Analytical specifications of *Siddha* herbomineral formulation *Manimandhirathi chooranam* by PLIM guidelines

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***Abstract*- In *Siddha* system of medicine, there are 32 types of internal medicines and 32 types of external medicines are mentioned in literature.Various sources of drug are mentioned that are Plants (mūlikai), Metals & Minerals (tātu) & Animal origin drugs (cīva). *Chooranam* is one of the internal medicines in which the raw drugs are pounded into fine powder. *Manimandhirathi chooranam* is one of the herbomineral *chooranam* preparation mentioned in *"Agasthiyar Mani 4000 ennum vaithiya sinthamani venba Muthal pagam"* for *Kuṉmam* (Acid peptic disorders). Aim of this study to standardize the drug *Manimandhirathi chooranam*, according to the analytical specifications of *chooranam* as per PLIM guidelines that includes Physicochemical, Phytochemical & Biochemical analysis, heavy metal analysis, pesticide residue, aflatoxin and microbial load analysis. The obtained results of these tests have been mentioned in this article.**

***Keywords*: Siddha, Manimandhirathi chooranam, Standardization, PLIM guidelines, Kuṉmam.**

INTRODUCTION

Traditional medicines are practiced by the people of various parts of the world, depending upon their habitual and availability of their needs. *Siddha* system of medicine is one of the traditional medicines that have been practiced widely in the southern part of India, mainly in Tamil Nadu. In *Siddha* system of medicine, *Chooranam* (Cūraṇam-medicinal powders) is one of the form of internal medicines in which, purified raw materials are pounded separately, sieved and mixed according to a given ratio; for certain preparations the purified raw materials are mixed as per the ratio prescribed, then powdered and sieved; shelf-life of three months[1,2]. Standardization is an important factor for internal medicines, in order to estimate the quality of the drugs based on the concentration of their active principle. In India, about 4.1% prevalence of Peptic Ulcer Disease (2% gastric ulcers and 2.1% duodenal ulcers) are rated[3]. The drug, ***Manimandhirathi chooranam*** is one of the *Siddha* herbomineral drug indicated for ***Kuṉmam (Acid peptic disorders)***, *Soolai*, *Moolam*, *Vayitru noi* in *Siddha* literature ***"Agasthiyar Mani 4000 ennum vaithiya sinthamani venba Muthal pagam"***[4]. In this study, the analytical specifications of *chooranam* mentioned in PLIM guidelines[5] that are, description of drug - Macroscopic & microscopic, Loss on drying at 105ºC , Total – ash, Acid – insoluble ash, Water-soluble extractive, Alcohol – soluble extractive, Particle size (80-100 mesh for Churna), Identification-TLC/HPTLC-with marker (wherever possible), Test for heavy/Toxic metals (Lead, Cadmium, Mercury, Arsenic), Microbial contamination (Total bacterial count,Total fungal count), Test for specific Pathogen (E. coli, Salmonella spp. S.aureus, Pseudomonas aeruginosa), Pesticide residue(Organochlorine pesticides, Organophosphorus pesticides, Pyrethroids), Test for Aflatoxins (B1,B2,G1,G2) are conducted and their results have been detailed.

MATERIALS AND METHODS

Selection of the drug

"Manimandhirathi chooranam" has been taken from the *Siddha* literature *"Agasthiyar Mani 4000 ennum vaithiya sinthamani venba Muthal pagam"*, Page No-192

Table.no: 01 - Ingredients of the drug MMC

S.No	Name of the drug	Scientific name	Quantity
1.	*Indhuppu*	*Sodium chloride impura salt*	*1 varagan (4.2 gm)*
2.	*Seeragam*	*Cuminum cyminum*	*2 varagan (8.4 gm)*
3.	*Asamadha omam*	*Carum copticum*	*3 varagan (12.6 gm)*
4.	*Sukku*	*Zingiber officinale*	*4 varagan (16.8 gm)*
5.	*Thippili*	*Piper longum*	*5 varagan (21 gm)*

6.	*Milagu*	*Piper nigrum*	*6 varagan (25.2 gm)*
7.	*Kadukkai*	*Terminalia chebula*	*21 varagan (88.2 gm)*

Collection of the raw drug

The raw drugs *Indhuppu (Sodium chloride impura salt), Seeragam (Cuminum cyminum), Asamadha omam (Carum copticum), Sukku (Zingiber officinale), Thippili (Piper longum), Milagu (Piper nigrum), Kadukkai (Terminalia chebula)* were bought from authenticated raw drug store, Chennai, Tamil Nadu.

