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Standardization of *Siddha* Poly-Herbal Formulation *Thirinethira Chooranam* by Modern Pharmaceutical Analytical Techniques

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ABSTRACT

Thirinethira Chooranam is a multi-herbal formulation indicated as hematinic and appetizer in Siddha literature. The aim of this study is to standardize *Thirinethira Chooranam* to ensure its quality, purity and safety through its physicochemical, microbiological, chromatographic, biochemical parameters and analysis of heavy metals, Pesticide residue and aflatoxins. This study determines the physico-chemical parameters that are loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive and pH as 2.187%, 6.9 %, 0.18%, 14.17%, 4.567% and 5.5 respectively denotes purity and quality of drug. From the study results it is ensured that the test drug *Thirinethira chooranam* is free from microbial contamination, heavy metal traces, Pesticide residue toxicity and aflatoxin toxicity. Qualitative preliminary Phyto-chemical analysis reveals that presence of major phytochemicals alkaloids, flavonoids, steroids, triterpenoids, coumarins, phenol, tannin, sugar and betacyanin. High-Performance thin layer chromatography finger printing analysis of the test drug reveals the presence of seven eminent modes relevant to presence of seven different phytocomponents present with in it. Rf value of the modes ranges from 0.01 to 085. Bio-chemical analysis reports the presence of carbonate, sulphides, phosphate, ferrous and magnesium. The obtained results of all the analyses of this study provide data on pharmacognostic (physical, chemical and biochemical) properties of siddha poly-herbal formulation *Thirinethira Chooranam*. In future these data can be utilized as references for the standardization of the drug *Thirinethira Chooranam*.

Keywords: *Thirinethira Chooranam*, Siddha, poly-herbal formulation, standardization

INTRODUCTION

Siddha is one of the oldest systems of medicine in the world. It contains mono-herbal, poly-herbal, herbal-mineral and animal based formulations.[1] Siddha medicines are getting more attention for its potential therapeutic values with lesser side effects. Standardization is most important to ensure the quality, safety and efficacy and bring herbal medicine into mainstream of today's healthcare system.[2]As herbal drugs are commonly mixtures of many components and the difference in the source and quality of plant material, herbal drugs are subjected to more influenceable factors than synthetic drugs which often affect the quality of herbal drugs. *Thirinethira chooranam* is a powder form of enteral medication indicated as hematinic and appetizer in siddha literature. The aim of this study is to standardize Siddha poly-herbal formulation *Thirinethira Chooranam* through organoleptic characters, Physico-chemical analysis, (loss on drying, acid

insoluble ash, water soluble extractive, alcohol soluble extractive, pH, solubility nature) determination of particle size, qualitative phytochemical analysis, High-Performance Thin Layer Chromatography (HPTLC), Heavy metal analysis, determination of Microbial load, Test for Specific pathogen and determination of Pesticide residue, Test for aflatoxin and Analysis of acid and basic radicals, according to Pharmacopoeial Laboratory of Indian Medicine (PLIM) guidelines.

MATERIALS AND METHODS

The test drug *Thirinethira Chooranam* is a poly-herbal formulation mentioned in the *Siddha* literature *"Koshaye Anuboga Vaithiya Bramma Ragasiyam* – part-2"[3] indicated as hematinic and appetizer.[3] Ingredients of *Thirinethira Chooranam* were tabulated in Table-1.

Table-1: Ingredients of Thirinethira Chooranam

S. No	Name of Drugs	Botanical Name	Part Used	Quantity
1	Dried Ginger	Zingiber officinale	Rhizome	35grams
2	Pepper	Piper nigrum	Fruit	35grams
3	Long Pepper	Piper longum	Fruit	35grams
4	PottralaiKaiyaanthakarai	Wedeliachinensis	Leaves	Q.S
5	Sugar Candy powder	Saccharum officinarum		300 grams

Collection of the Raw Drugs

Zingiber officinale, Piper nigrum, Piper longum and Sugar Candy powder were bought from authenticated country drug store in Chennai, Tamilnadu. *Wedeliachinesis* leaves were brought from Koyambedu Market.

