STANDARDIZATION AND ANALYTICAL EVALUATION OF TRADITIONAL SIDDHA FORMULATION THIRISOOTHA MEZHUGU: A MODERN ANALYTICAL APPROACH.

A. M. Dharsana*,1, P. Shanmuga Priya2, C. Parkavi1, J. Kanimozhi1, S. Vanathi1

1Post Graduate, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatorium, Chennai 600047, Tamil Nadu, India.

2Lecturer, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatorium, Chennai 600047, Tamil Nadu, India.

ABSTRACT

Siddha system of medicine has distinguished itself from the rest of the traditional medicines by its compassionate way of understanding the human disease and also by the unique way of treating the dreadful disease. As the practice of siddha system of medicine hikes day by day and now people around the globe started realizing the importance of siddha formulations and its applications towards various diseases. Though Siddha system has its own value the concern on availability of physicochemical and standardization data of most of the siddha formulation is high doubtful. Hence standardization of siddha drugs becomes highly essential in order to explore its potency and efficacy in the global market. As a consequence of focusing upon the current need of drug standardization the present work undertaken to standardize the traditional herbomineral siddha formulation Thirisootha mezhugu (TM) which has been used for the treatment of various ailment like hemiplegia and lumbar spondylosis. Still now there is no proper documentary evidence available on standardization aspect of this formulation this prompted us to peruse the systematic standardization of TM by qualitative, phytochemical and physiochemical evaluation by AYUSH guidelines, particle size distribution by Scanning Electron Microscope (SEM) and heavy metal by inductively coupled plasma optical emission spectrometry (ICP-OES) analysis. The results obtained from physiochemical analysis clearly reveals that total ash value of TM is about 19.63% in which acid insoluble ash is 0.62% and water soluble ash is about 1.42%. Extraction value of drugs
renders most significant information about partition of the active component the results of extract value study shows that alcohol soluble extract of TM is 31.80% and water soluble extract is 47.70%. Result obtained from the phytochemical analysis reveals the present of alkaloid and starch, further result of qualitative chemical analysis shows the presence of silicate, carbonate and chlorides. The results obtained from the SEM analysis shows that most of the particles present in TM are in micro size ranges from 1µm – 2 µm. The results of the present investigation render some valuable information about the siddha formulation Thirisootha mezhugu to the future researchers and also provide evidence based information on standardization part of this noble formulation.

KEYWORDS: Standardization, Siddha system, Thirisootha mezhugu, SEM, ICP-OES, Phytochemical, Physiochemical analysis.

INTRODUCTION
Traditional Medicine has played an important role in meeting the demands of primary health care in many developing countries and its use has expanded widely in many developed countries.1 Siddha system of medicine is one among them, which is flourished in the southern India especially Tamilnadu.[1] Siddha is a traditional healing holistic medicine of India which emphasizes the maintenance of a relaxed mind and body harmony and insists to keep pace with the laws of nature. In Siddha system, besides herbs, metal and mineral drugs were also used as medicines.[2]

Siddha system is the ancient and unique among the Indian system of medicines. The traditional systems of medicine have become significantly more popular all over the globe because of the curative property, less toxic and minimal side effects. It is more widely used for the human ailments from time immemorial. It has been estimated that 70-80% of world’s population relies on traditional healthcare. The mode of preparation and plant used in traditional medicine varies from place to place. In addition acceptance of traditional medicines, especially herbal medicines in the developed world is sharply increasing.[3,4,5]

Active research in the mechanism of curative actions of siddha system of medicine is very much essential, to have widespread acceptance of the ancient practice. International bodies like world health organization (WHO), provides guidelines for prevention, control, safety, efficacy as well as evaluation and standardisation of herbomineral preparations. Standardization of siddha preparations is highly essential and it becomes mandatory with
respect to regulatory concern. Standardization not only to mention its quality but also to maintain the standard of the finished product.\[6\]

As a measure of focusing upon the need of drug standardization the present investigation work undertaken to standardize the traditional herbomineral siddha formulation *Thirisootha mezhugu* (TM) which has been used for the treatment of various ailment like Pakkavatham (hemiplegia), thandagavatham (lumbar spondylosis). Still now there is no proper documentary evidence available on standardization aspect of this formulation this prompted us to peruse the systematic standardization of TM by chemical, phytochemical and physiochemical evaluation by AYUSH guidelines, particle size distribution by SEM and heavy metal by ICP-OES analysis.

