PHYSICO-CHEMICAL AND PHYTOCHEMICAL STANDARDIZATION OF A SIDDHA POLYHERBAL FORMULATION THALEESADI CHOORANAM

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Article History: **Received:** 30.05.2023 **Revised:** 02.07.2023 **Accepted:** 24.08.2023

Abstract

The present study aims to explore the phytochemical and physicochemical analysis of a Siddha classic polyherbal formulation -*Thaleesadi Chooranam* (T.S.C.). This *Thaleesadi Chooranam* contains the ingredients like *Thaleesadi*, black pepper and ginger. Its counterpart keeps the immune system and overall health and well-being. This study aims to estimate the quality of *Thaleesadi Chooranam* by conducting preliminary phytochemical analysis, with the help of following tests done with H.P.T.L.C. and U.V. -Vis spectrometric analysis, also to know physiochemical parameters like ash value, extractive value and loss on drying as per pharmacopoeial laboratory for Indian medicine guidelines. The T.S.C., upon successive extraction with ethyl acetate, gave a yield of (5: 1: 0.1, v/v). The results obtained after physiochemical analysis of the test drug showed 6.75 % Loss on drying at 105^0C, 4.18% of Total Ash, 0.572% of Acid insoluble ash, 1.853% of Water soluble ash and more. The results obtained after preliminary phytochemical analysis showed the presence of Alkaloids, Carbohydrates, Glycosides, Phenols, Terpenoids, Tannins, and Saponins. The results indicate that the drug is of standard quality and may use as a reference standard in laying pharmacopoeia standards.

Keywords: Siddha formulation, *ThaleesadiChooranam*, Standardization, Physicochemical analysis, Phytochemical analysis, HPTLC.

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DOI: 10.31838/ecb/2023.12.6.285

1. INTRODUCTION

Siddha system of Indian medicine is considered the science and art of healing and provides essential ailment for humankind through its novel medications. Healing by rejuvenation is one of the principles involved in Siddha medicine. The Siddha system has attained tremendous popularity due to its versatile preparations. However, most of the Siddha formulations are herbal and polyherbal components. However, the health benefits of herb and spice extract discussion going on for centuries. They are using in many branches of industry, such as medicine, pharmacy, cosmetology, and food production [1]. *Thaleesadi Chooranam* is a classic Siddha Polyherbal formulation chosen from the text *Agathiyar Ratnachurukkum*. It indicated Gastritis, Cholic, Flatulence, Excessive thirst, cough, indigestion, *vali* 80, *Azhal* 40, *Ayyam* 96 and asthma. The use of scientific tools is essential to validate the traditional claim. Though Siddha medicines are safe and effective, it is the utmost duty of the Siddha physicians to standardize the formulation to reach the scientific society [2]. The *Thaleesadi Chooranam* is a polyherbal drug; all the ingredients included effectively cure *Ayyam* diseases [3,4,5]. The main aim of this study is to evaluate the physiochemical and phytochemical characteristics of the Siddha drug *Thaleesadi Chooranam*.

2. MATERIALS AND METHODS

Material:

The *Thaleesadi Chooranam* (T.S.C.) selected for the proposed study procured from Pharmacy, Siddha Regional Research Institute (Under C.C.R.S., Ministry of A.Y.U.S.H., Govt. of India) Thiruvananthapuram, Kerala. Our Institute Medicines purchased from Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd., Chennai. (I.M.C.O.P.S.).

