



## PHYSICO – CHEMICAL STANDARDIZATION OF MAHAVALLATHY LEGIYAM – A PRELIMINARY STUDY

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### ABSTRACT:

Mahavallathy (MV) legiyam, a *Siddha* herbal formulation has the indication for 21 types of *megam* (Sexually transmitted disorders), 18 types of *kuttam* (skin disorders), *Puttru* (cancer), *Powthiram* (fistula), *Sori* (itchy skin lesion) and *Sirangu* (scabies). It is widely used for cancer by many *Siddha* practitioners. It is prepared from 30 medicinal plants and major ingredient of legiyam is *Serangottai* (*Semecarpus anacardium*) which possess the anti-cancer activity. Standardization is required to achieve batch-to-batch consistency as well as large-scale drug manufacturing. This study aims to develop preliminary physicochemical profile of Mahavallathy legiyam. The test drug was subjected to Physico-chemical analysis, Phytochemical Analysis, Biochemical analysis, Instrumental analysis such as heavy metal analysis, HPTLC Analysis, Aflatoxin, Pesticide residue, Specific pathogen and Microbial contamination. This study showed the presence of mercury, arsenic, iron, calcium, aluminium, sulphate and carbonate through bio chemical analysis. MV legiyam contains 0.7ppm mercury in inductively coupled plasma optical emission spectrometry (ICP-OES). This study revealed the data regarding the physicochemical characterisation of MV legiyam, which will be helpful in standardising the drugs and further comparison studies.

**Keywords:** anti-cancer drug, Mahavallathy legiyam, serangottai, standardisation, Siddha.

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**DOI:** - 10.31838/ecb/2023.13.si5.087

**INTRODUCTION:**

*Siddha* is one of the significant ancient Indian medical systems primarily practised in Tamil Nadu.<sup>1</sup> *Siddhars* are the poly-pharmacists incorporating the plants, animals, metals and mineral origin as the major ingredient of the drug<sup>2</sup>. Due to the growing popularity in India's ancient medical system, *Siddha* formulations are now in greater demand on a global scale. One of the major issues that *Siddha* practitioners encounter is the lack of formal quality management guidelines for herbal medicines and their formulations<sup>3</sup>. Hence, standardisation of *Siddha* drug is needed one for commercialisation of *Siddha* formulations<sup>4</sup>. This ensures the formulation's safety and quality, and it's used to explain all the steps required during the manufacturing process and quality control that results youin a reproducible quality<sup>5</sup>.

In *Siddha* system of medicine, there are 32 internal and 32 external medicines. *Legiyam* is one of the probable formulations that retain its effectiveness for six months mentioned in *Siddha* literature<sup>6</sup>. *Mahavallathy (MV) legiyam*, a semisolid *Siddha* herbal formulation has the indication for 21 types of *megam* (Sexually transmitted disorders), 18 types of *kuttam* (skin disorders), *Puttru* (cancer), *Powthiram* (fistula), *Sori* (itchy skin lesion) and *Sirangu* (scabies)<sup>7</sup>. It is prepared from 30 medicinal plants and major ingredient of *legiyam* is *Serankottai (Semecarpus anacardium)* which possess the anti-cancer activity<sup>8</sup>. Since cancer is a life-threatening disease, this medicine is widely used for treating cancer by many *Siddha* practitioners. As this medicine is a Sastric preparation, it is familiar in treating many diseases, but has not yet undergone any physico-chemical standardisation. Hence, in the present study, test drug was procured and its physico – chemical analysis, Phyto chemical Analysis, Biochemical analysis, Instrumental analysis such as heavy metal analysis, HPTLC Analysis, Aflatoxin, Pesticide residue, Specific pathogen and Microbial contamination were carried out.

**MATERIALS AND METHODS:****Preparation of Mahavallathy Legiyam:**

The ingredients and quantity of the test drug mentioned in Table 1. The raw drugs have been purified before the preparation of medicine. The purified raw drugs were made into powdered and sieved. Boil, purified marking nut in ghee till it floats, then filter and take the ghee. Prepare syrup using milk and sugar, then add the powder and mix well. Add the treated ghee. Then add honey to it. Finally add powders of saffron and korosanai.

Mix it thoroughly until it gets semisolid (*legiyam*) consistency<sup>7</sup>.