Recognition and Authentication of the drug :

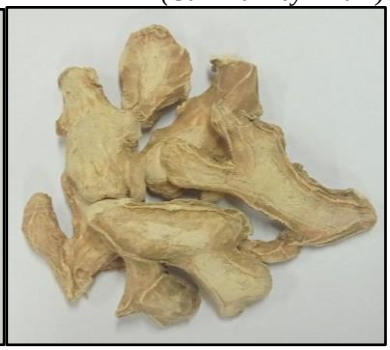
All drugs were recognized and authenticated by Gunapadam experts in Government Siddha Medical College, Arumbakkam, Chennai. Each sample has been labeled as 1060-1066/PGG/320220100503/GSMC-CH/2020-2023. The identified product samples were maintained in the PG Gunapadam laboratory for future references.

Fig. No: 01 - Ingredients of MMC

Indhuppu
(Sodium chloride impura salt)

Seeragam
(Cuminum cyminum)

Asamadha omam
(Carum copticum)



Sukku
(Zingiber officinale)

Thippili
(Piper longum)

Milagu
(Piper nigrum)

Kadukkai
(Terminalia chebula)

Purification of the drug:
Purification process were done as per classical *Siddha* literature (*Sarakkugalin suthi sei muraigal*)[6]

Method of Preparation :
The above given ingredients were taken in the mentioned quantity and pounded into fine powder. Sieved the powder in a thin cotton cloth, then stored in a clean air-tight container and named as *Manimandhirathi Chooranam - MMC*

Purification of the Chooranam:

***Pittaviyal murai* (Steaming process):**
The *Manimandhirathi chooranam* was purified by the *Pittaviyal* method as per *Siddha* literature. A mud pot was taken and it was half filled by a mixture of milk with equal quantity of water. The mouth of the pot was sealed with a cloth. This *chooranam* was placed over the cloth and tied firmly around the mouth of the mud pot by another pot. The gap between mud pots was tied with a wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk 3 /4 part was reduced in the lower pot. The same drug was later dried and powdered then sieved again. It was used for further study.

Storage of the drug:
The prepared test drug was stored in a clean, dried, air tight container. The contents were explored frequently to avoid moisture and microbes.

DRUG PROFILE :
Route of Administration : Oral route
Dose : 1 to 2 gram, twice a day
Adjuvant: Warm water
Indication: ***Kuṉmam (Acid peptic disorders)***, *Soolai, Moolam, Vayitru noi*

STANDARDIZATION OF THE DRUG
Standardization of the drug brings the validation of a medicine by subjecting the drug to many analyses and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug.

Organoleptic properties :
The state, nature, odor, feel, flow property, physical appearance, and taste were noted from the prepared drug MMC.

Physicochemical analysis[7,8]:
The following physicochemical analysis was done and their results were noted.

Loss on drying
An accurately weighed 1 gm of MMC formulation was taken in a tarred glass bottle. The crude drug was heated at 105ºC for 6 hours in an oven at a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

Total Ash
Weighing accurately 2 gm of MMC formulation was added in the crucible at a temperature 600ºC in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

Acid insoluble ash
Ash above obtained from MMC, was boiled for 5 min with 25 ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

Water soluble ash
Total ash of MMC, 1 gm was boiled for 5 min with 25 ml water and insoluble matter collected on an ashless filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450ºC in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

Water soluble extractive
5 gm of air dried drug, fine powdered MMC was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 100ºC and weighted. The percentage of water soluble extract was calculated with reference to the air dried drugs.

Acid soluble extractive

1 gm of air dried drug fine powdered MMC was macerated with 20 ml alcohol in a closed flask for 24 hours. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10 ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100ºC and weighted. The percentage of alcohol soluble extract was calculated with reference to air dried drug.

Solubility:

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

pH determination:

Required quantity of the test sample was mixed with distilled water and then subjected to screening using a pH meter.