English Name	**Tamil Name**	**Botanical Name**	**Active Compounds**
Yew Leaves	*Thaleesam*	*Abies spectabilis*	Abiesin, betuloside, limonene, a-pinene, abiesadine, and myricetin
Cinnamon Bark	*Lavangapatai*	*Cinnamomum verum*	Sabine,Myrcene,Cinnamyl acetate,Benzyl benzoate,Eugenol.
Cardamom	*Elam*	*Elettaria cardamomum*	Cineole,1,8-Cineole
Three pungent	*Thrikaduku(sukku,milagu,thippli)*	*Zingife rofficinale, Piper nigrum Piper longum*	6-gingerol,8-gingerol,Piperidine,Pyrrolidines,Piperine,Beta-caryophyllene,Piperolactam
Liquorice	*Athimadhuram*	*Glycyrrhizin glabra*	glycoside A, and 5-hydroxy-8-methoxyl-flavone-7-O-beta-D-glucuronide and glycoside B. Isoflavones: glabridin, galbrene, glabrone, shinpterocarpin, licoisoflavones A and B, formononetin, glycerin, kumatakenin, hispaglabridin A, hispaglabridin B, 4′-O-methylglabridin and 3′-hydroxy-4′-O-methylglabridin, glabroisoflavanone A and B glabroiso-flavanone B
Asafoetida	*perungayam*	*Ferula asafoetida*	*Umbelliprenin,Tadshiferin,Galbanic acid,Gummosin,Franesiferol A,B,C*
Costus	*Cottam*	*Costusspeciosus*	*Diosgenin, dioscin, Tricontanoic acids*

Cumin	*jeerakam*	*Cuminum cyminum*	cuminal, γ-terpinene, pinocarveol, linalool, 1-methyl-2-(1- -methyl ethyl) benzene, carotol, apigenin, luteolin, cumin aldehyde, cuminic alcohol, p-cymene, β-pinene
Dill seeds	*Sathakuppai*	*Anethum graveolens*	Chlorogenic acid, p-coumaric acid, benzoic acid, salicylic acid, and ellagic acid
Nigella seeds	*karumjeerakam*	*Nigella sativa*	*Thymoquinone, Thymol, Hederin*
Long Pepper Root	*Thippilimoolam*	*Piper longum*	*Piplartine*
Cloves	*Lavangam*	*Cinnamomum Verum*	cinnamaldehyde, camphor, cinnamyl-acetate, caryophyllene, *trans*-α-bergamotene, caryophyllene oxide, linalool, geraniol, bornyl acetate, α-cubebene, γ-element, α-copaene, guaiol, and eugenol
Mace	*Jathipathri*	*MyristicaFragrans*	*Myristicin, Saffrole, Mysristic acid*
Chinese Galls	*Karkidakasringi*	*Rhus succedanea*	*Palmitic acid,Stearic acid,Arachidic acid,Oleic acid,Linoleic acid.*
Nutmeg	*Jathikkai*	*Myristica Fragrans*	*Myristicin, Saffrole, Mysristic acid*
Indian spikenard	*Jadamanjil*	*NardostachysJatamansi*	*Sesquiterpenes,Jatamnsic acid,Jatamansinone,Nardosinone*
Cinnamon Buds	*Sirunagapoo*	*Cinnamomum Wightii*	
Chewbacca Buds	*Chembakamokku*	*MicheliaChampaca*	*Lignins, Benzoic acid,*
Embelia Fruits	*Vayvidangam*	*Embelia Ribes*	*Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone)*
Cinnamon Leaves	*LavangaPathiri*	*Cinnamomum Verum*	cinnamaldehyde, camphor, cinnamyl-acetate, caryophyllene, *trans*-α-bergamotene, caryophyllene oxide, linalool, geraniol, bornyl acetate, α-cubebene, γ-element, α-copaene, guaiol, and eugenol
Ajowan	*Omum*	*Trachyspermum Ammi*	*P-cymene,C-terpinene,alpha and beta -pinene*
Corriander	*Dhaniya - Malli*	*Coriandrum sativum*	*Linalyl-acetate,Decanal,Trideconol,*
Cane Sugar	*Sarkari*		

Table 1: Ingredients of *Thaleesadi Chooranam*: [5]

Table 2: Basic Information

Main Indication	Cough
Potential Action	Bronchodilatory, Mucolytic, Antitussive
Dosage	1 to 3 grams
Best Adjuvant	Honey in productive cough & Ghee (Clarified Butter) in dry cough

Physicochemical Analysis: [6,7,8,9,10,11,12, 13,14]

Extraction:
The T.S.C. was extracted successively with ethyl acetate by cold maceration method. The concentrated extracts are under reduced pressure at room temperature.
The sample tested for the following parameters per Pharmacopoeial laboratory for Indian Medicine guidelines: Loss of drying, Total ash, Acid insoluble ash, Water insoluble ash, Alcohol soluble extractives, and Water-soluble extractives. These data helped identify and ascertain the quality of the collected crude drug.