Identification and Authentication of Ingredients of the Drug

Identification and authentication of ingredients were done by Botanist, Government Siddha Medical College, Arumbakkam, Chennai. Specimens of Ingredients *Zingiber officinale, Piper nigrum, Piper longum, Wedelia chinensis and* Sugar candy powder labelled as 1046/PGG/601210544/GSMC-CHI/2019-2022 to 1050/PGG/601210544/GSMC-CHI/2019-2022 respectively. The labelled specimens were kept in *Gunapadam* Laboratory for future reference.

Preparation of the Drug-*Thirinethira Chooranam*

The test drug *Thirinethira Chooranam* was prepared in the *Gunapadam* Laboratory of Govt. Siddha Medical College, Chennai, Tamil Nadu, india. After the proper purification,[4]35gm of each ingredient *Zingiber officinale*, *Piper nigrum* and *Piper longum* were mixed together and ground in a stone mortar. *Wedelia chinensis* juice obtained from *Wedelia chinensis* leaves which were processed in steam water for 2 minutes. Dissolved the powdered compound in the *Wedelia chinensis* juice (1.4 ltr) and grinded to make pellets. After the pellets were got dried, it was powdered and filtered. Finally, 300gm of sugar candy powder was mixed to that filtered content and kept in an air tight container and labelled as THC.

Laboratory of Analysis

All the analyses were done at Noble Research Solutions, Chennai.

ORGANOLEPTIC CHARACTERS

Colour, odour, taste and consistency of the drug were noted.

PHYSICO-CHEMICAL EVALUATION. [5], [6]

Percentage of Loss on Drying

10gm of THC was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed.

Percentage loss on drying = Loss of weight of sample/Weight if the sample ×100

Determination of Total Ash

Test drug THC was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it changes to the colour of white which denotes absence of carbon. Total ash

percentage will be evaluated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/Weight of the crude drug taken ×100

Determination of Acid Insoluble Ash

The ash of THC was used in the determination of Acid Insoluble Ash which is obtained from aforementioned total ash test. The ash was subjected to boiling with 25 ml of dilute hydrochloric acid for 6minutes. Then the insoluble matter was collected in crucible and washed with hot water and ignited to constant weight. Percentage of acid insoluble ash evaluated with reference to the weight of air-dried ash.

Acid insoluble ash = weight of ash/ weight of the crude drug taken ×100

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 then it was allowed 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 in comparison with air-dried drug.

Alcohol soluble extract= Weight of extract/weight of the sample taken ×100

Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for one day, shaking frequently for 3 to 4 times and it was allowed to cool 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 in comparison with air-dried drug.

Determination of pH

About 5gm of test sample was dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 minutes and then subjected to pH evaluation.

Solubility Test

In a dry test tube one gram of sample was taken and to it 2ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done in solvents Chloroform, Ethanol, Water, Ethyl Acetate, Hexane and Dimethyl sulfoxide (DMSO). The results are observed individually.

Particle Size Determination [7]

Particle size determination was carried out by optical microscopic method. In which the sample were dissolved in the sterile distilled water (app $1/100^{th}$ 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.

PHYTOCHEMICAL EVALUATION [8]

This evaluation is used to detect the sort of phytochemicals present in the test drug.

Test for Alkaloids-Mayer's Test

To the 5 ml of aqueous extract of THC, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for Coumarins

To the 5 ml of aqueous extract of THC, 1ml of 10% sodium hydroxide was added. Yellow colour inference indicates the presence of coumarins.

Test for Saponins -Foam's Test

To the 5 ml of aqueous extract of THC, 5ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

Test for Tannins-FeCl3 Test

To the 5 ml of aqueous extract of THC, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of Tannins.

Test for Glycosides-Bontrager'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 2ml of filtered hydrolysate, 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour inference indicates presence of glycosides.

