STANDARDIZATION AND QUALITY CONTROL PARAMETERS OF SIDDHA POLY-MINERAL FORMULATION ‘DHASALAVANA DHRAVAGAM’

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ABSTRACT

According to the traditional Siddha literature Kannusamiyam Ennum Vaidhiya Saegaram, the Siddha poly-mineral formulation of the tested drug Dhasalavana Dhraavagam has unique properties being a specialized liquid form of medicine indicated for Poly Cystic Ovarian Syndrome. The aim of this present study was to standardize the purity, quality and safety of the tested drug Dhasalavana Dhraavagam. The physico-chemical characterization of the tested drug was revealed by qualitative biochemical analysis and modern instrumental techniques such as Fourier Transformation Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM). Physico-chemical parameters revealed that the tested drug is a clear colourless liquid with no characteristic odour and specific gravity at 25°C is 1.214, acidic (pH 5.7) in nature. The qualitative biochemical analysis revealed that the presence of Sulphate, Chloride, Phosphate, Calcium, Sodium, Potassium, Magnesium, Ammonium, Iron, Zinc and Copper. The FT-IR study revealed the presence of functional groups like phenols and alcohols, amine, primary amines, nitro groups, nitro methane, alkyl halides in Dhasalavana Dhraavagam. The SEM analysis showed that the size of the particles to be 119nm, 122nm, 150nm, 159nm, 166nm in the tested drug. So it was concluded that the Dhasalavana Dhraavagam contains essential elements which are responsible for curing PCOS. Also the drug possesses no toxic metals which ensures its safety in therapeutic usage.

KEYWORDS: Dhasalavana Dhraavagam, Siddha, PCOS, Physico-chemical, instrumental analysis, poly-mineral formulation.
INTRODUCTION
The standardization of the drugs is a key factor in assessing the quality control of the drugs to establish the medicine in a advantageous way. Standardization of drug is essential to exhibit conformation of its identity and determination of its purity, quality and quantity.[1] *Dhasalavana Dhravagam* is a traditional Siddha poly-mineral formulation consists of 10 salts used in the treatment of PCOS as per the classical Siddha text.[2] Siddha medicine consists of 64 forms of medicines (32 internal and 32 external). Among thirty two kinds of internal medicine, *Dhravagam* is a special type of liquid drug acquired through destructive distillation of salts and alkalies with or without any addition of fluids in a specific process. It has a characteristic property which enables higher absorption rate even at a minimal dose and also enhances the therapeutic potential.[3] Even few drops of dhravagam had curative properties. Even though *Dhasalavana Dhravagam* preparation is used for therapeutic purpose to treat PCOS traditionally, the drug is not validated in a scientific way to prove its safety and efficacy, hence the author is attempted to validate the standardization of *Dhasalavana Dhravagam* scientifically through bio-chemical, physico-chemical and instrumental analysis.

MATERIALS AND METHODS
Selection of drug
*Dhasalavana Dhravagam* has been selected from the Siddha classical literature, “Kannusamiyam Ennum Vaithiya Saekaram”.[2]

Ingredients of the test drug are *Vediyuppu* (Salt petre), *Padikaram* (Alum), *Kalluppu* (Rock salt), *Indhuppu* (Halite), *Navacharam* (Sal ammoniac), *Kariuppu* (Common salt), *Venkaram* (Borax), *Annabedi* (Green vitriol), *Pooneeru* (Fullers earth) and *Thurusu* (Blue vitriol).

Collection of the drugs
All the raw materials except fuller’s earth were obtained from Country drug shop, Parrys Corner, Chennai. *Pooneeru* (Fuller’s earth) is said to be oxides of calcium and potassium which was collected from the soil of Maamandore area, Kanjeeapuram district on the full moon night of April 2014.

Identification and Authentication
All the raw drugs were identified and authenticated by the faculties of Gunapadam (Pharmacology) at Government Siddha Medical College, Arumbakkam, Chennai. The
specimen samples of the identified raw drugs were preserved in the Post graduate laboratory of Gunapadam for future references.