Physico-Chemical Parameters: [15,16,17,18]
The authors executed the tests at the department of Chemistry, Siddha Regional Research Institute, Thiruvananthapuram, Kerala.

The results of the physicochemical parameters are given in Table 3.

Table 3. Physicochemical evaluation of *Thaleesadi Chooranam*

Sl. No.	Parameters	Result of *Thaleesadi Chooranam* (TSC) (%)
1.	L.O.D. at 105^0C	6.75
2.	Total Ash	4.18
3.	Acid insoluble ash	0.572
4.	Water soluble ash	1.853
5.	Alcohol soluble extractives	21.13
6.	Water soluble extractives	20.44

Preliminary Phytochemical analysis:
Test for Saponins
To 5ml test sample *T.S.C.*, 5 ml of Water was added, and the tube was shaken vigorously. Copious lather formation shows the presence of saponins.

Test for Tannins
To 5ml test sample *T.S.C.*, Ferric chloride was added; a dark blue or greenish-black colour formed, showing the presence of tannins.

Test for Terpenoids
Liebermann-Burchard test
To 5ml of the test sample, *T.S.C.* was mixed with chloroform solution, and a few drops of acetic anhydride were added and mixed well. 1 ml of concentrated sulphuric acid was added from the sides of the test tube, and the appearance of a red ring showed the presence of terpenoids.

Test for Phenols:
Lead Acetate test
To 5ml test sample *T*.S.C., 3 ml of 10% lead acetate solution was added. A bulky white precipitate showed the presence of phenolic compounds.

Test for Steroids
To 5ml test sample *R.E.C.*, 2ml of chloroform was added with a few drops of concentrated Sulphuric acid (3ml) and shaken well. The upper layer in the test tube turned red, and the sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Test for Glycosides
T.S.C. test sample was Mixed with the bit of anthrone on a watch glass, then added one drop of concentrated H_2SO_4 and made paste form and warmed gently over a water bath. A dark green colour showed the presence of Glycosides.

Test for Carbohydrates:

Barfoed's Test
5ml of reagent was added to 5ml of T.S.C. test solution, mixed & kept in a boiling water bath for 1 min. The red precipitate formed indicates the presence of sugar.

Test for Alkaloids:
Dragendorff's Test
5ml of T.S.C. test solution, the filtrate was added 1ml of Dragendorff's reagent along the side of the tube. The formation of an orange-reddish-brown precipitate revealed the presence of alkaloids.

Test for Flavonoids
To 5ml test sample *T.S.C.,* about 5 ml of dilute ammonia solution was added, followed by a few drops of concentrated Sulphuric acid. The appearance of yellow colour shows the presence of Flavonoids.

Test for Coumarins
To 5ml of sample T.S.C., 1 ml of 10% sodium hydroxide was added, and a dark yellow colour shows the presence of coumarins.

Test for Proteins
Biuret Test
To 3ml of *T.S.C.* extracts, 1ml of 1% solution of copper sulphate was added, followed by 5% solution of sodium hydroxide; the formation of violet purple colour shows the presence of proteins.

Test for Quinones
To the 5ml of T.S.C. test sample, added with NaOH. A red colour indicates the presence of Quinones.

High-Performance Thin Layer Chromatography Analysis (H.P.T.L.C.)
H.P.T.L.C. is a micro-analytical separation and determination method widely applied in herbal drug analysis. The standard procedure[4] carried out H.P.T.L.C.

Preparation of extract:
1 g of the chooranam was soaked in 10 ml alcohol and kept overnight. The solution was boiled and filtered. The concentrated filtrate was 1 ml, and the extract was used for the H.P.T.L.C. study.

