

STANDARDIZATION OF SIDDHA HERBOMINERAL FORMULATION "LINGA MATHIRAI"

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ABSTRACT

Objective: *Linga Mathirai* is traditional Siddha medicine. The medicinal plants and the herbal preparations are preferred; nowadays, due to minimal side effects and the presence of abundant antioxidants and micronutrients which possesses a better therapeutic efficiency. According to the World Health organization, the herbal medicines have been defined as those containing plant parts or plant materials in raw state or processed form containing active principles. The Siddha system of medicine encompasses around 600 medicinal plants in *materia medica*.

Methods: Physicochemical analysis such as pH, ash values, and loss on drying is done and biochemical analysis of acid and basic radicals. Instrumental analysis of Fourier transform-infrared (FT-IR) for analyze the functional groups in the test drug, scanning electron microscope (SEM) for determine the particle size of the drug, and inductively coupled plasma-optic emission spectrometry (ICP-OES) for heavy metal analysis.

Result: For standardization, organoleptic characters were done in that pH is 7.6, ash value is 1.379%, water-soluble ash is 0.294%, acid-insoluble ash is 0.871%, loss on drying in 105 is 0.483%, and disintegration time is 23 min. The functional group were analyzed by FT-IR. Then, in SEM, it has nano and microparticles. Finally, in ICP-OES, heavy metals are in permissible limits.

Conclusion: This standardization helps this drug to develop in future and also to evaluate these drug scientifically by determine the toxicity of the drug and pharmacological effect.

Keywords: Standardization, Physicochemical, Fourier transform-infrared, Scanning electron microscope, Inductively coupled plasma-optic emission spectrometry.

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INTRODUCTION

The Siddha system of medicine is a prestigious system belonging to South India. According to Siddha system, medicine is a substance that helps to alleviate or eradicate the disease, gives strength to the body, and normalizes the functions of the body [1]. This ancient system of herbal medicines is being utilized by Indians and has also gained attention worldwide due to its long-term benefits in terms of overall wellness with no side effects [2]. Although many Siddha formulations mentioned in the literature have witnessed for the treatment for various diseases, there is striving for global acceptance due to lack of scientific validation and documentation, to overcome the limitations and ensure the quality, safety, and therapeutic efficacy modern methods can be incorporated [3]. Normally, raw drugs are submitted to series of processes such as purification, trituration, incineration, and calcination to get the end product. The standardization of the drugs will assess the quality control of the drugs. Standardization of drug is essential to exhibit conformation of its identity and determination of its purity, quality, and quantity [4]. The aim of this paper is to validate standardization of *Linga Mathirai* through organoleptic, biochemical, physicochemical, microbial load, and instrumental analysis.

METHODS**Standardization of the drug**

The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care. The process of evaluating the quality and purity of herbomineral drugs by means of various parameters such as physical, chemical, and biological observation is called standardization. Standardization of this drug comes under the following categories:

- Physicochemical analysis.
- Phytochemical analysis.
- Biochemical analysis.

Organoleptic evaluation

The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its color, odor, size, etc.

Physicochemical investigation

Physicochemical studies such as total ash, water-insoluble ash, acid-insoluble ash, loss on drying at 105°C, and pH were done at Central Research Institute, Chennai.

Biochemical analysis

The biochemical analysis was done to identify the acid and basic radicals present in the *L. mathirai*. Acid radicals are potassium, calcium, magnesium, ammonium, sodium, iron, Zinc, aluminum, copper, lead, mercury, and arsenic. Basic radicals are sulfate, phosphate, chloride, carbonate, nitrate, fluoride, and oxalate.

The extract is prepared by 5 g of *L. mathirai* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Microbial load**Availability of bacterial load**

By agar plating technique

The plate count technique is one of the most routinely used procedures because of the enumeration of viable cells by this method [5].