**Dose:** 3 grams, twice a day for 40 days.

**Procurement of test drug:**

The test drug MV *legiyam* was purchased from GMP certified pharmaceutical company and the analytical studies were carried out.

**Physico – chemical analysis:**

The "Pharmacopeial Laboratory for Indian Medicine" (PLIM guidelines) standard procedures were followed for all physico-chemical evaluations of MV *legiyam*.<sup>9</sup> The study was carried out at Noble Research Solutions, Perambur, Chennai, 600011.

**Phytochemical analysis:**

The presence of phytochemicals in MV *Legiyam* such as alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins, phenol, quinones, protein, amino acids, gum, mucilage, fixed oil, and fat were carried out by using the standard methods<sup>10</sup>.

**High Performance Thin Layer Chromatography Analysis:**

A recent, sophisticated, and automated separation technology derived from TLC is the HPTLC method. To achieve precise, sensitive, significant separation, pre-coated HPTLC graded plates and auto sampler were used both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a potential method for evaluating the quality of botanical materials effectively and affordably.

**Chromatogram Development:**

MV *Legiyam* was subjected to HPTLC analysis. It was done in chambers called CAMAG Twin Troughs. According to the component's adsorption capacity, sample elution was performed. The plates were removed out from the chamber after elution and dried.

**Chromatographic Scanning:**

The developed Plates were screened under UV at 366nm. Through CAMAG software, the data collected from scanning were integrated. The identification of phytocomponents contained in each specimen was performed using a chromatographic fingerprint, and the corresponding Rf values were tabulated.

**RESULTS & DISCUSSION:****Siddha specification of Mahavallathy Legiyam:**

According to literature, the legiyam must be in semi-solid preparation nature with dark brown in colour. MV Legiyam has characteristic odour and greasy in nature. Organoleptic characters of MV Legiyam were evaluated and showed in Table 2.

**Physico-Chemical Properties of Mahavallathy Legiyam:**

The loss on drying at 105°C is 23.7%, indicating a high moisture content that includes volatile compounds<sup>11</sup>. The pH of MV Legiyam was 4.87. The drug's acidic nature is represented by its pH value. The key factor in a drug's absorption is its pH. The stomach's weak acid is absorbed more quickly than other acids<sup>12</sup>. The fact that the medicine contains only 0.2% of total ash indicates that 99.8% of the substance is organic. Ash value is a strong indicator of medication contamination, adulteration, substitution, or negligence in preparation<sup>13</sup>. It has 0.4% acid-insoluble ash. The extractive value of MV Legiyam in alcohol is 18.71%, according to Table 3, whereas its solubility in water was 8.56%. Due to presence of ghee in Legiyam, alcohol extractive value is more compared to water soluble extractive<sup>14</sup>.

**Phytochemical analysis of Mahavallathy Legiyam:**

Alkaloids, carbohydrates, proteins, glycosides, amino acids, quinones, fixed oil, lipids, and saponins are only a few of the phytochemical components listed in Table 4. The results of the phytochemical testing showed that the medicine is abundant in all types of phytochemicals suggesting a wide range of potential therapeutic applications.

**High Performance Thin Layer Chromatography Analysis:**

The sample's HPTLC finger printing analysis revealed the presence of five distinct peaks, each of which corresponds to one of the sample's five diverse phyto components. The R<sub>f</sub> value of the peaks varies from 0.06 to 0.79. Moreover, peak 3 takes up the majority of the area of 53.68, indicating the compound's widespread presence. For routine quality control analysis, this method can be conveniently adopted. It offers chromatographic fingerprints of phytochemicals that are useful for establishing the authenticity and purity of phytotherapeutics<sup>15</sup>. HPTLC fingerprinting can be employed for the purpose of creating quality standards for polyherbal compositions<sup>16,17</sup>.

**Heavy metal analysis of Mahavallathy legiyam:**

This instrumental analysis revealed that the sample had no residues of heavy metals like Arsenic, Lead and Cadmium. Mercury, a heavy metal, is present in the sample at 0.7 ppm, which may be within the advised limit. (Table 6) This study clearly showed Mahavallathy legiyam is safe for human consumption<sup>18</sup>.