Application of extract and development of plates:
The alcohol extract was spotted in the form of bands of width 8 mm on silica gel 60 F254 pre-coated aluminium sheets with C.A.M.A.G. microliter syringe attached with Automatic T.L.C. Sampler 4 (ATS4). 5µl and ten µl of the extract were applied in two tracks as bands. After sample application, the plate was introduced vertically in a C.A.M.A.G. developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase, Toluene: Ethyl acetate: Formic acid (5: 1: 0.1, v/v) which gave the maximum resolution.

Documentation:
The air-dried developed plate was kept in a C.A.M.A.G. visualizer and captured images under U.V. light at 254 nm and 366 nm. The plate was scanned at 254 nm and 366 nm using T.L.C. Scanner 4 and documented the fingerprint profiles. The R_f values and fingerprint data were recorded with winCATS software associated with the scanner. The derivatization of the plate by using a vanillin-sulphuric acid reagent, heated at 105°C until the appearance of coloured bands, visualized under white light and documented the chromatogram. The plate was then scanned at 575 nm to obtain the densitometric profile and R_f values.

Ultra Violet-Visible (UV-Vis) spectrophotometric analysis
The analysis was conducted on the alcohol extract of the *Thalesaadi Chooranam* (*T.S.C.*) using a UV/VIS spectrophotometer (Model: UV3120). The extract was examined under U.V., visible light in the wavelength ranging from 200 to 800 nm, and the characteristic peaks were detected and recorded.

3. RESULTS AND DISCUSSION:

Results of physicochemical analysis of TSC
The results from the physicochemical analysis reveal that the value of Loss on drying value of the formulation is 6.75 %. The total ash value of the TSC was 4.18 %, in which the acid-insoluble ash value was 0.572 % in which, the water-soluble ash value was 1.853 %, and the alcohol-soluble extractive value was 21.13 %. Water soluble extractive value was 20.44 %, respectively.

Table 3 shows the results. The results derived from the physicochemical analysis divulge that the Loss of drying value of TSC was 6.75%, indicating that low moisture content could increase the stability and shelf life of the sample drug suitable for medicinal properties. The Total Ash value of TSC was 4.18%, indicating the sample drug's purity. The acid insoluble ash value of TSC indicates the sample drug is not contaminated with siliceous material like sand or dust. The water-soluble values indicated presents easy facilitation of diffusion and osmosis mechanism. The Alcohol soluble extractive values were indicating the test drug TSC has good quality, purity, and no adulteration.

Preliminary Phytochemical evaluation of TSC

The preliminary phytochemical analysis results indicate that the formulation of TSC shows the presence of biologically significant phytochemicals such as saponins, tannins, terpenoids, phenols, glycosides, carbohydrates, and alkaloids. Table 4 shows the results.

Table 4: Preliminary Phytochemical evaluation of TSC

Sl. No.	Tests	Result
1.	Saponins	+
2.	Tannins	+
3.	Terpenoids	+
4.	Phenols	+
5.	Steroids	-
6.	Glycosides	+
7.	Carbohydrates	+
8.	Alkaloids	+
9.	Flavanoids	-
10.	Coumarins	-
11.	Proteins	-
12.	Quinones	-