Phytochemical analysis[7,8] :

The following tests were carried out for the phytochemical analysis. Preliminarily the sample was dissolved with dilute Hydrochloric acid for several tests.

Test for Alkaloids:

Mayer's test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow color precipitate indicates the presence of alkaloids.

Dragendroff's test

Filtrates were treated with Dragendroff's reagent (Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.

Wagner's test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for Carbohydrates:

Molisch's test

To 2 ml of plant sample extract, two drops of alcoholic solution of α-naphthol are added. The mixture is shaken well and a few drops of concentrated sulphuric acid are added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

Benedict's test

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Saponin :

Foam test

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Test for Phenols :

Ferric chloride test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Test for Tannin :

Gelatin test

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

Test for Flavonoids

Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless in addition to dilute acid, indicates the presence of flavonoids.

Lead acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Test for Diterpenes :

Copper acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicating the presence of Quinones.

Gum and Mucilage

To 1 ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examined for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

Biochemical analysis:

5 gm of MMC was dissolved with 50 ml of distilled water. Boiled well for 10 minutes and cooled. Filtered the extract and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Analysis of Specific acid radicals :

Test for Carbonates :

1 ml of the test solution was added with 1 ml of concentration (conc.) HCl. Formation of brisk effervescence indicates the presence of carbonates.

Test for chlorides

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

Test for sulfates

1 ml of the test sample was added to dilute H_2SO_4 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 sulfides

1 ml of the test sample about 2 ml of HCl was added with slight warming the mixture. Formation of colorless gas with the smell of rotten egg indicates the presence of sulfides.

Test for phosphates

2 ml of test solution treated with 2 ml of Ammonium molybdate solution followed by addition of 2 ml of concentrated nitric acid. Formation of yellow precipitate Indicates the presence of phosphates.

Test for Fluoride and Oxalate

2 ml of the test solution about 2 ml of dil acetic acid and 2 ml 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 Indicates 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 Indicates the presence of Nitrates.

Analysis of Specific basic radicals :

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 (NH_4OH) solution. Formation of blue precipitate indicates the presence of copper.

Test for Ferric

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

1 ml of test solution, about 1 ml of potassium ferricyanide 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. HCL followed by the appearance of curdy white precipitate. Boil the precipitate with water. It does not dissolve. Add NH_4OH solution in it and add 1 ml dilute 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.

Particle size determination[9] :

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

Identification - TLC / HPTLC :

TLC Analysis[10,11]

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60 F 254, 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-microliter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm

HPTLC Analysis :

Pre-coated HPTLC graded plates and auto sampler were used to achieve precision, sensitive, significant separation both qualitatively and quantitatively.

Instrument - CAMAG TLC SCANNER III

TLC Plate - Aluminium Coated Silica Gel – Merck

Mobile Phase - Chloroform: n-Butanol: Methanol: Water: Acetic Acid (4:1:1:0.5:0.5)

Chromatogram Development - It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

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

Heavy metal analysis[12] :

The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determine the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion - Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the samples were digested with 1 mol/L of HNO3.

Standard preparation : As & Hg- 100 ppm sample in 1 mol/L HCl

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

Sterility test :

Pour plate method[13]

MMC was mixed with sterile distilled water and the mixture was used for the sterility evaluation. About 1 ml of the MMC extract was inoculated in sterile petri dish to which about 15 ml of molten agar 45ºC was added. Agar and MMC 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 37ºC for 24 - 48 hours and further extended for 72 hours for fungal growth observation. Grown colonies of organisms were then counted and calculated for CFU.

Table.no: 02 - Microbial load

Test	Specification
Total bacterial count	NMT 10^5CFU/g
Total fungal count	NMT 10^3CFU/g

Test for specific pathogens[14] :

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

Table.no: 03 - Specific Medium and their abbreviation

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

Pesticide residue[15,16,17] :

Test sample was extracted with acetone and followed by homogenization for a brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of the test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent had 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 a membrane filter.

Aflatoxins[18,19] :

Standard - Aflatoxin B1

Aflatoxin B2

Aflatoxin G1

Aflatoxin G2

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.

Standard aflatoxin was applied on the surface to pre-coated TLC plates 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 unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

RESULTS

Organoleptic properties :

Fig.no: 02 - Prepared MMC

MMC was soft and brown in color, smells as strongly aromatic, tastes as pungent and slightly salty.