**Aflatoxin of Mahavallathy legiyam:**

The fungi *Aspergillus flavus* and *Aspergillus parasiticus* produce the harmful aflatoxins as by products of their microbial growth. The table 7 showed that Aflatoxin B1, B2, G1, and G2 were not present in the sample of the MV legiyam.

**Pesticide residue of Mahavallathy legiyam:**

Due to agricultural spraying techniques and the application of fumigants during storage, pesticide residues are easily impacted on herbal constituents. This method revealed that the given sample were free from pesticides residues like Organochlorine, Organo phosphorus, Organo carbamates and pyrethroids mentioned in Table 8<sup>19,20</sup>.

**Test for specific pathogen of MV Legiyam:**

Typically, medicinal plants have larger concentrations of soil-derived bacteria and mould<sup>21</sup>. This study confirms that no of the plates with Mahavallathy legiyam inoculation had any growth or colonies showed in Table 9.

**CONCLUSION:**

Standardization of *Siddha* herbal formulations and development of effective quality protocols employing contemporary analytical methods are extremely important. In order to standardise or conduct routine quality checks on *Siddha* formulations, the heavy metal analysis, HPTLC fingerprint profile, and physical properties that were generated will be employed as a consistent analytical instrument. A modest effort has been made using this research to establish Standard Manufacturing Procedures (SMP) for Mahavallathy legiyam.

**ACKNOWLEDGEMENT:**

Authors are very thankful to Tamilnadu Dr. MGR Medical University, Chennai, National Institute of Siddha, Chennai and Noble research solutions, Chennai.

**CONFLICT OF INTEREST:**

The authors declare that there is no conflict of interest.

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**Table 1: Ingredients of Mahavallathy Legiyam**

S.No	Tamil Name	Botanical Name	Quantity
1.	Serangottai	<i>Semecarpus anacardium</i>	700g
2.	Parangipattai	<i>Smilax china</i>	350g
3.	Nellivatral	<i>Phyllanthus emblica</i>	350g
4.	Thandrikai	<i>Terminalia bellarica</i>	350g
5.	Chukku	<i>Zingiber officinale</i>	260g
6.	Sitrarathai	<i>Alpinia galanga</i>	260g
7.	Sadamaanjil	<i>Nardostachys grandiflora</i>	260g
8.	Lavangapattai	<i>Cinnamomum verum</i>	260g
9.	Kadukuroghini	<i>Picrorhiza scrophulariiflora</i>	70g
10.	Vetpalai	<i>Wrightia tinctoria</i>	70g
11.	Sivanarvembu	<i>Indigofera aspalathoides</i>	70g
12.	Nilapanai	<i>Curculigo orchoides</i>	70g
13.	Jathikkai	<i>Myristica fragrans</i>	35g
14.	Lavangam	<i>Syzygium aromaticum</i>	35g
15.	Lavangapathiri	<i>Cinnamomum tamala</i>	35g
16.	Milagu	<i>Piper nigrum</i>	35g
17.	Elam	<i>Elettaria cardomomum</i>	35g
18.	Omam	<i>Carum copticum</i>	35g
19.	Thippili	<i>Piper longum</i>	35g
20.	Sevviyam	<i>Piper nigrum</i>	35g
21.	Thippilimoolam	<i>Piper longum</i>	35g
22.	Kaarpogarisi	<i>Psoralea corylifolia</i>	35g
23.	Kothamalli	<i>Coriandrum sativum</i>	35g
24.	Vaavidangam	<i>Embelica ribes</i>	35g
25.	Karunjeeragam	<i>Nigella sativa</i>	35g
26.	Chithiramoolam	<i>Plumbago indica</i>	35g
27.	Vaaluluvaiaarisi	<i>Celatrus paniculatus</i>	35g
28.	Amukkara	<i>Withania somnifera</i>	35g
29.	Kungumapoo	<i>Crocus sativus</i>	8g
30.	Korosanai	<i>Felbovinum purifactum</i>	8g
31.	Nei	Ghee	3kg
32.	Thaen	Honey	3kg
33.	Naattusarkarai	Palm Sugar	10.5kg
34.	Pasumpaal	Cow's milk	3kg

**Table 2: Organoleptic characters of Mahavallathy Legiyam**

Texture	Semi solid
Odour	Characteristic
Touch	Greasy
Flow Property	Non- free flowing
Appearance	Dark brownish