This method is based on the principle that when material containing bacteria are cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies, therefore, is the same as the number of organisms contained in the *L. mathirai*.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 repeats)}}{\text{Amount of plated} \times \text{dilution}}$$



Fig. 1: 10⁻⁴ dilution (Bacteria)

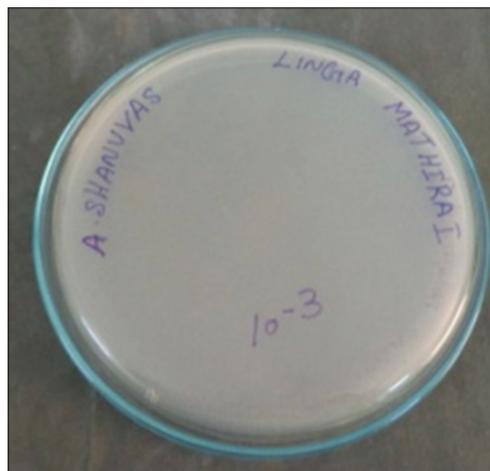


Fig. 4: 10⁻³ dilution (Fungi)

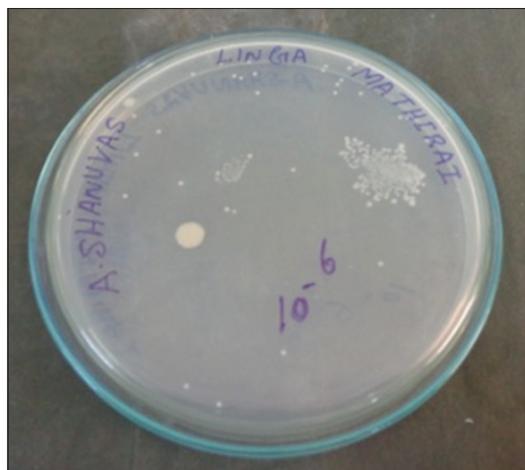


Fig. 2: 10⁻⁶ dilution (Bacteria)

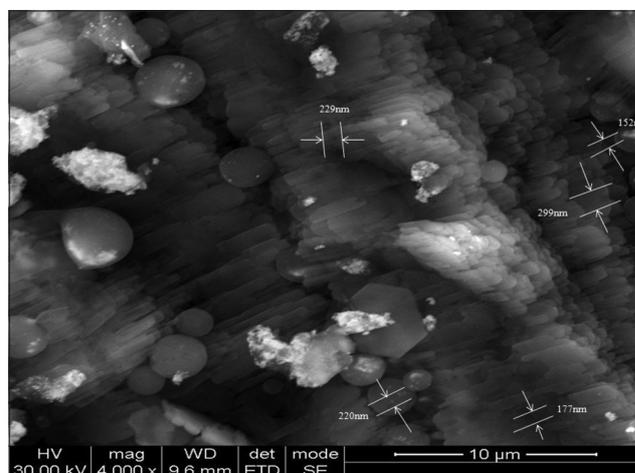


Fig. 5: Scanning electron microscope image of 10 µm



Fig. 3: 10⁻² dilution (Fungi)

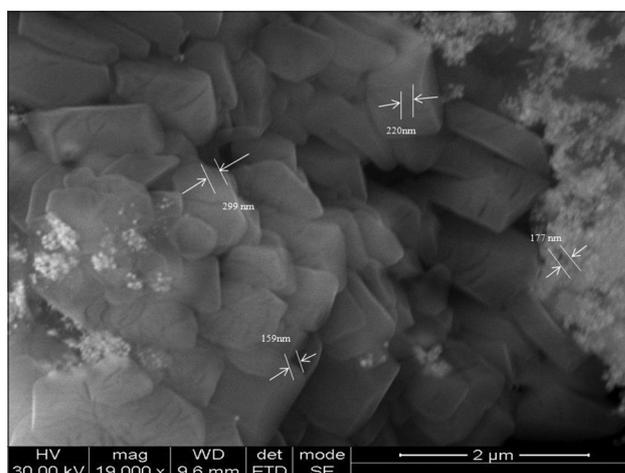


Fig 6: Scanning electron microscope image of 2 µm

sample material and can determine the amount of compounds present in the sample. It was an excellent tool for quantitative analysis [6].