Test for Flavonoids-Ammonia Test

To the 5 ml of aqueous extract of THC, about 5ml of dilute ammonia solution were been added followed by addition of few drops of concentrated sulphuric acid. Yellow colour inference indicates the presence of flavonoids.

Test for Steroids

To the 5 ml of aqueous extract of THC, 2ml of Chloroform was added with few drops of concentrated 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. This inference showed the presence of Steroids.

Test for Triterpenoids- Lieberman-Burchard's Test

To the5 ml of aqueous extract of THC, 2ml Chloroform solution few drop acetic anhydride was added then mixed well. 1ml of concentrated sulphuric acid was added from the sides of the test tube. Appearance of red ring indicates the presence of triterpenoids.

Test for Phenols-Lead Acetate Test

To the 5 ml of aqueous extract of THC, 3ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of Phenolic compounds.

Test for Anthocyanin

To the 5 ml of aqueous extract of THC, 1ml of 2N sodium hydroxide was added and heated for 5min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Betacyanin

To the 5 ml of aqueous extract of THC, 2mlof Hydrochloric acid (HCl) was added and heated for 5 minutes at 100°C. Formation of pink colour indicates the presence of betacyanin.

Test for Sugar-Benedict's Test

To the 5 ml of aqueous extract of THC, about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2minutes. A characteristic coloured precipitate indicates the presence of sugar.

Test for Proteins-Biuret Test

To the 5 ml of aqueous extract of THC, 1% solution of copper sulphate was added after adding 5% solution of sodium hydroxide. Appearance of violet purple colour indicates proteins presence.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY [9]

HPTLC method is a modern sophisticated and automated separation technique derived from Thin Layer Chromatography (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 herbal products efficiently and cost effectively. HPTLC method provides high level of selectivity, sensitivity and rapidity along with just an only one step sample preparation. Thus this method can be conveniently used for routine quality control test. 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 analysed.

After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

HEAVY METAL ANALYSIS BY ATOMIC ABSORPTION SPECTROMETRY (AAS)[10]

STANDARD: HG, AS, PB AND CD – SIGMA

Methodology

Atomic Absorption Spectrometry (AAS) is a very familiar and reliable analytical 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 like mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion

Test sample was digested with 1mol/L Hydrochloric acid (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 [11]

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 for 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 [12]

Test sample was directly inoculated into the specific pathogen medium of EMB Agar, Deoxycholate Agar, Mannitol Salt Agar and Cetrimide Agar for the detection of *Escherichia coli*(EC),*Salmonella*(SA), *Staphylococcus Aureus*(ST)and by *Pseudomonas Aeruginosa* (PS) respectively by pour plate method. The plates were incubated at 37ºC for 24 - 72h for observation. Present of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.

PESTICIDE RESIDUE [13], [14]

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 milliliters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

AFLATOXIN ASSAY BY Thin Layer Chromatography (TLC)[15]

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 365nm.

BIOCHEMICAL ANALYSIS OF ACID RADICALS [16]

Test for Carbonates

To 1 ml of the test solution about 1 ml of concentration (conc.) Hydrochloric acid (HCl) was added. Formation of brisk effervescence denotes the presence of carbonate.

Test for Chlorides

To 2 ml of test solution, about 1 ml of silver nitrate solution was added. Appearance of white precipitate indicates the presence of chlorides.

Test for Sulphates

To 1 ml of the test sample add diluted Sulfuric acid (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.

Test for Sulphides

To 1 ml of the test sample about 2 ml of Hydrochloric acid (HCl) was added with slight warming the mixture, Formation of colourless gas with the smell of rotten egg indicates the presence of sulfides.

Test for Phosphates

To 2 ml of test solution treated with 2 ml of ammonium molybdate solution followed by addition of 2ml of concentrated nitric acid, Formation of yellow precipitate Indicate the presence of phosphates.

Test for Fluoride and Oxalate

To 2 ml of the test solution 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.

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 Indicate the presence of Borates.

Test for Nitrates

0.5 ml of test solution heated with copper turning followed by addition of sulphuric acid, Appearance of reddish brown gas Indicate the presence of Nitrates.

