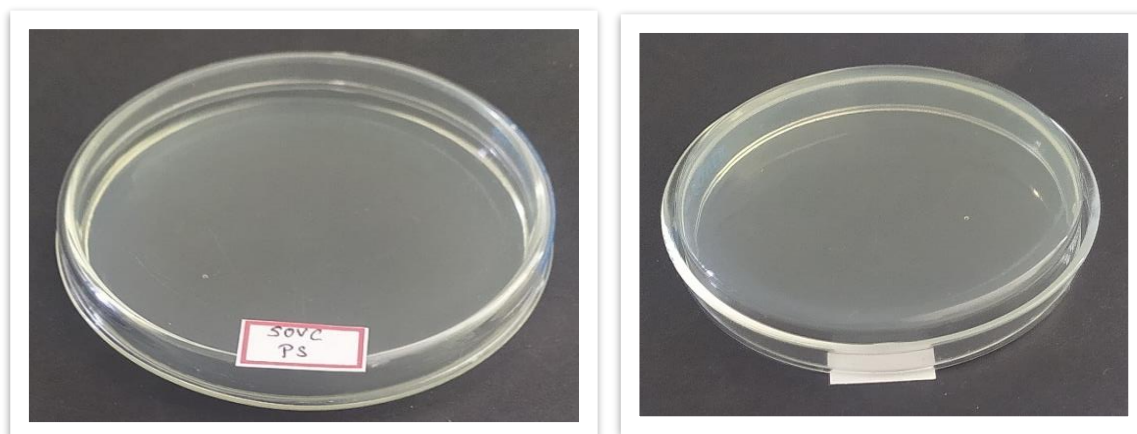


Fig 10. Culture plate with *Pseudomonas Aeruginosa* (PS) specific medium

Results for Pesticide Residue

Analysis of pesticide residues reveals that SOVC contains no traces of residues of organo chlorine, organo phosphorus, Organo carbamates and Pyrethroids. The observed results of pesticide analysis were tabulated in table-11.

Table no 11: Result of Pesticide Residue of SOVC

Pesticide Residue	Sample SOVC	AYUSH (mg/kg)	Limit
1. 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
11. Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2mg/kg
Dichlorovos	BQL	1mg/kg
3. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
4. Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL-Below Quantification Limit**Result for Aflatoxin by TLC (B1, B2, G1, G2)**

The results showed that no spots were identified when *SOVC* loaded on TLC plates compared to the standards, indicating that the *SOVC* was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2.

Table 12: Aflatoxin Assay for *SOVC*

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

Result for Acid and Basic Radicals**Result for Acid Radicals**

The biochemical analysis reveals the presence of carbonates and phosphates in *SOVC*. These were tabulated in table-13.

Table no: 13 Result of Acid Radicals of *SOVC*

S.NO	SPECIFIC RADICALS	TEST REPORT
ACID RADICALS		
1.	Carbonates	Positive
2.	Phosphates	Positive

Results for Basic Radicals

The biochemical analysis of the *SOVC* reveals the absence of essential radicals.

4. DISCUSSION

The drug *SOVC* was fine powdered with soft texture and pale brownish colour with aromatic odour. The drug *SOVC* soluble in specific solvent like Dimethyl sulfoxide, Ethanol, water thereby it proves its efficiency of solubility increasing in bio-availability in the stomach [18]. The loss on drying was found to be 9.04% which indicates the moisture content of the drug. Total ash value was found to be 16.37% which notes the presence of inorganic components. Acid insoluble ash was 6.67% which indicates that the drug contains minimum amount of siliceous matter. The water soluble ash was 3.70%, water soluble extractive and Alcohol soluble extractive values were found to be 19.15% and 7.053% which prove that the secondary metabolites are extractable with above solvents and it shows the high polar secondary metabolites such as Alkaloids, Carbohydrates, Tannins, Saponin, Phenols, Flavonoids, Diterpenes, Quinones, Gum and Mucilage etc [19]. HPTLC finger printing screening of the sample reveals the

appearance of two prominent peaks corresponds to presence of two versatile phytochemicals present within it. Rf value of the peaks ranges from 0.02 to 0.24. Heavy metal screening shows that the sample has no traces of heavy metals such as Cadmium, Lead, Arsenic and Mercury. These results indicate that the trial drug is very safe as it contains heavy metals within specified limits which reveals the safety of the drug. Results obtained from the test for specific pathogen reveals that No growth/colonies were seen in any of the plates inoculated with the test sample *SOVC* 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 Organo chlorine, Organo phosphorus, Organo carbamates and Pyrethroids in the *SOVC*. The results obtained from the test for Aflatoxin shown that there were no spots were identified in the *SOVC* 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 *SOVC* reveals the presence of Carbonates and Phosphates. Carbonates is needed for the generation of muscle nerve conduction, muscle contraction and release of hormones. Presence of carbonates in the test sample, it may be given for the treatment hormonal and metabolic disorders and also used for the management of low carbonates levels such as Hypoparathyroidism, Osteoporosis, Osteomalacia/ Rickets and certain muscle disease. Due to the presence of Phosphates, it performs physiological functions in various systematic activities and possess beneficial activity towards healthy lifestyle. Phytochemical screening indicates that the formulation *SOVC* indicates the presence of **Alkaloids** which possess anti-inflammatory, anti-tumour and used as a pain relief and local anesthetic [20]. **Carbohydrates** play an important role in the human body. They act as an energy source, participate in cholesterol and triglyceride metabolism, help control blood glucose and insulin metabolism, and help with fermentation. Presence of **Tannin** helps to reduce inflammation of mucous membrane and inhibition of carcinogenesis [21]. Presence of **Saponins** acts as an immunological adjuvant by increasing the immune response [22]. Presence of **phenols** act as antioxidant activity of phenolic compounds is attributed to the capacity of Scavenging free radicals, donating hydrogen atoms, chelate metal cations. The trial drug contains **Flavonoids** which exhibits anti-diabetic, anti-oxidative, anti-inflammatory and anti-Tumour Activity [23]. Thus presence of Flavonoids in the test sample it may protect cells from oxidative stress and reduces blood glucose levels. **Diterpene** has an anti-oxidant effect. It also has tumour inhibitory properties as well as a stimulating effect on the immune system. It is used widely as a stomachic (100)Quinones. **Quinones** are small electron transfer molecules, found in virtually all cells that undergo aerobic metabolism, that play an important role in oxidative stress. Quinones have a role in anti-tumour agents and anti-aging. can be **Gum & Mucilage** is used as a bulk laxative and used for their demulcent properties for cough suppression. Phyto-chemicals can be used as a major tool for obtaining a quality control of drug. However the presence of these phyto constituents proved that the trial drug will be very effective in treating various disorders.

5. CONCLUSION

Results obtained from the above discussion; Finally concluded that the *Siddha* polyherbal formulation *SOVC* possess 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 *SOVC*. Hence this present investigation had generated an evidence-based data with respect to purity, standards, phyto chemical, physico chemical and biochemical nature of the medication *SOVC*.

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