+ Positive and - Negative

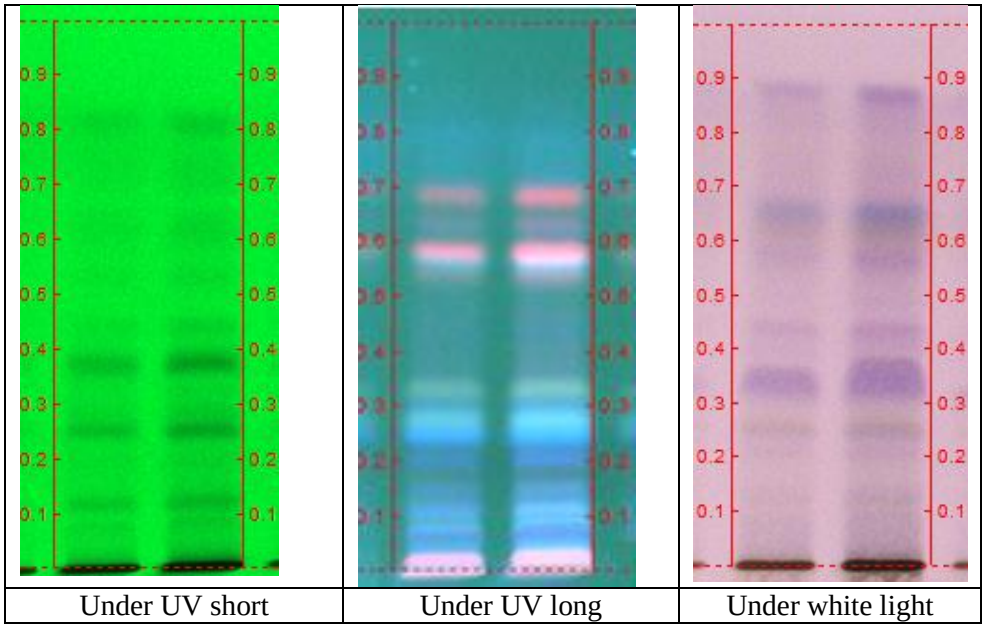
HPTLC analysis of TSC

The HPTLC analysis of the sample drug TSC reveals the presence of 10 prominent peaks corresponding to 10 different compounds with Rf values ranging from 0.06 to 0.81 with a percentage area of 2.66 to 21.54 %. The bands revealed the presence of six green, two blue, and one fluorescent yellow bands showing the presence of saponins, tannins, terpenoids, phenols, glycosides, carbohydrates, and alkaloids. The results were tabulated in Table 5 and illustrated in Figure 1,2&3

Alcohol extract

Solvent system: Toluene: Ethyl acetate (5: 1: 0.1)

Track 1- 5µl, Track 2- 10µl



Under UV short	Under UV long	Under white light
ג= 254 nm	**ג=366 nm**	**ג=575nm**

Figure 1: HPTLC Chromatogram of *Thaleesadi Chooranam*

Figure 2: HPTLC Chromatogram of *Thaleesadi Chooranam*

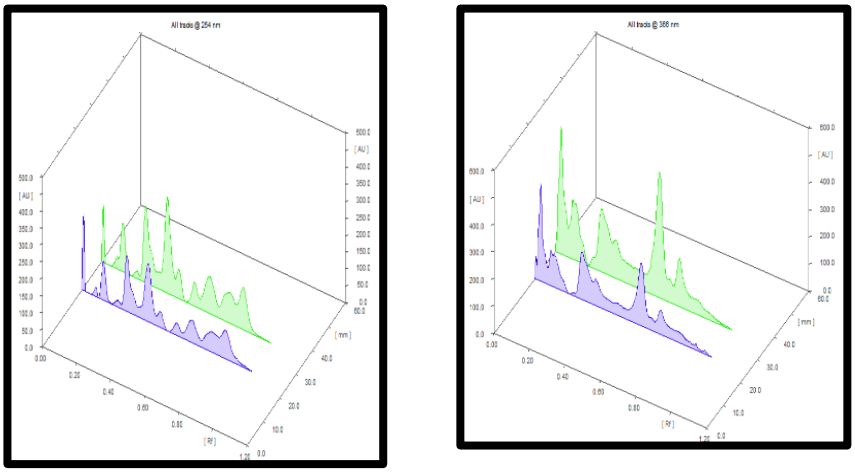
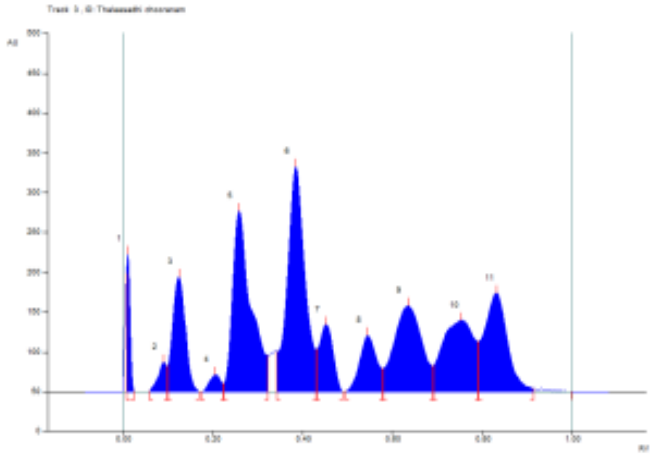