**MATERIALS AND METHODS**

As per the siddha literature the drug Thirisootha mezhugu includes the following ingredients such as veeram, pooram, lingam, ilavangam, elam, chukka, nervaalam, chithiramoola verpattai and those drugs were purified and prepared by the following process

**Collection of Raw Materials**

The drugs Pooram (Hydrargyrum subchloride), Lingam (Red sulphide of mercury) Veeram (Hydrargyrum perchloride) Ilavangam (Syzygium aromaticum) Elam (Elattaria cardamom) Chukku (Zingiber officinale) Nervalam (Croton tiglium) Chithiramoola verpattai (Plumbago indica) were collected from a reputed raw drug shop, Chennai.

**Authentication**

The drugs Veeram (Mercuric trichloride), pooram (Hydrargyrum subchloride) Lingam (Red sulphide of mercury) were identified and authenticated by Siddha Central Research Institute (Chemist), Arumbakkam, Chennai.

The drugs Ilavangam (Syzygium aromaticum) Elam (Elattaria cardamom) Chukku (Zingiber officinale) Nervalam (Croton tiglium) Chithiramoola verpattai (Plumbago indica) were identified and authenticated by Botanist, National Institute of Siddha, Chennai-47 (Certificate No: NISMB 2112015).
METHOD OF PURIFICATION

Veeram
Perchloride of mercury was soaked in cow’s milk in a porcelain vessel. It was insolated till the milk is completely dried to get the purified form.

Pooram
The poultice made of betel leaf (Piper betel) and pepper (Piper nigrum) each 8.75 gm was taken and dissolved in 1.3 litre of water, calomel 35 gm was tied with a cloth and immersed in the liquid from the cross bar (Thulayanthram process) and heated. After the water reduced to ¾ of its volume, the calomel was taken out, washed with water and dried to get it in purified form.

Lingam
Lime juice, cow’s milk and the Indian acalypha juice are mixed in equal proportion and allowed to fuse cinnabar so as to get it in a consolidated potency.

Elavangam
Removed all the impurities and dried it in the sunlight.

Ealam
Removed all the impurities and dried it in the sunlight.

Chithiramoola verpattai
After removing the outer portion of the bark, powdered, then took milk in a bowl, tied a cotton cloth around the mouth of the bowl and kept the above powder and again covered it with a bowl and boiled the milk in low flame for 3 hours, dried the powder and ground it in a kalvam once again.

Nervaalam
Soaked the nervaalam in lime juice, then washed, and dried it in the sunlight.

Chukku
Soaked the chukku in lime water.
Method of preparation
The above mentioned purified drugs are powdered, mixed together in a kalvam with honey for about 3 to 4 saamam (9 to 12 hours) in mezhugu patham.\(^7\)

**Therapeutic dose** : 1 to 1 ½ milau alavu (60 to 90mg)

**Adjuvant** : Palm jaggery

**Indication** : Pakkavatham (hemiplegia), thandagavatham (lumbar spondylosis).

**Organoleptic characters**

**Color**
About 10g of mezhugu was taken in a clean glass beaker and tested for its color by viewing again a white opaque background under direct sunlight.\(^8\)

**Odour**
About 10 gm of the *Thirisootha mezhugu* was placed in 100 ml of beaker and tested for its odour by wafting the air above the beaker. Further organoleptic characteristic features of the finished product *Thirisootha mezhugu* was done by visual observation such as touch, smell and tasting the test dug and respective observation were detailed.

**Percentage Loss on Drying**\(^9\)
10gm of *Thirisootha mezhugu* was accurately weighed in evaporating dish and was air dried at 105°C for 5 hours and then weighed.

**Determination of Total Ash**\(^10\)
3 g of *Thirisootha mezhugu* was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC 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.

**Determination of Acid Insoluble Ash**
About 0.5gm of 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.

**Determination of Water Soluble Ash**
About 0.5gm of 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 15 minutes 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.