BIOCHEMICAL ANALYSIS OF BASIC RADICALS [16]

Test for Lead

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

Test for Arsenic

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

Test for Mercury

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

Test for Copper

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

Test for Ferric

To 1 ml of test solution, about 2 ml of potassium ferrocyanide was added; Formation of blue precipitate indicates the presence of ferric.

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.

Test for Zinc

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

Test for Silver

1 ml of the test solution was added with 1 ml of conc. Hydrochloric acid (HCl) followed by appearance of curdy white precipitate. Boil the precipitate with water. It does not dissolve. Add Ammonium

hydroxide (NH_4OH) solution in it and add 1 ml dilute Nitric acid (HNO_3), Formation of curdy white precipitate indicates the presence of silver.

Test for Magnesium

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

RESULTS AND DISCUSSION

ORGANOLEPTIC CHARACTERS

Organoleptic characters reveal that THC is fine powder, pale brownish in colour, aromatic in odour and sweet with pungent in taste.

Test drug THC shown in fig.-1. The inferences are tabulated in table-2.

Fig.-1 Thirinethira chooranam (THC)

Table-2: Organoleptic characters

State	Solid
Nature	Fine
Odour	Aromatic
Touch	Soft
Flow Property	Non Free flowing
Appearance	Pale Brownish

PHYSICO-CHEMICAL PARAMETERS

The analysis of physicochemical parameter determines that the percentage of loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive and pH as 2.187%, 6.9 %, 0.18%, 14.17%, 4.567% and 5.5 respectively.

The observed results of physicochemical analysis were tabulated in Table-3.

Table-3: Physicochemical Properties

Loss on Drying at 105 °C (%)	2.187 ± 0.4981%
Total Ash (%)	6.9 ± 1.67%
Alcohol insoluble Ash (%)	0.18 ± 0.06245%
Water soluble Extractive (%)	14.17 ± 1.106 %
Alcohol Soluble Extractive (%)	4.567 ± 2.511 %
pH	5.5
THC-Soluble Solvents	Ethanol, Water and DMSO
THC-Insoluble Solvents	Chloroform and Ethyl acetate

PARTICLE SIZE DETERMINATION

Microscopic observation of the particle size analysis reveals that the average particle size of the sample was 30.18 ± 7.32 µm further the sample has particle with the size range of lowest 23 µm to highest 44 µm.

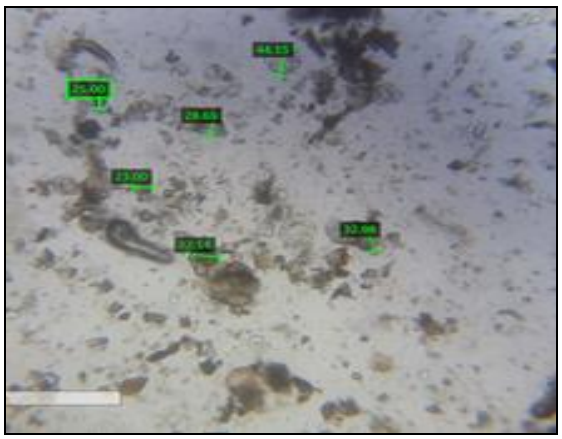

Fig.-2: Microscopic Observation of Particle Size for the sample THC

PRELIMINARY PHYTOCHEMICAL TESTS

Preliminary Phytochemicals test result reveals the presence of alkaloids, flavonoids, steroids, triterpenoids, coumarin, phenol, tannin, sugar and betacyanin (Fig-2). The observed results were tabulated in Table-4. These phytochemicals are responsible for potential therapeutic properties of THC.