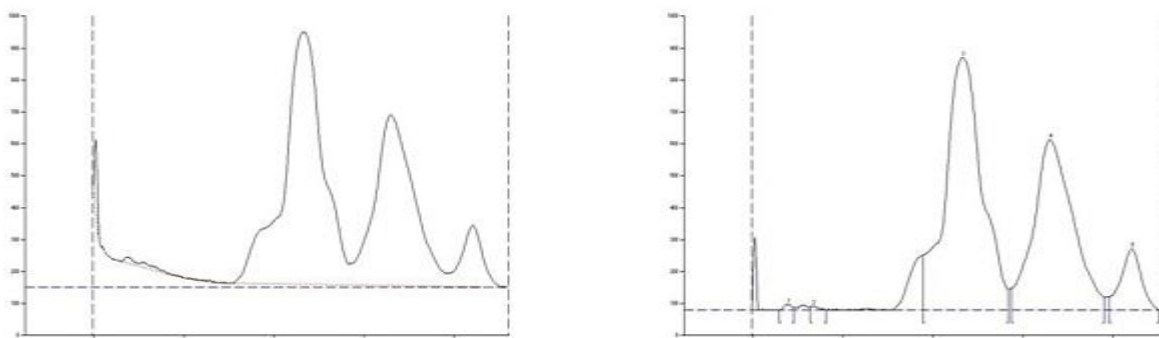
**Table 3: Physico-Chemical Properties of Mahavallathy Legiyam**

S. No	Parameter	Percentage
1.	Loss on Drying at 105 °C (%)	23.7%
2.	Total Ash (%)	0.2%
3.	Acid insoluble Ash (%)	0.4%
4.	Water Soluble Extractive (%)	8.56%
5.	Alcohol Soluble Extractive (%)	18.71%
6	pH at 25°C (1% w/v Solution)	4.87



**Table 4: Phytochemical analysis of Mahavallathy Legiyam**

S.no	Phytochemicals	RESULTS
1.	Alkaloids	Present
2.	Carbohydrate	Present
		Present
3.	Flavonoids	Absent
		Absent
4.	Glycosides	Present
		Present
5.	Saponins	Absent
6.	Tannins	Absent
7.	Phenol	Absent
8.	Protein & Amino acids	Present
9.	Quinones	Present
10.	Gum & Mucilage	Absent
11.	Fixed oil & Fat	Present

**Table 5: HPTLC fingerprint profile of MV legiyam at 366nm**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.06	0.1	0.08	18.7	1.21	0.09	8.9	249.7	0.28
2	0.13	11.0	0.13	11.9	0.77	0.16	1.1	179.8	0.20
3	0.38	172.9	0.47	790.4	51.09	0.57	65.7	48610.6	53.68
4	0.58	68.5	0.66	534.0	34.52	0.78	41.2	34915.6	38.56
5	0.79	41.4	0.84	191.9	12.41	0.90	0.9	6603.5	7.29

**Table 6: Heavy Metals of Mahavallathy legiyam**

Name of the Heavy Metal	Absorption Max	Result Analysis	Maximum Limit
Mercury	253.7 nm	0.7 ppm	1 ppm
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

\*BDL- Below Detection Limit Report and Inference

**Table 7: Aflatoxin of Mahavallathy Legiyam**

Aflatoxin	Sample MVL	AYUSH Specification Limit
B1	Not Detected – Absent	0.5 ppm
B2	Not Detected – Absent	0.1 ppm
G1	Not Detected – Absent	0.5 ppm
G2	Not Detected – Absent	0.1 ppm

**Table 8:** Pesticide Residue of Mahavallathy legiyam

Pesticide Residual	Sample MVL	AYUSH Limit (mg/kg)
Organo Chlorine 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	0.1 mg/kg
Endosulphan	BQL	0.1 mg/kg
Organo Phosphorus Pesticides		
Malathion	BQL	0.1 mg/kg
Chlorpyriphos	BQL	0.1 mg/kg
Dichlorovos	BQL	0.1 mg/kg
Organo carbamates		
Carbofuran	BQL	0.1 mg/kg
Pyrethroid		
Cypermethrin	BQL	0.1 mg/kg

\*BQL- Below Quantification Limit

**Table 9: Test for Specific Pathogen**

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