Table.no: 04 - Organoleptic properties of MMC

S.No	Parameter	Result
1.	State	Solid
2.	Nature	Fine
3.	Odour	Strongly aromatic
4.	Touch	Soft
5.	Flow property	Free flowing
6.	Appearance	Brown in color
7.	Taste	Pungent and slightly salty

Physicochemical analysis :

The physicochemical analytical results of MMC were tabulated below.

Table.no: 05 - Results of Physicochemical analysis of MMC

S.No	Parameter	Result
1.	Loss on drying	10.49%
2.	Total Ash	5.93%
3.	Acid insoluble ash	0.77%
4.	Water soluble ash	3.62%
5.	Water soluble extractive	43.446%
6.	Acid soluble extractive	34.73%
7.	pH	7.4

Solubility:

Table.no: 06 - Results of Solubility test of MMC

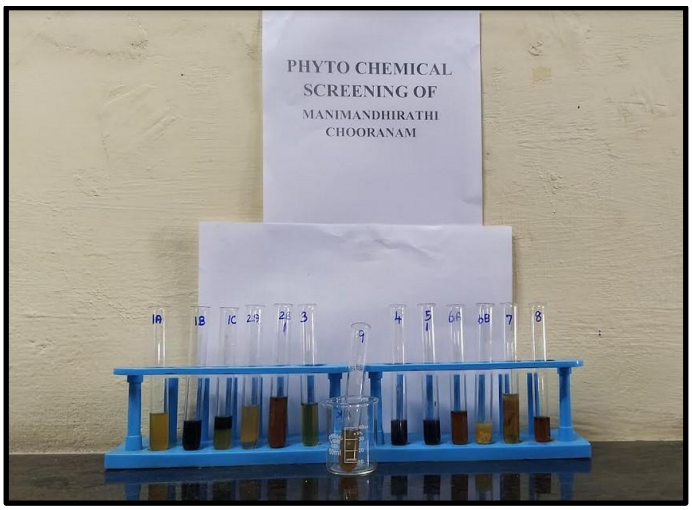
S.No	Solvent Used	Solubility / Dispersibility
1.	Chloroform	In Soluble
2.	Ethanol	Soluble
3.	Water	Soluble
4.	Ethyl acetate	In Soluble
5.	Hexane	In Soluble
6.	DMSO	Soluble

Phytochemical analysis :

Table.no: 07 - Results of Phytochemical analysis of MMC

S.No.	Phytochemicals	Test Name	H_2O Extract
1	Alkaloids	Mayer's Test Dragendroff's Test Wagner Test	-ve +ve +ve
2	Carbohydrates	Molisch's Test Benedict Test	+ve +ve
3	Saponin	Foam Test	+ve
4	Phenols	Ferric Chloride Test	+ve
5	Tannins	Gelatin Test	+ve
6	Flavonoids	Alkaline Reagent Test Lead acetate	-ve +ve
7	Diterpenes	Copper Acetate Test	-ve
8	Quinones	Test for Quinones	+ve
9	Gum & Mucilage	Test for Gum & Mucilage	+ve

Fig.no: 03 - Phytochemical screening of MMC



Biochemical analysis :

Table.no: 08 - Results of Test for acid radicals

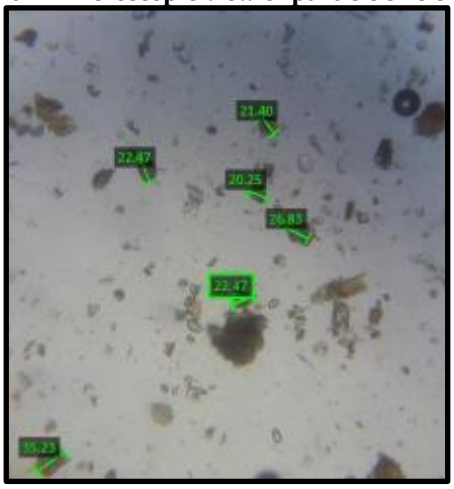
S.No	Specific radical	Observation	Test report
1.	Test for carbonates	Presence of brisk effervescence	Presence
2.	Test for chlorides	Absence of White precipitate	Absence
3.	Test for sulfates	Presence of white precipitate	Presence
4.	Test for sulphides	Absence of rotten egg smell	Absence
5.	Test for phosphates	Absence of yellow precipitate	Absence
6.	Test for Fluoride & Oxalate	Absence of white precipitate	Absence
7.	Test for Borates	Absence of green flame	Absence
8.	Test for Nitrates	Absence of reddish brown color	Absence