**Purification of raw drugs**

All the ingredients of *Dhasalavana Dhravagam* were purified according to the Siddha classical texts.\[4\]

1. **Salt petre** was soaked in lemon juice, dried in sunshine until the moisture content was lost.\[4A\]
2. **Alum** was dissolved in pure water and filtered. Then it was allowed to boil in a pan until its moisture content was lost. When the mixture attained a thick molten consistency, it was allowed to cool and stored.\[4B\]
3. **Borax** was powdered well and fried in a pan to complete dehydration.\[4C\]
4. **Sal ammoniac** was ground with cow’s urine for three hours and allowed to dry.\[4D\]
5. **Common salt** was dissolved in sea water and filtered. The filtrate was boiled till it reaches semi consistency state. It is dried in sunlight to attain the solid state as purified salt.\[4E\]
6. **Blue vitriol** was fried, till it turns whitish.\[4F\]
7. **Fuller’s earth** According to Bogar, was dissolved in lemon juice and filtered. The filtrate was boiled till the water completely evaporates to get purified form.\[4G\]
8. **Halite** was kept soaked in vinegar for three days and insolated to get a purified and detoxified form.\[4H\]
9. **Rock salt** was dissolved in vinegar and cleaned with a cloth, dried in sunshade.\[4I\]
10. **Green vitriol** was dissolved in water. A small quantity of sulphuric acid was added to it filtered and heated till it attained the consistency of dry salt.\[4J\]

**Preparation of Dhasalavana Dhravagam**

After Purification, Salt petre (120 g), Alum (120 g), Rock salt (40gm), Halite (40gm), Sal ammoniac (20g), Common salt (20g), Borax (15g), Green vitriol (50gm), Fullers earth (5gm), Blue vitriol (5gm) were ground well and transferred to the *Valai Iyanthiram* (Distillation apparatus) made of earthen distillation set up and intensely heated. During the process of heating, the salts were completely decomposed and the acidic fumes were expelled. The fumes were condensed at the condenser deluged in cold water and collected in the vessel. It is stored in an air tight glass container and labeled as DLD.
Physico-chemical parameters: The DLD was evaluated for various physico-chemical parameters such as physical characters like colour, odour, pH, specific gravity and clarity of dhravagam according to the procedures described in the text.\cite{5} The determination of acid-basic radicals was done as per the methodologies described in the text.\cite{6}

Instrumental analysis

Fourier Transformation Infrared Spectroscopy (FT-IR)

Identification of functional groups present in DLD was determined by Spectrum one FT-IR Spectrometer Scan Range MIR 450-4000 cm\(^{-1}\) at a resolution of 1.0 cm\(^{-1}\) and the procedure was carried out at Anna University, Chennai. For sampling technique, KBr method was adopted.\cite{7} IR interacts with the sample and the bond between atoms in the molecule stretch, bends and absorbs infrared energy and creates the infrared spectrum. The computer display revealed spectrum of graphs with peaks and the results were printed on the graphs.

Scanning electron microscopy (SEM): Topography and morphologic information of DLD determined by SEM-Hitachi, scan range S-3400n at a resolution of 1.2 nm with gold particle separation on a carbon substrate and magnification from a min of 12 x to greater than 1, 00, 000 X was done at Anna University, Chennai. The electron beam passed over the sample, scans the surface and gave sufficient data.

RESULTS

The classical Siddha poly-mineral formulation DLD has unique properties being a specialised liquid form of medicine indicated for PCOS. This study deals with the scientific analysis of DLD to standardize the drug through scientific parameters like physico-chemical and elemental analysis to validate safety and efficacy.

Physical characterization: The physical parameters like colour, odour, pH, volatile matter, specific gravity and clarity test are summarized in Table no.1. It indicates that DLD is clear, colourless fluid. It has no characteristic odour and acidic (pH 5.7) in nature.

