

**STANDARDIZATION OF SIDDHA HERBAL DRUG – “MAHA MANJISHTATHI KASHAYAM****Dr. Nandhini E.<sup>1</sup>, Mohamed Musthafa M.<sup>2</sup> and Nivetha G.<sup>3</sup>**<sup>1</sup>PG Scholar, PG Dept of Sirappu Maruthuvam, Govt Siddha Medical College, Chennai, Tamilnadu, India.<sup>2</sup>Professor and Head of the Department of Sirappu Maruthuvam, Govt Siddha Medical College, Chennai, Tamilnadu, India.<sup>3</sup>PG Scholar, PG Dept of Varma Maruthuvam, Govt Siddha Medical College, Chennai, Tamilnadu, India.**\*Corresponding Author: Dr. Nandhini E.**

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Article Received on 25/01/2019

Article Revised on 15/02/2019

Article Accepted on 05/03/2019

**ABSTRACT**

Standardization of Siddha formulaion drugs is very essential to order to justify their acceptability in the modern system of medicine. A siddha herbal drug “Maha manjishtathi kashayam” from the siddha text have vital importance in standardization which will encompass the entire field of study from the cultivation of medicinal plants to its clinical application. Herein standardization parameters like Organoleptic characters, Physicochemical analysis, Heavy metal analysis, TLC and HPTLC analysis, Phytochemical analysis and Sterility test are carried out as per Ayush guidelines. The outcome of this study clearly proves the quality, purity, safety and potency of the drug which will help the medicine to survive and succeed in future researches on both clinically and economically.

**KEYWORDS:** Maha manjishtathi kashayam, Organoleptic characters, HPTLC.**INTRODUCTION**

Siddha system is the most ancient and divine medical system meant for its uniqueness. According to the tradition it was Shiva who unfolded the knowledge of Siddha system of medicine to his concert Parvati who handed it down to Nandhi devar and he in turn to the 18 siddhars. Therefore, it is called ‘Shiva Sampradayam, (tradition of Siva), or ‘Siddha Sampradayam. The medicines were prepared by the Siddhars on herbs, metals and minerals. The father of Siddha Medicine is the primordial Guru, Agasthiar. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. Treatment with medicinal plants is considered very safe as there is no or minimal side effects, they are comparatively safe, eco-friendly and locally available. These remedies are in sync with nature, which is the biggest advantage. Although synthetic drugs exhibit quicker efficacy, there are at present, unsubstantiated opinion of higher incidents of adverse reactions following the use modern drugs when compared As a result of increasing demand for herbal medicines, the need for standardization of herbal products is very essential for the global acceptance. Standardization confirms the identity of the product; determine its quality and purity and detection of the nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observations.

I have chosen the herbal drug “Maha manjishtathi kashayam” from the Siddha text of “Agasthiyar vaidhiya pillar tamil.<sup>[5]</sup> for treating thethru kuttam (Psoriasis),<sup>[3]</sup> a skin disease. Hence my aim of this study is to evaluate the Qualitative and Quantitative analysis of “Maha manjishtathi kashayam” which may also help the medicine for the widespread acceptance of globally, scientifically and economically. Organoleptic characters, Physicochemical analysis, Heavy metal analysis, TLC and HPTLC analysis, Phytochemical analysis and Sterility test were carried out as the standardization parameters (as per AYUSH guidelines)

**MATERIALS AND METHODS**

Selection of Drugs for “Maha manjishtathi kashayam” consists of Manjitti (*Rubia cordifolia*)<sup>[1]</sup>, Kadukai thol (*Terminalia chebula*), Thandrikai (*Terminalia bellarica*), Nellimulli (*Phyllanthus emblica*), Kadugurohini (*Picrorhiza scrophuleriflora*), Vasambu (*Acorus calamus*), Maramanj (*Coscinium fenestratum*), Veppam marapattai (*Azadirachta indica*), Seenthil kodi (*Tinospora cordifolia*) were purchased from the raw drug shop R.N.RAJAN & CO, Paris. After getting proper authentication from the Head of the Department of Medicinal Botany and Pharmacology (Gunapadam), GSMC, Chennai-106 the medicines were prepared.