**Determination of Alcohol Soluble Extractive**

About 5 g of the air dried *Thirisootha mezhugu* 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 tared 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.

**Determination of Water Soluble Extractive**

About 5 g of the air dried *Thirisootha mezhugu* 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 for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared 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.

**Determination of PH**

About 5 g of *Thirisootha mezhugu* 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 using PH meter.

**Preliminary phytochemical Evaluation**

*Thirisootha mezhugu* was subjected to class of preliminary phytochemical screening of the following components

**Test for flavonoid**

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Shows the presence of yellow color indicates the presence of Flavonoids.
Test for Steroids
When the sample reacted with chloroform, acetic acid and conc. H2SO4 and formed a blue and green colour. Which indicates confirmed the presence of steroids.

Test for Alkaloid
Test drug was extracted with 2ml of HCl was added. To this acidic medium 1ml of dragendroffs reagent was added on, orange or red precipitate produced immediately indicate the presence of alkaloids.

Test for Phenol
To test drug a few drops of alcohol and ferric chloride solution was added. Bluish green or red indicates the presence of phenol.

Test for tannins
Test drug was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Glycosides
Test drug were mixed with a little anthrone reagent on a watch glass. One drop of concentrated sulfuric acid was added and made into a paste, warmed gently over water bath. The presence of glycosides was identified by dark green coloration.

Test for Starch
Test sample was added with iodine solution. Appearance of bluish black color denotes the presence of starch.

Chemical Analysis

Test for Chloride
Identification test for chloride in which sample added with 1 N sodium hydroxide and appearance of white precipitate indicates the presence of chloride.

Test for carbonates
Test sample was added with dil HCl. Appearance of CO2 effervescence denotes the presence of carbonates.
Test for Silicates
About 0.5 gm of the test sample was added to 0.2 gm of sodium carbonate and 2 gm of potassium carbonate, heat the mixture and add 5 ml of water and transfer in to beaker with 50 ml water. To this add HCl gradually until effervescence ceases. Add 10 ml of HCl and 20 ml water. Evaporates an residue will obtain and that is in soluble in water and ethanol.

Scanning Electron Microscope Analysis (SEM)
The surface morphology of *Thirisootha mezhugu* was analyzed with a Zeiss Gemini Supra 55 and SEM with Oxford instrument X-act. The copper disc was pasted with carbon tape and the sample was dispersed over the tape. The disc was coated with gold in ionization chamber before microscopic analysis. The sample was mounted on specimen stub, placed inside the microscope’s vacuum column evaporator and a beam of electrons passed from an electron gun, travelled through a series of magnetic lenses. The electrons are counted by the detector and the signals are sent to the amplifier. The number of electrons dispersed from each spot of the sample builds up the resultant image. The micrographs obtained give sufficient data about the topography of the sample. Energy used for SEM analysis is 0.5 – 20 kV with magnification range of 5000 to 40,000X. [13]

Inductively Coupled Plasma optical emission Mass Spectrometry (ICP-OES) [14]
ICP-OES is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals, several non-metals and inorganic substance at concentration below one part per trillion. Samples are decomposed to neutral elements in high temperature argon plasma and analyzed based on their mass to charge ratios. It is an automated, simple and unique quantitative and qualitative analysis. It measures elemental isotopes ratio. When the test sample is exposed to the plasma from outside the atoms present with in the test sample was excited to high energy state and then each atom while returning to low energy state will release spectral rays corresponds to the photons will be measured.

Procedure
Test drug *Thirisootha mezhugu* was digested by transforming 0.5 gm of the sample into a closed beaker and 5 ml of concentrated 2% w/v of HNO3 was added and digested to near dryness. 16 M nitric acid was further added each time to the sample and digested until the clear solution was obtained. 5 ml of 12 M Hydrochloric acid was added to ensure complete digestion. The digested solution was cooled to room temperature and made to the final volume of 100 ml with deionized water. Sample solutions were then filtered through
membrane (0.45micron) filter. Finally, the digested samples were used for metal analysis using inductively coupled plasma optical emission spectrometry (Perkin Elmer optima 5300 DV Model).