Table-4: Preliminary Phytochemical Tests

S. No	Phytochemicals	Result
1	Alkaloids	Present
2	Flavonoids	Present
3	Steroids	Present
4	Triterpenoids	Present
5	Coumarin	Present
6	Phenol	Present
7	Tannins	Present
8	Sugar	Present
9	Betacyanin	Present

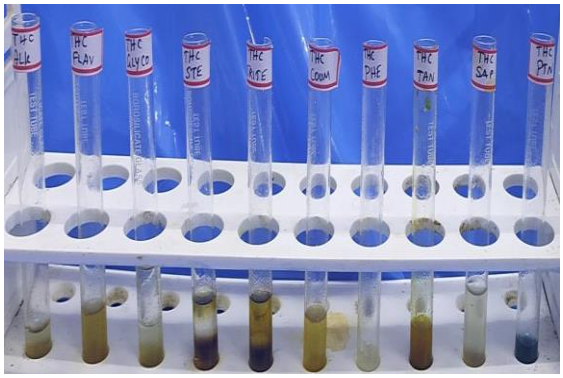

Fig.-2: Preliminary Phytochemical screening

HPTLC finger printing analysis of the sample reveals the presence of seven eminent modes relevant to presence of seven different phytocomponents present with in it. Rf value of the peaks ranges from 0.01 to 0.85.

The result of HPTLC finger printing analysis of the sample THC tabulated in Table-5 with Rf value of the peaks ranges from 0.01 to 085. Fig.3 shows the graph of HPTLC finger printing.

PERFORMANCE THIN LAYER CHROMATOGRAPHY

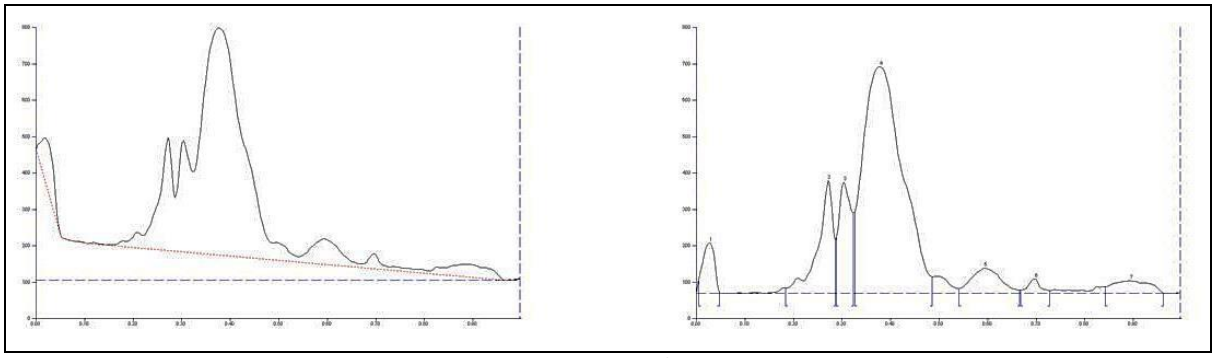

Fig.-3: HPTLC finger printing

Table-5: HPTLC Peak Table

Peak	Start Rf	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area%
1	0.00	38.4	139.3	9.14	0.05	4.4	1596.8	4.30
2	0.19	15.1	310.2	20.35	0.29	146.6	4517.3	12.17
3	0.29	154.9	305.8	20.06	0.32	221.6	3709.2	10.00
4	0.33	225.6	623.3	40.90	0.49	46.0	23502.9	63.34
5	0.54	13.5	69.7	4.57	0.67	9.4	1995.1	5.38
6	0.67	9.9	40.4	2.65	0.73	7.9	496.6	1.34
7	0.85	19.2	35.4	2.32	0.96	1.1	1288.2	3.47

HEAVY METAL ANALYSIS BY AAS

Results of the present investigation have clearly shown that the sample had no traces of heavy metals such as Arsenic, Mercury, Lead and Cadmium.

The observed results of heavy metals analysis were tabulated in Table-6.

Table-6: Result of Heavy Metal Analysis

Heavy Metal	Absorption 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 Detective Level

MICROBIAL CONTAMINATION TEST BY POUR PLATE METHOD

Microbial contamination test reveals that no growth / colonies in any of the plates inoculate with the test sample THC. The results of microbial contamination test were tabulated in Table-7. Plates are shown in (Fig.-4).

