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 L. 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.
- Phytomedicine 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. 2: 10^{-6} dilution (Bacteria)**

**Fig. 3: 10^{-2} dilution (Fungi)**

**Fig. 4: 10^{-3} dilution (Fungi)**

**Fig. 5: Scanning electron microscope image of 10 µm**

**Fig. 6: Scanning electron microscope image of 2 µm**

**Instrumental analysis**

**Fourier transform-infrared (FT-IR)**

It was the preferred method of infrared spectroscopy. FT-IR was an important and more advanced technique. It was used to identify the functional group, to determine the quality and consistency of the
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 fingerprint, 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 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 1: Physical characterization of L. mathirai

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

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

<table>
<thead>
<tr>
<th>Weight of each Mathirai (mg)</th>
<th>% of weight variation</th>
<th>Maximum weight variation with in±7.5%</th>
<th>Maximum weight variation with in±15.0%</th>
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</thead>
<tbody>
<tr>
<td>138</td>
<td>5.423</td>
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<tr>
<td>137</td>
<td>4.660</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>132</td>
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<td>Yes</td>
<td>Yes</td>
</tr>
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<td>130</td>
<td>−0.687</td>
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</tr>
<tr>
<td>139</td>
<td>6.187</td>
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<tr>
<td>135</td>
<td>3.132</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>126</td>
<td>−3.743</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>129</td>
<td>−1.451</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>125</td>
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</tr>
<tr>
<td>126</td>
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<td>Yes</td>
</tr>
</tbody>
</table>

Average: 130.95 g. 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 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.

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. mathrari 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 10^2 dilution is 4 and in 10^4 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 (Ultracefine 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. mathiri 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. mathrari, 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. mathrari 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.

REFERENCES