In FT-IR, infrared was passed from a source through a sample (*L. mathirai*). This infrared was absorbed by the sample (*L. mathirai*) according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the *L. mathirai*. Like the finger print, there were no two unique molecular structures producing the same infrared spectrum. It was recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present [7].

Scanning electron microscope (SEM)

In scanning electron microscope, high-energy electron beam was focused through a probe toward the sample (*L. mathirai*). Variety of signals was produced on interaction with the surface of the sample (*L. mathirai*). This results in the emission of electrons or photons, and it was collected by an appropriate detector [8].

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, and chemical composition, and it displays the shape of the sample [9].

Inductively coupled plasma-optic emission spectrometry (ICP-OES)

Mechanism

In plasma emission spectroscopy (OES), a *L. mathirai* solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this

Table 1: Physical characterization of *L. mathirai*

Parameter	Results
Color	Brick red
Odor	Odorless
Consistency	Hard
Shape	Spherical
State of matter	Solid
pH	7.6
Total ash (%)	1.379
Water-soluble ash (%)	0.294
Acid-insoluble ash (%)	0.871
Loss on drying at 105°C (%)	0.483
Disintegration time (min)	23

L. mathirai: Linga Mathirai

temperature, all elements become thermally excited and emit light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values [10].

The ICP-OES analysis was done in SAIF, IIT MADRAS, Chennai-36, using Perkin Elmer Optima 5300 DV.

Sample preparation

About 100 mg *L. mathirai* was occupied in a clean, dry test tube. To this, 3 ml nitric acid was added and mixed well and allowed for few minutes until the reactions were completed. And then, 25 ml of refined water was added to prepare digested solution. The digested *L. mathirai* solution was shifted into plastic containers and labeled properly. It was completed in Biochemistry Laboratory, Government Siddha Medical College, Chennai-106.

RESULT AND DISCUSSION

Organoleptic character

Organoleptic character like pH, water soluble ash, acid insoluble ash, loss on drying and disintegration time were tabulated [see Table 1]

Discussion

- pH of *L. mathirai* is 7.6. It is slightly alkaline in nature. The alkaline medium enhances the mineral storage to buffer, reduces aging process, and increases the utilization of oxygen level in body [11].
- The amount of minerals and earthy materials present in the drug is represented by total ash value. The value of *L. mathirai* is 1.379%; it determines the purity of the drug.
- Water-soluble ash represents easy facilitation of diffusion and osmosis mechanism. Here, the value of *L. mathirai* is 0.294% will denote its diffusion capacity.
- The amount of siliceous matters in the drug is represented by acid-insoluble ash value. The acid-insoluble ash value of *L. mathirai* is 0.871%, which determines the superior quality of the *L. mathirai*.
- The moisture content of the drug is determined by loss on drying. These will also indicate stability and shelf life of the drug. Here, the percentage denotes the higher stability of the *L. mathirai*.
- *L. mathirai* is formulated according to classical Siddha text; disintegration indicates the better solubility and absorbability of drug.

Table 2: Uniformity weight variation test result of *L. mathirai*

Weight of each Mathirai (mg)	% of weight variation	Maximum weight variation with in±7.5%	Maximum weight variation with in±15.0%
138	5.423	Yes	Yes
137	4.660	Yes	Yes
132	0.840	Yes	Yes
130	-0.687	Yes	Yes
139	6.187	Yes	Yes
135	3.132	Yes	Yes
126	-3.743	Yes	Yes
129	-1.451	Yes	Yes
125	-4.507	Yes	Yes
133	1.604	Yes	Yes
127	-2.979	Yes	Yes
128	-2.215	Yes	Yes
136	3.896	Yes	Yes
124	-5.271	Yes	Yes
129	-1.451	Yes	Yes
131	0.076	Yes	Yes
135	3.132	Yes	Yes
120	-8.326	No	Yes
122	-6.799	Yes	Yes
126	-3.743	Yes	Yes