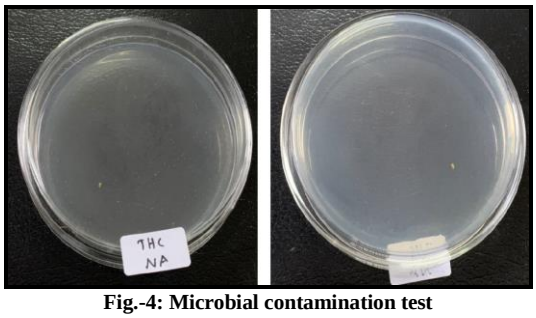

Fig.-4: Microbial contamination test

Table-7: Result For Microbial Contamination Test

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

TEST FOR SPECIFIC PATHOGEN

The test results of specific pathogen screening for *E.coli*, *Salmonella*, *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in test drug THC reveals that absence of the fore mentioned specific pathogens. The results were tabulated in Table-8. Culture plates in Fig 5.1, Fig 5.2, Fig 5.3, and Fig 5.4

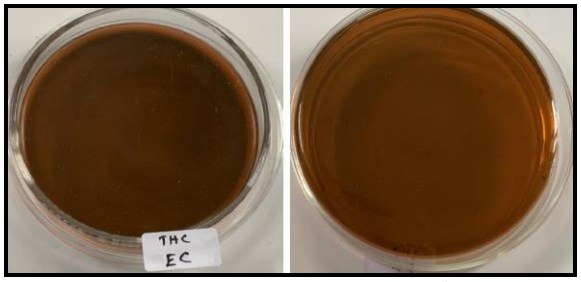

Fig.5.1-Culture plates with E-coli (EC) specific medium

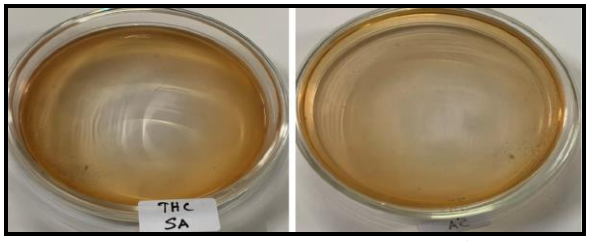

Fig.5.2.-Culture plate with Salmonella (SA) specific medium

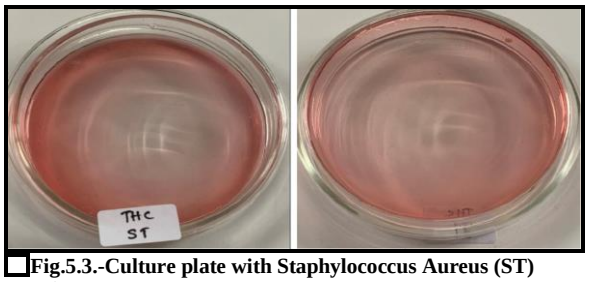

Fig.5.3.-Culture plate with Staphylococcus Aureus (ST) specific medium

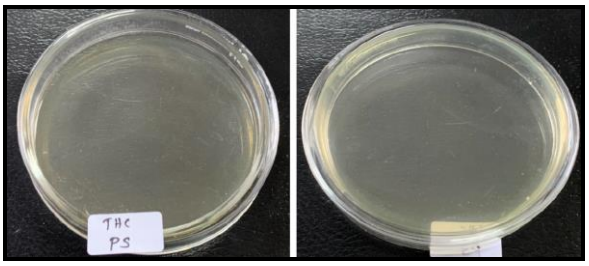

Fig.5.4.-Culture plate with Pseudomonas Aeruginosa (PS) specific medium

Table -8: Result of test for specific Pathogen

S. No	ORGANISM	SPECIFICATION	RESULT
1	E.coli	Absent	Absent
2	Salmonella	Absent	Absent
3	Staphylococcus Aureus	Absent	Absent
4	Pseudomonas Aeruginosa	Absent	Absent

ANALYSIS OF PESTICIDES RESIDUES- ORGANOCHLORINE, ORGANOPHOSPHORUS AND PYRETHROIDS

Analysis of pesticides residues reveals that THC contains no traces of residues of organochlorine, organophosphorus and pyrethroids.