**Method of Purification**

Herbal Drugs Purified and dried under the classical text.<sup>[2]</sup>

**Kadukaai:** Seed of Kadukaai is removed and the outer skin is used.

**Neelimulli:** The seeds are removed, and the outer part is used.

**Thandrikaai:** The seeds are removed, and the outer part is used.

**Manjitti:** The dust particles are removed and then used.

**Kadugurohini:** Fried in a low flame and then used

**Vasambu:** The rhizome is burnt in flame; it is buried under the sand and allow it to cool. It is powdered and then used.<sup>[4]</sup>

**Maramanjil:** The dust particles are removed and then used.

**Veppam mara pattai:** External skin is removed and then used.

**Seendhil kodi:** The dust particles are removed and then used.

**Method of preparation<sup>[5]</sup>**

All ingredients are dried and coarsely powdered. Powder is added in boiling water and heated. Then it is reduced as kashayam. After cooling, pour it into a sterilized container or bottle.

**Dosage:** 30 ml, twice a day for 48 days.

**Indication:** All kinds of "KUTTAM".

**Organoleptic Characters****State**

Water Extraction

**Appearance**

Dark Brownish Reddish Brown in color.

**Nature**

Woody bark Consistency Clear Liquid

**Taste**

Astringent

**Odor**

Mild Aromatic

**Physicochemical Evaluation****Percentage Loss on Drying**

10gm of test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed. Percentage loss in drying = Loss of weight of sample/Wt of the sample X 100

**Determination of Total Ash**

3 g of test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 °C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference

to the weight of air-dried drug. Total Ash = Weight of Ash/Wt of the Crude drug taken X 100.

**Determination of Acid Insoluble Ash**

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Acid insoluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

**Determination of Water Soluble Ash**

The ash obtained by total ash test will be boiled with 25 ml of water for 5 mins. The insoluble matter is collected in crucible and will be washed with hot water and ignite for 15mins at a temperature not exceeding 450°C. Weight of the insoluble matter will be subtracted from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air-dried drug.

Water Soluble Ash = Weight of Ash/Wt of the Crude drug taken X 100.

**Determination of Alcohol Soluble Extractive**

About 5 g of test sample will be macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing 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 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Alcohol sol extract = Weight of Extract/ Wt of the Sample taken X 100

**Determination of Water Soluble Extractive**

About 5 g of the test sample will be macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand and 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 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Water soluble extract = Weight of Extract/ Wt of the Sample taken X 100

**Determination of pH**

About 5 g of test sample will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the subjected to pH evaluation

**Heavy Metal Analysis By aas**

Standard: Hg, As, Pb and Cd – Sigma

### Methodology

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample KN was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test sample.

### Sample Digestion

Test sample digested with 1mol/L HCl for determination of arsenic and mercury. Similarly for the determination of lead and cadmium the sample were digested with 1mol/L of HNO<sub>3</sub>.

### Standard preparation

As & Hg- 100 ppm sample in 1mol/L HCl. Cd & Pb- 100 ppm sample in 1mol/L HNO<sub>3</sub>.

### TLC and HPTLC Analysis

TLC Analysis Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10- micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system Toluene: Ethyl Acetate: Acetic Acid (1.5:1:0.5) After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long wave UV light 365 nm

### High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. In addition it is a reliable method for the quantization of nano grams level of samples. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of photochemical which is suitable for confirming the identity and purity of medicinal plant raw materials.

### 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 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 extract and Rf values were tabulated.

### Phytochemical Analysis

#### Extraction

Sample Extraction were carried out with water and the resulting extract was utilized for the photochemical analysis.

#### Test for alkaloids

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

#### Test for coumarins

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

#### Test for saponins

To the test sample, 5 ml of water was added, and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

#### Test for tannins

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

#### Test for glycosides

Borntrager's Test Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

#### Test for flavonoids

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

#### Test for phenols

**Lead acetate test:** To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

#### Test for steroids

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

**Triterpenoids-Liebermann–Burchard test**

To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

**Test for Cyanins: Aanthocyanin**

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

**Test for Carbohydrates - Benedict's test**

To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.