Figure 3: HPTLC Peak of *Thaleesadi Chooranam*



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	208.2 AU	0.01 Rf	228.3 AU	22.42 %	0.03 Rf	0.0 AU	1132.4 AU	4.52 %
2	0.06 Rf	0.2 AU	0.08 Rf	27.2 AU	2.67 %	0.10 Rf	0.5 AU	327.8 AU	1.31 %
3	0.10 Rf	0.9 AU	0.13 Rf	115.0 AU	11.29 %	0.17 Rf	0.1 AU	1976.5 AU	7.89 %
4	0.18 Rf	0.2 AU	0.21 Rf	19.3 AU	1.89 %	0.23 Rf	12.6 AU	387.6 AU	1.55 %
5	0.23 Rf	12.6 AU	0.26 Rf	163.2 AU	16.03 %	0.33 Rf	28.4 AU	4522.4 AU	18.06 %
6	0.33 Rf	28.8 AU	0.39 Rf	171.2 AU	16.82 %	0.44 Rf	28.1 AU	5272.3 AU	21.05 %
7	0.44 Rf	28.4 AU	0.46 Rf	46.2 AU	4.54 %	0.50 Rf	0.0 AU	1054.1 AU	4.21 %
8	0.51 Rf	0.2 AU	0.56 Rf	37.1 AU	3.65 %	0.58 Rf	14.5 AU	939.4 AU	3.75 %
9	0.59 Rf	14.6 AU	0.65 Rf	65.8 AU	6.46 %	0.70 Rf	19.9 AU	2840.1 AU	11.34 %
10	0.70 Rf	19.9 AU	0.77 Rf	61.0 AU	5.99 %	0.80 Rf	53.3 AU	2943.5 AU	11.75 %
11	0.81 Rf	53.3 AU	0.84 Rf	83.9 AU	8.24 %	0.94 Rf	7.0 AU	3646.1 AU	14.56 %

Table 3: Peak Table HPTLC analysis of *Thaleesadi Chooranam* Ultra Violet- Visible (UV-Vis) spectrophotometric analysis of *Thaleesadi Chooranam*

To record the UV-Vis spectra of the powder sample of "Thaleesadi Chooranam," the sample drug was scanned in the wavelength 200-800 nm by using a UV-Vis spectrometer (Model: UV 3120). Fig.4 exhibits the UV-Vis spectra of the sample of TSC. Absorption peaks with their absorbance are shown. The UV-Vis profile of the TSC sample showed peaks at 294 nm, 312 nm, and 380 nm with absorption. The results obtained in UV-Vis spectra revealed the existence of several medicinally essential phytoconstituents.