Table.no: 09 - Results of Test for basic radicals

S.No	Specific radical	Observation	Test report
1.	Test for Lead	Presence of yellow precipitate	Presence
2.	Test for Arsenic	Absence of brownish red precipitate	Absence
3.	Test for Mercury	Presence of yellow precipitate	Presence
4.	Test for Copper	Absence of blue precipitate	Absence
5.	Test for Ferric	Absence of blue precipitate	Absence
6.	Test for Ferrous	Absence of blue precipitate	Absence
7.	Test for Zinc	Absence of white precipitate	Absence
8.	Test for Silver	Absence of curdy white precipitate	Absence
9.	Test for Magnesium	Absence of white precipitate	Absence

Particle size determination :

Fig.no: 04 - Microscopic view of particle size of MMC



Microscopic observation of the particle size analysis of MMC discloses that the mean average particle size of the sample was sized up to be 24.36 ± 5.15 µm, range of lowest 20µm to highest 35µm.

Identification - HPTLC :

HPTLC fingerprinting analysis of the sample reveals the presence of eight prominent peaks corresponds to the presence of eight versatile phyto components present within it. Rf value of the peaks ranges from 0.02 to 0.91.

Fig.no: 05 - TLC Visualization of MMC at 366 nm

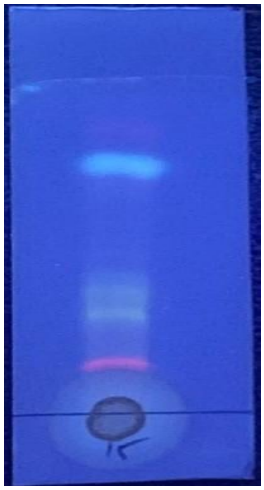


Fig.no: 06 - 3D chromatogram

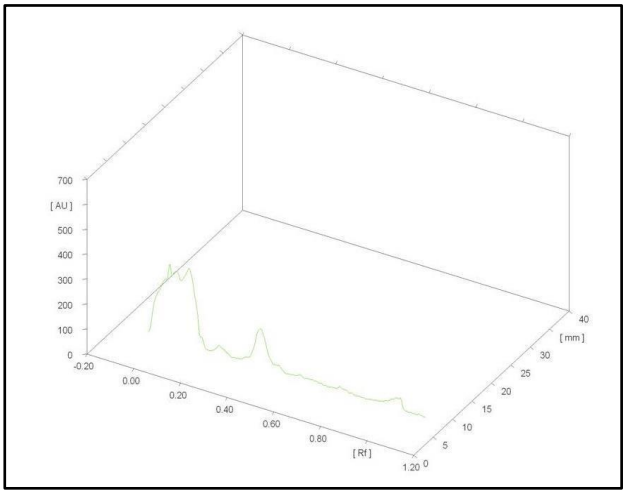


Fig.no: 07 - HPTLC fingerprinting of MMC

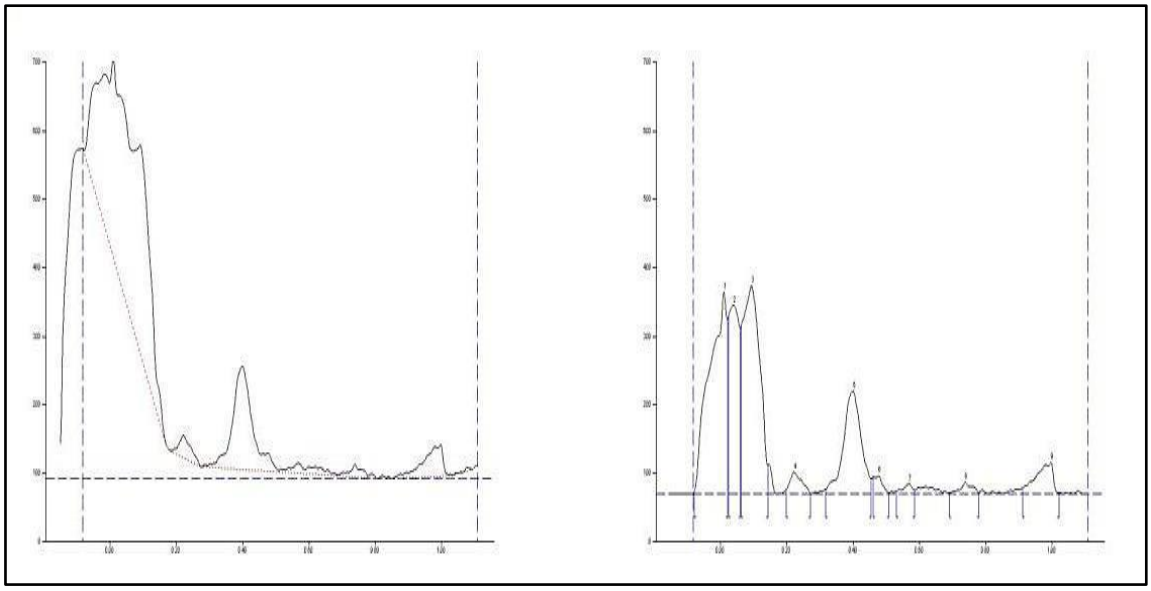