**Proteins (Biuret Test)**

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

**RESULTS AND DISCUSSIONS****Organoleptic Characters****Table 1: Organoleptic character of MMK.**

S. No.	Characters	Results	RESULTS
1	State	Solid- Mixed Crude raw Material- Mostly Woody/ Hard solid	Decoction- Water Extraction-
2	Appearance	Dark Brownish	Reddish Brown
3	Nature	Woody bark Consistency	Clear Liquid
4	Odor	Mild	Mild Aromatic
5	Taste	Astringent	Astringent

**Physicochemical Evaluation****Table 2: Physicochemical evaluation of MMK**

S. No.	Parameter	Mean (n=3) SD
1.	<i>Loss on Drying at 105 °C (%)</i>	9.76 ± 0.85
2.	<i>Total Ash (%)</i>	8.51 ± 1.35
3.	<i>Acid insoluble Ash (%)</i>	15.27 ± 0.83
4.	<i>Water Soluble Ash (%)</i>	13.07 ± 0.70
5.	<i>Alcohol Soluble Extractive (%)</i>	34 ± 2.88
6.	<i>Water soluble Extractive (%)</i>	23.37 ± 0.94
7.	<i>PH</i>	4.4

**Heavy Metal Analysis****Table 3: Heavy Metal Analysis of MMK.**

S. No.	Heavy Metal	Traces
1	Mercury	No detectable
2	Lead	No detectable
3	Arsenic	No detectable
4	Cadmium	No detectable
5	Chromium	No detectable

**Sterility test by pour plate method for MMK****Objective**

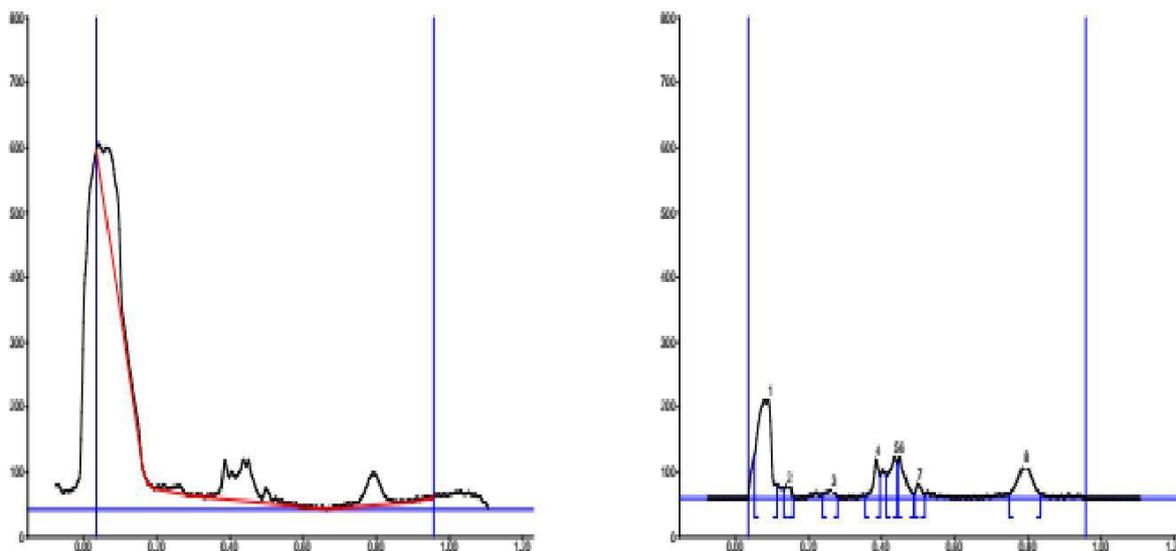
The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

**Methodology**

About 1ml of the test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45oC were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37o C for 24-48 hours. Grown colonies of organism was then counted and calculated for CFU.

**TLC and HPTLC Analysis****Result And Inference**

Results of the present investigation (Table.3) has clearly shows that the sample SC has no traces of Mercury and further shows the presence of Arsenic at 0.268 ppm level and hence it was considered that the heavy metals mercury was absent in the sample MMK.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	66.0	0.09	151.9	34.03	0.12	20.4	4244.5	46.24
2	0.13	14.8	0.14	20.2	4.52	0.16	1.1	258.3	2.81
3	0.24	5.6	0.26	12.7	2.84	0.28	2.2	243.5	2.65
4	0.36	3.8	0.39	60.6	13.59	0.40	35.2	709.9	7.73
5	0.41	36.4	0.44	63.9	14.32	0.44	54.7	1031.2	11.23
6	0.45	55.1	0.45	64.7	14.49	0.49	7.1	889.7	9.69
7	0.49	7.5	0.50	23.3	5.21	0.52	5.5	276.9	3.02
8	0.75	5.9	0.79	49.1	10.99	0.84	6.9	1525.3	16.62

TLC Analysis at 254 nm



TLC Analysis at 366nm



**Report and Inference**

HPTLC finger printing analysis of the sample MMK (Table.4) reveals the presence of three prominent peaks corresponds to presence of three versatile phytocomponents present within it. Rf value of the peaks ranges from 0.9 to 0.79. Further the peak 1 occupies the

major percentage of area of 46.24 % which denotes the abundant existence of such compound. Followed by this peak 5 occupies the percentage area of 11.23 %.