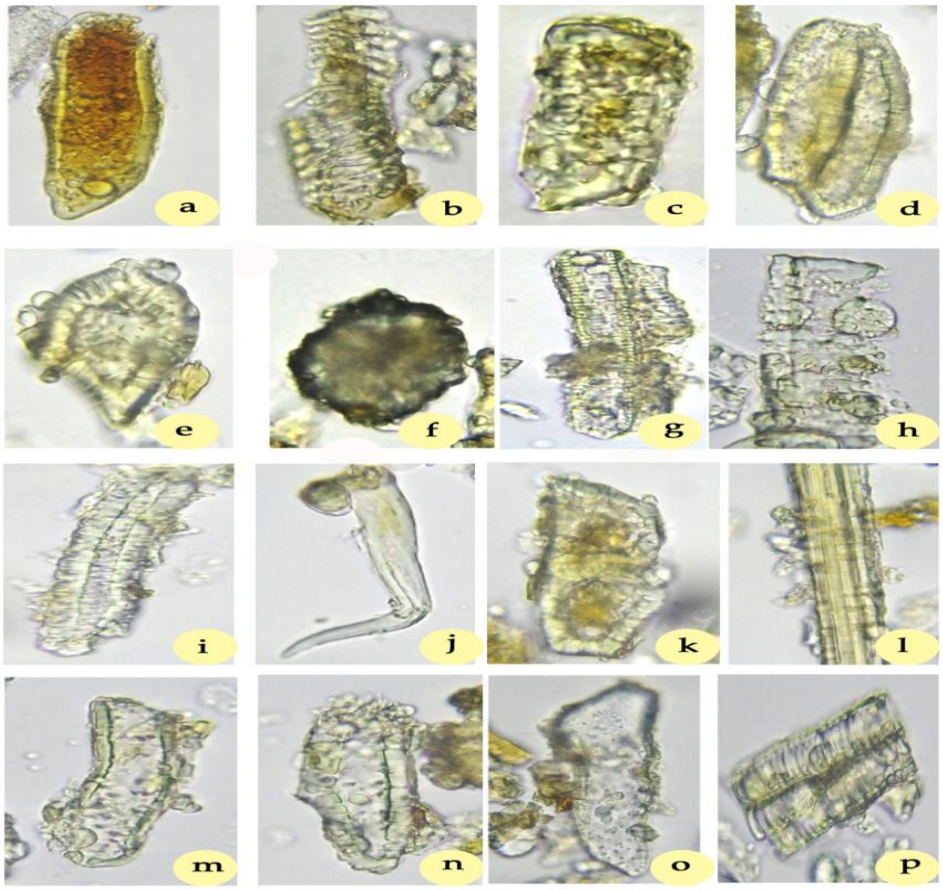
Powder microscopy Test:

Methodology: About 0.5gm of the powdered sample was mounted in glycerin at room temperature for two h and observed under 10X and 40X objectives of a bright field microscope (Meswox, India) for powder characteristics. Photomicrographs of diagnostic characters were captured using the attached camera.

Result: The observation for cellular characters in the sample is that Secondary thickenings of xylem vessels, such as pits and spirals, were observed. Stone cells of various morphotypes and sclereids with inclusions also were seen in the sample. Rosette and prismatic Calcium oxalate crystals, simple as well as compound starch grains, and smooth trichomes were the other diagnostic characteristics.