Average: 130.95 g. *L. mathirai*: Linga Mathirai

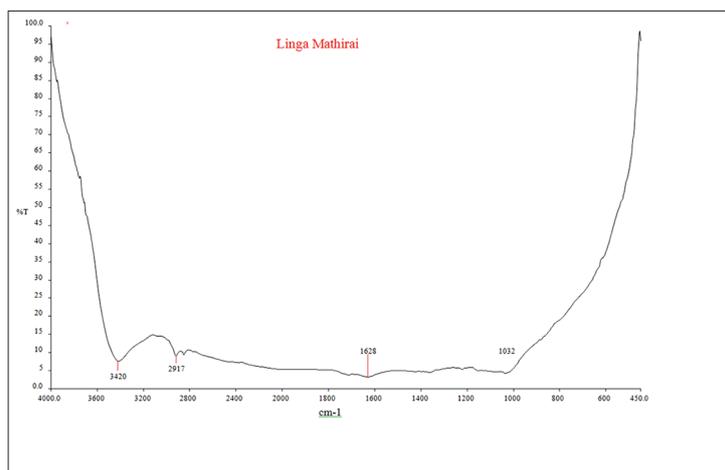


Table 3: Results for basic radicals

Parameter	Observation	Result
Test for potassium	Yellow color precipitate	+ve
Test for calcium	-	-ve
Test for magnesium	-	-ve
Test for ammonium	-	-ve
Test for sodium	-	-ve
Test for iron (ferrous)	-	-ve
Test for zinc	-	-ve
Test for aluminum	-	-ve
Test for lead	-	-ve
Test for copper	-	-ve
Test for mercury	-	-ve
Test for arsenic	-	-ve

Table 4: Results for acid radicals

Parameter	Observation	Result
Test for sulfate	Formation of white precipitate	+ve
Test for chloride	-	-ve
Test for phosphate	-	-ve
Test for carbonate	-	-ve
Test for fluoride and oxalate	-	-ve
Test for nitrate	-	-ve

Table 5: FTIR result of *L. mathirai*

Characteristic absorption (s) (cm ⁻¹)	Functional groups
3420	Alcoholic group normal "polymeric" OH stretch
2917	Amine group of N-H Bending
1628	Alkene C-H stretch, acid group of O-H stretch
1032	Alkene group of C=C stretch, amide group of N-H Bending
	Alkyl halide C-F stretch, ether C-O stretch, ester C-O stretch

FTIR: Fourier transform-infrared, *L. mathirai*: Linga Mathirai

Weight variation test

The result of weight variation has been tabulated [see Table 2]

Discussion

- Average weight of the *Mathirai* was noted as 130.95 g. Of 20 tablets tested, 19 tablets of them lie within ±7.5% weight variation (1 tablet

Table 6: ICP-OES result of *L. mathirai*

Elements	Detected levels
Sulfur (mg/L)	52.514
Potassium (mg/L)	20.821
Calcium (mg/L)	12.120
Phosphorus (mg/L)	08.541
Mercury (mg/L)	0.674
Iron (mg/L)	0.380
Sodium (mg/L)	03.110
Arsenic	BDL
Cadmium	BDL
Nickel	BDL
Lead	BDL

ICP-OES: Inductively coupled plasma-optic emission spectrometry, *L. mathirai*: Linga Mathirai

above the limit) and all 20 tablets lie within ±15% weight variation.

- According to the limits of weight test cited in the Indian pharmacopoeia, *L. mathirai* passed the uniformity weight test.
- The uniformity test resembles uniformly distribution of this tablet helps good absorption and distribution.

Traditional test for pill

Character	Inference
Non-sticky on rolling	+
No cracks over the surface after drying	+
Shall be rolled uniformly over the plane surface	+

Biochemical analysis

Basic radicals

Result of the basic radicals shows the presence of potassium and absence of other basic radicals which has been tabulated [see Table 3]

Interpretation

The basic radical test shows the presence of potassium and absence of heavy metals.

Potassium

The K+ inwardly rectifier channel is one of the two subunits of the pancreatic islet ATP-sensitive potassium channel complex (IKATP). It has a key role in glucose-stimulated insulin secretion and thus is a potential candidate for a genetic defect in Type II (non-insulin-dependent) diabetes mellitus [12].