**Phytochemical Analysis****Table 5: Phytochemical analysis of MMK.**

S. no.	Test	Observation
1	Alkaloids	-
2	Flavanoids	-
3	Glycosides	+
4	Steroids	-
5	Triterpenoids	+
6	Coumarin	-
7	Phenol	+
8	Tanin	+
9	Protein	-
10	Saponins	+
11	Sugar	+
12	Anthocyanin	-
13	Betacyanin	-

+ indicates Presence and - indicates Absence of the Phytocomponents

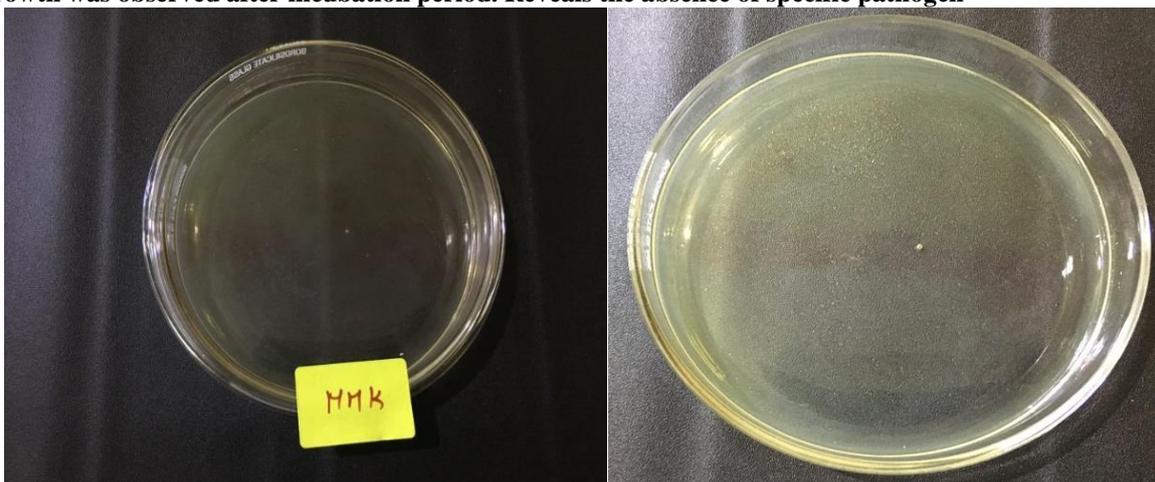
**Test for Alkaloids****Test for Flavanoids****Test for Glycosides****Test for Steroids****Test for Triterpenoids****Test for Coumarins**

**Test for Phenols****Test for Proteins****Test for Carbohydrates****Test for Anthocyanins/ Beta cyanins****Report and Inference**

Table. 5 shows the Maha manjishtathi kashayam indicates the presence of rich Glycosides, Phenol, Tannin, Triterpenoids, phenol, saponins and Sugar.

**Sterility Test by Pour Plate Method****Observation**

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



**RESULT**

No growth / colonies were observed in any of the plates inoculates with the test sample.

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 105CFU/g	Asper AYUSH specification
Total Fungal Count	Absent	NMT 103CFU/g	As per AYUSH specification

**CONCLUSION**

Heavy Metal Analytical study clearly shows that the metal mercury was absent in the sample Maha manjishtathi kashayam. Thus the drug “Maha manjishtathi kashayam” is very safe in recommending for the clinical trial. Phytochemical study indicates the presence of rich Glycosides, Phenol, Tannin, Triterpenoids, phenol, saponins and Sugar in “Maha manjishtathi kashayam” which may help to reduce the redness, scaling and patches in Psoriasis. Sterility test indicates that there no growth/colonies were observed in the plates inoculates with the test sample of MMK. It shows the purity of the drug “Maha manjishtathi kashayam”. This experimental study clearly demonstrates the Qualitative and Quantitative analysis of “Maha manjishtathi kashayam” which will help to conduct further clinical studies and standard researches.

**ACKNOWLEDGEMENT**

My Sincere thanks to Noble Research Solutions, Chennai for their guidance and support in doing Organaoleptic characters, Physico-chemical analysis, Heavy metal analysis, TLC & HPTLC analysis and Sterility method for my clinical trial medicine.

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