The observed results of pesticides analysis were tabulated in table-9.

Table-9: The Results of Pesticides Analysis

I.	Organo Chlorine Pesticides	RESULT	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.	**Organo carbamates**		
	Carbofuran	BQL	0.1mg/kg
IV.	**Pyrethroid**		
	Cypermethrin	BQL	1mg/kg

BQL- Below quantified limit

AFLATOXIN ASSAY BY TLC (B1,B2,G1,G2)

Result of aflatoxin assay reveals that test drug THC is free from Aflatoxin B1, B2, G1, and G2. The observed results of Aflatoxin assay analysis tabulated in Table-10.

Table No-10: Results For Aflatoxin Assay By Tlc

Aflatoxin	Sample THC	AYUSH Specification Limit
B1	Not Detected-Absent	0.5ppm
B2	Not Detected-Absent	0.1ppm
G1	Not Detected-Absent	0.5ppm
G2	Not Detected-Absent	0.1ppm

ANALYSIS FOR ACID AND BASIC RADICALS

Acid and basic radicals analysis reports that the test drug THC contains carbonates, sulphides, phosphates in acid radicals and ferric, magnesium in basic radicals. The observed results of acid and basic radical analysis were tabulated in Table-11 & 12 respectively.

Table No- 11: Result of Test for Acid Radicals

Acid Radicals	Result
Test for carbonates	Present
Test for sulphides	Present
Test for phosphates	Present

Table No-12: Result of Test for Basic Radicals

Basic Radicals	Result
Test for Ferric	Present
Test for Magnesium	Present

DISCUSSION

The drug THC was a fine powder pale brownish in colour with strongly aromatic in odour. Organoleptic properties colour, texture, taste and fine powder nature indicates the genuinity, purity and quality of the test drug THC. The loss on drying value indicates the moisture content of the drug which was evaluated as 2.187 ± 0.4981%. Moisture content of the herbal medicine should be minimized as it encourages the growth of living organism, presence of fungi or insects, and cause deterioration following hydrolysis.[17] For herbal drug the moisture content have to be less than 14%.[18] The total ash value of the test drug THC was 6.9 ± 1.67%. The value of acid insoluble ash was 0.18 ± 0.062453% which indicates that the drug contains only negligible amount of siliceous matter. Ash value is one of the most significant measurable factors for the quality control of herbal drug. A high ash value represents presence of more inorganic residues such as phosphates, carbonates and silicates.[19] The water soluble extractive value and alcohol soluble extractive value were determined as 14.17 ± 1.106 % and 4.567 ± 2.511% respectively. Extractive values are useful in the determination of amount of the phyto-constituents present in herbal drug and helpful in estimating the chemical proportions soluble in a particular solvent.[20] The pH value is calculated as 5.5 which indicates that the drug is weekly acidic. Solubility property impacts bioavailability and pharmacological efficacy of a drug.THC is soluble in water, ethanol, dimethyl sulfoxide and insoluble in chloroform and ethylacetate. Drugs that poorly soluble in water often need enhanced doses to attain therapeutic plasma concentration after intake.[21]. Particle size determination confirms that the test drug THC is fine powder with average particle size of 30.18µm. Solubility, processing properties, bioavailability, product uniformity, stability and therapeutic efficiency are affected by particle size.[22] Qualitative Preliminary Phytochemicals test result reveals the presence of alkaloids, flavonoids, steroids, triterpenoids, coumarin, phenol, tannin, sugar and betacyanin in the test drug THC. Phytochemicals are chemical compounds present in plants and act as pharmacologically active substance which useful in treating human diseases as medicinal ingredients and nutrients. These phytochemicals are responsible for potential therapeutic properties of THC.[23] Alkaloids are nitrogen containing base which is used as anti-cancerous, sedatives, anti-microbial, insecticidal. Basically flavonoids are consist of polyphenolic compound which has an efficient anti-oxidant activity, anti-microbial, cytotoxic, anti-inflammatory and anti-tumour activity.[24] Plant steroids has several pharmacological activities like