Table.no: 10 - Peak table of phytocomponents of MMC

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.08	2.8	0.01	295.8	25.40	0.02	255.9	8085.3	29.32
2	**0.02**	259.4	0.04	275.9	23.69	0.06	244.1	4493.5	16.30
3	0.06	246.5	0.09	304.4	26.14	0.14	43.1	8070.5	29.27
4	0.20	6.4	0.22	32.6	2.80	0.27	0.1	598.2	2.17
5	0.32	6.7	0.40	150.2	12.89	0.46	23.0	4175.4	15.14
6	0.46	25.0	0.48	26.4	2.26	0.51	1.9	358.6	1.30

7	0.53	3.8	0.57	14.7	1.26	0.59	6.5	228.7	0.83
8	0.69	1.8	0.74	18.1	1.55	0.78	2.5	332.3	1.21
9	**0.91**	9.0	1.00	46.6	4.00	1.02	1.6	1231.6	4.47

Heavy metal analysis :
Results of the present investigation have clearly shown that the sample has no traces of heavy metal such as Arsenic and Cadmium, whereas the sample shows the presence of Lead and Mercury at 2.88 and 0.29 ppm.

Table.no: 11 - Heavy metal analysis report

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	2.88 ppm	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.29 ppm	1 ppm

BDL- Below Detection Limit ppm - Parts per million

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

Fig.no: 08 - Results of sterlity test by pour plate method for MMC

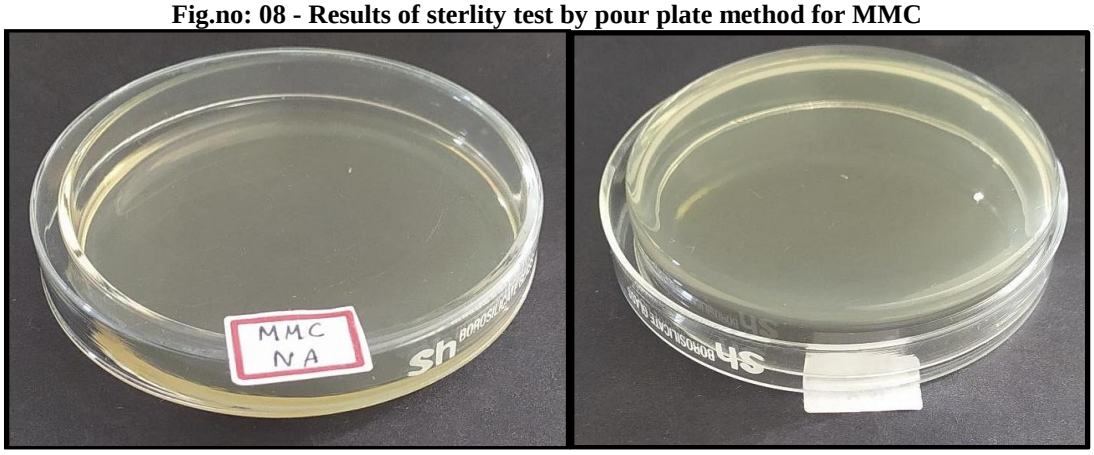


Table.no: 12 - Results of sterility test by pour plate method for MMC

Test	Result	Specification
Total bacterial count	Absent	NMT 10^5 CFU/g
Total Fungal Count	Absent	NMT 10^3 CFU/g