RESULTS

Physico-chemical Evaluation of *Thirisootha mezhugu*

Organoleptic property of the *Thirisootha mezhugu* justifies the genuinity of the raw drug and finished formulation with respect to its dark brown colour, and sour taste by its identity. The results obtained from the physicochemical evaluation revels that the total ash value of TM was found to 2.39 %. Similarly acid insoluble and water soluble ash value was fond to be 0.62 % and 1.42 % w/w respectively.

Extractive values are representative of the presence of polar and non-polar compounds in a finished product. The water soluble extractive value can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the process of drying; storage etc. The result of the study shows that alcohol and water soluble extractive value TM was found to be of 31.80 and 47.70% w/w respectively. The loss on drying at 105°C in TM was found to be 19.63 % w/w. pH of the formulation plays a significant role in the living biological system with respect to aid in absorption and distribution through systemic circulation. pH of the TM was found to be 4.5. The results of physiochemical analysis were tabulated in Table 01.

Table: 1 Physico-chemical Evaluation of *Thirisootha mezhugu*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Physico-chemical Parameter</th>
<th>% in W/W (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Organoleptic characters</td>
<td></td>
</tr>
<tr>
<td>a.colour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b.taste</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Loss on drying at 105°C</td>
<td>19.63 %</td>
</tr>
<tr>
<td>3.</td>
<td>Ash Values</td>
<td></td>
</tr>
<tr>
<td>Total Ash</td>
<td></td>
<td>2.39 %</td>
</tr>
<tr>
<td>Acid Insoluble Ash</td>
<td></td>
<td>0.62 %</td>
</tr>
<tr>
<td>Water soluble Ash</td>
<td></td>
<td>1.42%</td>
</tr>
<tr>
<td>4.</td>
<td>Extract Values</td>
<td></td>
</tr>
<tr>
<td>Alcohol soluble</td>
<td></td>
<td>31.80 %</td>
</tr>
<tr>
<td>Water soluble</td>
<td></td>
<td>47.70%</td>
</tr>
<tr>
<td>5.</td>
<td>pH</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Preliminary phytochemical analysis of *Thirisotha mheezhugu*

Preliminary phytochemical analysis of TM reveals the presence of phytocomponents such as alkaloids and starch. The results of phytochemical analysis were tabulated in Table 02.

**Qualitative chemical Analysis of Thirisootha mheezhugu**

Qualitative chemical analysis of TM reveals the presence of chlorides, carbonates and silicates. The results of qualitative chemical analysis were tabulated in Table 02.

**Table: 2 Qualitative chemical and preliminary phytochemical analysis of Thirisootha mezhugu**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of Chemical/ Phyto Components</th>
<th>Presence or Absence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>Absence</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>Presence</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>Absence</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>Absence</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>Absence</td>
</tr>
<tr>
<td>6</td>
<td>Glycosides</td>
<td>Absence</td>
</tr>
<tr>
<td>7</td>
<td>Starch</td>
<td>Presence</td>
</tr>
<tr>
<td>8</td>
<td>Chlorides</td>
<td>Presence</td>
</tr>
<tr>
<td>9</td>
<td>Carbonates</td>
<td>Presence</td>
</tr>
<tr>
<td>10</td>
<td>Silicates</td>
<td>Presence</td>
</tr>
</tbody>
</table>

**SEM analysis of Thirisootha mezhugu**

The particles of TM observed spherical in shapes and sizes are in the range from 1 micron to 2 microns. Although the particle sizes of different batches showed similarity, it seems that these particles are aggregates of much smaller particles. When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gives these particles a tendency to aggregate together to form larger particles. This Mezhugu exhibited larger sizes and agglomeration of the particles. As shown in Figure 01.
Elemental Analysis of *Thirisootha mezhugu* formulation

The results obtained from qualitative elemental analysis of TM by ICP-OES showed the presence of Calcium (Ca), Mercury (Hg), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P) and Zinc (ZN), Whereas the following elements are found below the detective level such as Arsenic (As), Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb). The results were tabulated in Table 03.