immunosuppressive, anti-tumor, hepatoprotective, antibacterial, plant, anthelminthic, cardiotonic and cytotoxic activity. Plant steroids has several pharmacological activities like immunosuppressive, anti-tumor, hepatoprotective, antibacterial, plant, anthelminthic, cardiotonic and cytotoxic activity.[25] Triterpenoids are one of the secondary plant metabolites of plants which are isopentenyl pyrophosphate oligomers. Triterpenoids has efficacy in the chemoprophylaxis and chemotherapeutic approach to decrease the burden of breast cancer. [26] As triterpenoids have therapeutic activities of anti-inflammatory, analgesic, antipyretic, hepatoprotective, cardiotonic and sedative, it is used in Asian countries.[27] Coumarins has anti-inflammatory, anti-spasmodic, anti-edematous and vascular tonic effects.[28] Phenols possess anti-bacterial and anti-fungal activity. [29] Tannins are poly-phenolic compounds which regulates glucose level in blood by stimulating receptor cells to bind carbohydrate.[30] Betacyanins has potential therapeutic efficacy in the treatment of dyslipidemia, hypertension, cancer, and cardiovascular diseases.[31] HPTLC finger printing analysis of the sample THC reveals the presence different phytocomponents. Heavy metal analysis shows that the test drug THC has lead, arsenic, cadmium and mercury in below detection limit. Results of the present investigation have clearly showed that the sample THC has no traces of heavy metal lead. The term heavy metal represents to a metallic chemical elements that has a relatively high density and poisonous even at minute concentration. Some metals like mercury, lead, arsenic and cadmium cause poisonous and carcinogenic effects when administrated even at low concentration. Heavy metals may be present in the siddha preparation as an additive and trace by knowing or unknowingly. But chronic exposure towards some heavy metals greatly affects the respiratory, cardiovascular, renal and nervous system by cause inflammation and further leads to loss of normal body's physiology.[32] No growth / colonies were observed in any of the plates inoculated with the test sample THC indicates that the test drug is sterile and free from bacterial and fungal contamination. THC has pesticide in below quantify limit which means there is no contamination of pesticides. Pesticides are chemical compounds used to control or eradicate pest. According to their chemical structure, they are classified into Organo Chlorine, Organo Phosphate and Pyrethroids. When these pesticides are used on herbal plants during agricultural practices will remain in plants that become a significant source of contamination of herbal medicines. As pesticides cause several health issues it is necessary to determine the quantity of pesticides in herbal medicines.[32] The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were not contaminated from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 .Aflatoxins are toxic fungal secondary metabolites produced by Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius. As aflatoxins are highly toxic, carcinogenic, mutagenic, teratogenic and hepatotoxic, it is important to determine the aflatoxins in herbal medicines. [33] Test for Acid radicals reveals that presence of carbonates, sulphides and phosphates. Carbonate, as bicarbonate ions maintains pH and acid balance in several parts of the body. Sulfur is an important constituent of vitamins and coenzyme. Phosphorus is essential for healthy physiological function of heart, bone growth, renal function and regulates blood sugar level.[34] Test for basic and reveals that presence of ferric and magnesium. When Ferric is reduced to the ferrous state by the apical membrane-bound enzymes present in human enterocytes,[35] it is important for erythrocyte production. Magnesium act as cofactor in more than 300 enzyme systems that control versatile biochemical functions in the human body,

including protein production, muscle and nerve function, blood sugar control and blood pressure regulation.[36]

CONCLUSION

Through the results of this study, it was concluded that the siddha poly-herbal formulation THC has essential biologically active therapeutics and will act therapeutically in treating diseases. The obtained results of all the analyses of this study provide data on pharmacognostic properties of the Drug THC. In future these data can be used as references for the standardization of the drug THC.

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