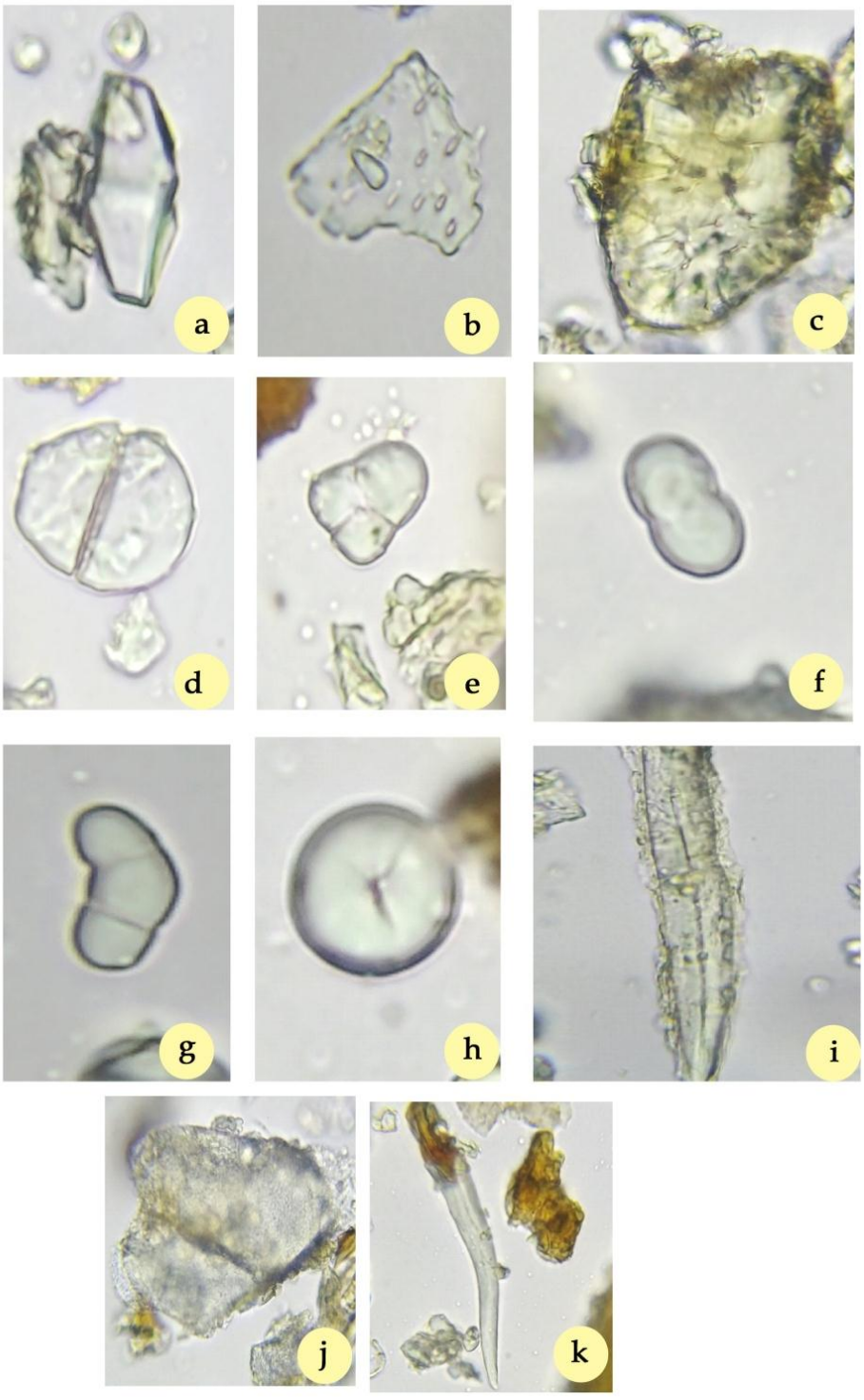
Figure 4. UV-Vis spectrum of *Thaleesadi Chooranam*

Plate 1: Powder microscopy study of Talisadi Choornam



a.Sclereid filled with tannin ; b.Fragment of xylem vessel with spiral thickening; c-e. Stone cell ; f.Rosette Calcium oxalate crystal; g-h.Vessel with pitted thickening ; i.Sclereid with narrow lumen; j.Uniseriate smooth trichome; k.Isodiamtric stone cells filled with tannin; l.Fibre bundles; m-o.Sclereid; p.Vessels with spiral-thickening.

Plate 2: Powder microscopy study of Talisadi Choornam



a.Prismatic Calcium oxalate crystal; b. Fragment of pitted vessel; c.Stone cell; d-g. Compound starch grain; h.Simple starch grain ; i.Fibre with narrow lumen; j.k.Perisperm cell; k.Smooth trichome.

4. CONCLUSION

The standardization process of *Thaleesadi Chooranam* was completed as per PLIM guidelines and standardized procedure. The results of standardization of Siddha polyhedral formulation TSC by different parameters such as physicochemical analysis, preliminary

phytochemical analysis, HPTLC fingerprinting analysis, UV-Vis spectrum analysis, and Powder microscopy will be helpful to as a tool for authentication and analysis of their safety and quality of the herbal drug. Various standardization parameters established in the present study will help control the standards and quality of the raw material of TSC.

Conflict of Interest
The authors declare that they have no conflict of interest.

Acknowledgement
The authors sincerely thank the Central Council for Research in Siddha, Director-General in Siddha, Chennai, Tamil Nadu.

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