Acid radical

Interpretation

The acidic radicals test shows the presence of sulfate [see Table 4].

Microbial load

Availability of bacterial and fungal load in *L. mathirai* was estimated by agar plating method, the results were discussed below

- The contaminated toxins present in the drug will produce adverse effect, which develops unwanted diseases. They are unfit for humans [13].
- Here, the contamination of *L. mathirai* has been examined by bacterial and fungal load.
- Total bacterial load in 10^{-4} dilution is 4 and in 10^{-6} dilution is nil.
- Total fungal load in 10^{-2} dilution is nil and in 10^{-3} dilution is nil [See Figs. 1 and 2].
- The load of bacterial and fungal is within the limits of the WHO norms [See Figs. 3 and 4].

Instrumental analysis**FTIR**

The function group was analyzed by FTIR which has been tabulated [see Table 5].

Discussion

- The above table shows the presence of alcoholic group, amine group, alkane group, and alkyl halide group is the organic functional groups, also ether and ester group of carbonyl functional groups.
- OH group has higher potential toward inhibitory activity against microorganisms.

Scanning electron microscopy

For the SEM result see Figs. 5 and 6.

Discussion

SEM picture shows nano and microparticle (Ultrafine particle) size of the sample. Moreover, the picture shows various sizes of the particles such as 152 nm, 177 nm, 220 nm, 229 nm, and 299 nm.

Sizes are ranging from 179 nm to 304 nm. The surface of the sample grains is uniformly arranged. These are microparticles presenting as 152 nm, 177 nm, 220 nm, 229 nm, and 299 nm. The difference in morphology as evident from the micrograph is due to the presence of chemicals in the samples.

Microparticles - significance

- Microparticles are defined as particulate dispersion or solid particles with a size in the range of 100–1000 nm in diameter.
- Size and surface of microparticles can be easily manipulated to achieve both passive and active drug targeting.
- They control the release of drug during the transportation and at the site of localization, alter drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy, thereby bioavailability and reduced side effects [14].

ICP-OES

Heavy metals was analyzed by ICP-OES, results has been tabulated [see Table 6].

Discussion

- From the above results, the heavy metals such as arsenic, cadmium, and lead are below detectable limit.
- Mercury was in permissible limit.
- Hence, the safety of the drug *L. mathirai* is ensured.
- Furthermore, the drug contains calcium, iron, potassium, sodium, sulfur, and phosphorus.

Calcium [15]

- Calcium was associated with the lower risk of diabetes mellitus.
- Calcium is necessary in normalizing the glucose tolerance.
- Abnormal regulation of intracellular calcium affecting both insulin sensitivity and insulin release.

Iron

- The heme containing enzymes such as catalase and peroxidase protect cell against potentially damaging highly reactive species.

- Iron is essential for many numbers of biological functions such as growth, reproduction, healing, and immune function.

Sodium

- Recently, sodium glucose cotransporter 2 reabsorbs most of the glucose filtered by the kidneys. Thereby, lowering the blood glucose levels and has been approved as new antihyperglycemic drug [16].
- A synergistic effect of all these calcium, iron, potassium, sodium, phosphorus, and sulfur increases the potency of the drug against diabetes mellitus.

CONCLUSION

For standardization of *L. mathirai*, physical characterization such as pH, total ash, water-soluble ash, acid-insoluble ash, loss on drying, disintegration is done. In acid and basic radical analysis, the presence of potassium and sulfate has been identified. In microbial load of bacterial and fungal is within the limits. In FT-IR, functional groups are identified. In SEM, the drug has both nano and microparticles which has increased drug efficacy and reduced side effects. In ICP-OES, safety of this drug is ensured by analysis of heavy metals which are in permissible limits. These results that *L. mathirai* have maximal efficacy and reduced adverse effect which helps in development of drug. Hence, the standardization of the drug is the first step for further assessing toxicological and validating pharmacological activities.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

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