Test for specific pathogen :
The results of tests for specific pathogens showed the absence of *E.coli, Salmonella spp, Staphylococcus aureus, Pseudomonas aeruginosa* in MMC.

Table.no: 13 - Results of test for specific pathogen in MMC

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

Fig.no: 09 -Culture plate with E-coli (EC) specific medium

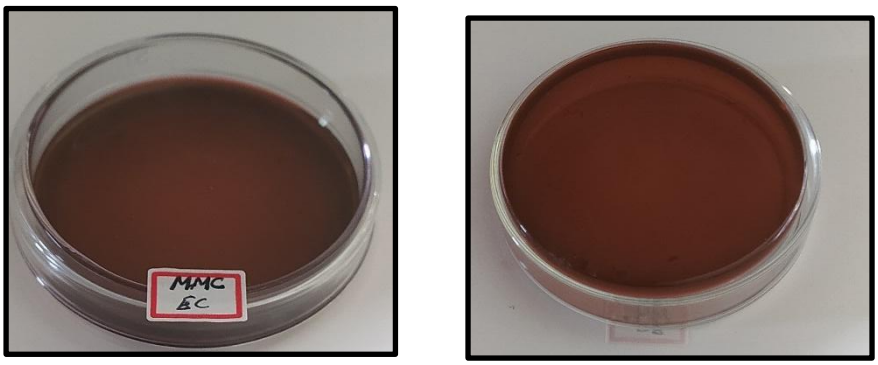


Fig.no: 10 -Culture plate with Salmonella (SA) specific medium

Fig.no: 11 - Culture plate with Staphylococcus Aureus (ST) specific medium

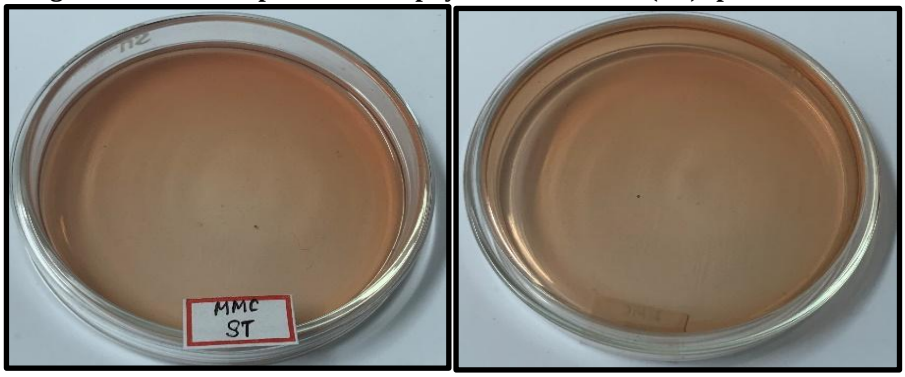
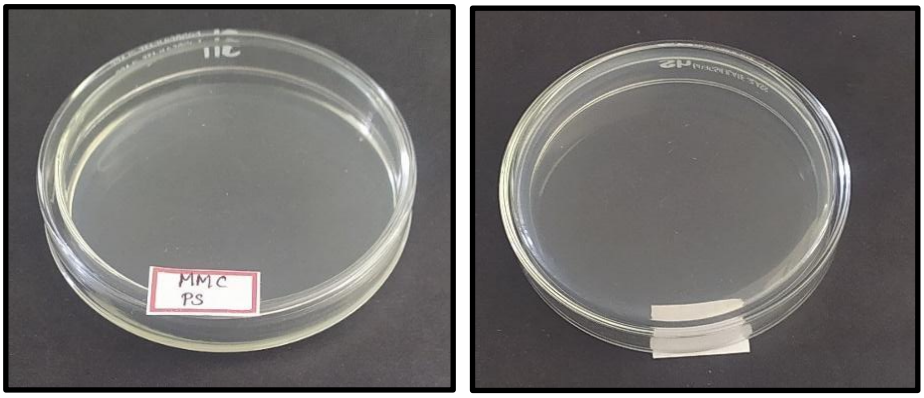


Fig.no: 12 - Culture plate with Pseudomonas Aeruginosa (PS) specific medium



Pesticide residue :

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids .

Table.no: 14 - Test Result Analysis of MMC

Pesticide Residue	Sample MMC	AYUSH Limit (mg/kg)
I.Organochlorine Pesticides		
Alpha BHC	BQL	0.1 mg/kg
Beta BHC	BQL	0.1 mg/kg
Gamma BHC	BQL	0.1 mg/kg
Delta BHC	BQL	0.1 mg/kg
DDT	BQL	1 mg/kg
Endosulfan	BQL	3 mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1 mg/kg
Chlorpyrifos	BQL	0.2 mg/kg
Dichlorvos	BQL	1 mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1 mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1 mg/kg

BQL- Below Quantification Limit

Aflatoxins :

The results showed that there were no spots being identified in the test sample loaded on TLC plates when compared to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

Table.no: 15 - Results of Aflatoxin test

Aflatoxin	Sample MMC	AYUSH specification limit
B_1	Not Detected - Absent	0.5 ppm (0.5 mg/kg)
B_2	Not Detected - Absent	0.1 ppm (0.1 mg/kg)
G_1	Not Detected - Absent	0.5 ppm (0.5 mg/kg)
G_2	Not Detected - Absent	0.1 ppm (0.1 mg/kg)

DISCUSSION

The Preclinical study focuses on the safety and efficacy of a drug that includes invitro and in vivo studies. One of the foremost steps, standardizing the drug MMC that ensures the quality of the drug. The drug MMC was soft and brown in color, smells as strongly aromatic, tastes as pungent and slightly salty. The loss on drying of MMC was 10.49% that denotes the moisture content of a drug, which should not be more than 0.01 gm. MMC had the total ash value - 5.93%, Acid insoluble ash value - 0.77%, water soluble ash value of 3.62% that assures that the drug contains less amount of siliceous compounds. The water soluble extractive value - 43.446% and the alcohol soluble extractive - 34.73% were useful to determine the phytoconstituents of the drug MMC.

Phytochemical tests result that MMC contains Alkaloids, carbohydrates, Saponin, Phenols, Flavonoids, Quinones, Gum & Mucilage. Tests for acid radicals show that carbonate and sulfate are present. Test for basic radicals shows that Lead and Mercury are present. HPTLC fingerprinting analysis of MMC reveals the presence of eight prominent peaks corresponds to the presence of eight versatile phyto components present within it.

Heavy metal toxicity of herbal products depends upon their bioavailability, cation exchange of soil in which the plant is grown. The bioaccumulation of these heavy metals causes oxidative stress to a broad spectrum of toxicity effects in plants. The drug MMC contains Arsenic and Cadmium in below detection limit, whereas Lead and Mercury at 2.88 and 0.29 ppm respectively, within their mentioned limit. Sterility test showed that no growth / colonies of any microbial growth was observed, which ensured the microbial sterility of MMC. Tests for specific pathogens showing the absence of microbial growth in their medium explains that the drug was not infected by *E.coli, Salmonella spp, Staphylococcus aureus, Pseudomonas aeruginosa.*

Tests for pesticide residue of a drug showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids - the most important parameter in plant based herbal drugs, which in turn contaminated by various agricultural practices.

Aflatoxins are the lethal mycotoxins present in agricultural crops whereas the herbomineral drug MMC was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 shows the safety assurance of the drug.

CONCLUSION

Standardizing a drug is the foremost important step in the drug developing process. It has been followed according to analytical specifications for *chooranam*(Fine powder), mentioned in PLIM guidelines. From the above test results, the drug MMC is highly safe to recommend for oral administration. Whereas the preclinical in-vitro studies have been conducted on the drug *Manimandhirathi chooranam*, furthermore studies that are In-vivo and clinical studies want to be done to prove its therapeutic efficacy.

Declaration by Authors :

Ethical approval

Approved

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None

Conflict of Interest

No conflict of interest

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