Table 3: Elemental Analysis of *Thirisootha mezhugu* by (ICP-OES)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Elements</th>
<th>Wavelength In nm</th>
<th>Concentration in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>As</td>
<td>188.979</td>
<td>BDL</td>
</tr>
<tr>
<td>2.</td>
<td>Ca</td>
<td>315.807</td>
<td>22.012 mg/L</td>
</tr>
<tr>
<td>3.</td>
<td>Cd</td>
<td>228.802</td>
<td>BDL</td>
</tr>
<tr>
<td>4.</td>
<td>Cu</td>
<td>327.393</td>
<td>BDL</td>
</tr>
<tr>
<td>5.</td>
<td>Hg</td>
<td>253.652</td>
<td>3.715 mg/L</td>
</tr>
<tr>
<td>6.</td>
<td>K</td>
<td>766.491</td>
<td>23.421 mg/L</td>
</tr>
<tr>
<td>7.</td>
<td>Mg</td>
<td>285.213</td>
<td>01.503 mg/L</td>
</tr>
<tr>
<td>8.</td>
<td>Na</td>
<td>589.592</td>
<td>25.610 mg/L</td>
</tr>
<tr>
<td>9.</td>
<td>Ni</td>
<td>231.604</td>
<td>BDL</td>
</tr>
<tr>
<td>10.</td>
<td>Pb</td>
<td>220.353</td>
<td>BDL</td>
</tr>
<tr>
<td>11.</td>
<td>P</td>
<td>213.617</td>
<td>16.741 mg/L</td>
</tr>
<tr>
<td>12.</td>
<td>Zn</td>
<td>206.2</td>
<td>01.121 mg/L</td>
</tr>
</tbody>
</table>

(BDL- Below Detection Limit)

**DISCUSSION**

The quality assessment of herbo-mineral formulation is of paramount importance in order to justify their acceptability in modern system of medicine. World Health Organization (WHO)
encourage, recommend and promotes traditional remedies in natural health care programmes, because safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of herbo-mineral products by using modern techniques and applying suitable standards.

The inherent proposition of Siddha medical system is based on three humours which are the Vatham, Pitham and Kapham. The potency of the prepared Siddha drugs has high range of therapeutic value. There is no appropriate technique for the standardization of Indian medicine especially for the Siddha drug. From the results of the present investigation it was evident that *Thirisootha mezhugu* a siddha herbomineral preparation consist of potential phytocomponents like alkaloids a biologically functional components which is effective against various disorder’s. Further elemental analysis results shows that most of the elements like Calcium (Ca), Mercury (Hg), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P) and Zinc (ZN) present in the formulation and the following elements such as Arsenic (As), Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb) are absent in the formulation. The results obtained from physiochemical analysis clearly reveals that total ash value of TM is about 19.63% in which acid soluble ash is 0.62% and water insoluble ash is about 1.42%. Extraction value of drugs renders most significant information about partition of the active component the results of extract value study shows that alcohol soluble extract of TM is 31.80% and water soluble extract is 47.70%.

One of the key factors controlling the absorption of the drug is surface area, decrease in size of the particle directly increases the surface area and thereby aids in increased absorption and bioavailability.\(^{[15]}\) Interestingly, in modern science, several researchers have demonstrated enhanced bioavailability of nanoparticles as compared to their bulk form. For example, Ishihara et al. reported higher bioavailability of micronized zinc oxide as compared to standard zinc oxide.\(^{[16]}\) The results obtained from the SEM analysis shows that most of the particles present in TM are in micro size ranges from 1µm – 2µm. This micro particle size range helps to attain the bio-availability of the drug TM and aid in reaching the target site of action.

**CONCLUSION**

From the data’s of the present investigation it was concluded that the siddha drug *Thirisootha mezhugu* was prepared and analyzed according to the standard procedures. Results of the study generated an evidence based data with respect to the chemical, phytochemical,
physiochemical parameters of the study drug TM. Findings of elemental analysis revealed the absence of elements such as Arsenic (As), Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb) but mercury was present above the permitted level. There were notable changes in SEM analysis that is most of the particle in the formulation are in 1-2 µm size range. Further studies has to be carried with special emphasis on molecular biology aspect of the drug and its target receptor in the biological system in near future.

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REFERENCE