Table no: 1. Physical characterization of DLD.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Colourless</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>No characteristic odour</td>
</tr>
<tr>
<td>3</td>
<td>pH</td>
<td>5.7</td>
</tr>
<tr>
<td>4</td>
<td>Volatile matter</td>
<td>0.907 %</td>
</tr>
<tr>
<td>5</td>
<td>Specific gravity at 25°C</td>
<td>1.214</td>
</tr>
<tr>
<td>6</td>
<td>Clarity test</td>
<td>Clear</td>
</tr>
</tbody>
</table>
Chemical analysis of Dhasalavana Dhravagam

The results of Acid-Basic radical study of DLD is summarized in Table No.2 and Table No.3 respectively. It showed that DLD had Sulphate, Chloride, Phosphate, Calcium, Sodium, Potassium, Magnesium, Ammonium, Iron, Zinc and Copper and absence of heavy metals such as lead, arsenic and mercury.

Basic and acidic radical studies

Table No: 2 Basic radicals studies of DLD.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameter</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test for Potassium</td>
<td>Yellow colour precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Test for Calcium</td>
<td>White colour precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Test For Magnesium.</td>
<td>White colour precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>Test For Ammonium.</td>
<td>Reddish brown precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>5</td>
<td>Test For Sodium</td>
<td>Yellow colour</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td>Test for Iron (Ferrous)</td>
<td>Blood red colour</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td>Test For Zinc</td>
<td>Formation of white precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>8</td>
<td>Test for Copper</td>
<td>Bluish green flame</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Table No: 3 Acid radical studies of DLD.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameter</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test for Sulphate</td>
<td>Formation of white precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Test for Chloride</td>
<td>Formation of white precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Test for Phosphate</td>
<td>Yellow colour precipitate</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Instrumental analysis

Fourier Transform InfraRed spectroscopy (FTIR)

The study revealed the presence of functional groups like phenols and alcohols, amine, primary amines, nitro groups, nitro methane, alkyl halides in DLD. It was tabulated in Table no. 4 and represented graphically in Figure no. 1.

Table No: 4 Result of FT-IR analysis of DLD.

<table>
<thead>
<tr>
<th>Absorption peak cm⁻¹</th>
<th>Stretch</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3453.9</td>
<td>O-H stretch; H- bonded</td>
<td>Phenols and alcohols</td>
</tr>
<tr>
<td>2364.0</td>
<td>N-H bond</td>
<td>Amine</td>
</tr>
<tr>
<td>2345.6</td>
<td>N-H bond</td>
<td>Amine</td>
</tr>
<tr>
<td>1638.1</td>
<td>N-H bond</td>
<td>Primary amines</td>
</tr>
<tr>
<td>1381.3</td>
<td>N=O bond</td>
<td>Nitro groups Nitro methane</td>
</tr>
<tr>
<td>681.4</td>
<td>C-Br stretch</td>
<td>Alkyl halides</td>
</tr>
</tbody>
</table>
FT-IR (Fourier Transform Infra-Red)

![FT-IR Graph]

Fig. No. 1. Showing The Graph of Edax Fourier Transform – Infra Red Spectroscopic Analysis (FTIR).

DLD, 15-12-14.pk

DLDF944~1.SP 3601 4000.0 400.0 3.1 100.0 4.0 % T 4 0.3

Sunder sample C 08/12/2014

REF 4000 99.9 2000 93.3 600

3453.9 3.1 2364.0 86.6 2345.6 88.0 2079.3 91.5 1638.1 60.2

1381.3 94.5 681.4 87.5 END 7 PEAK(S) FOUND

RESULTS OF SCANNING ELECTRON MICROSCOPE ANALYSIS

The SEM picture (Fig No.2) showed that the particles were all in stabilized form having irregular morphology and distributed unevenly.

![SEM Image]

Fig no: 2. SEM images showing the shape and size of the particle size of the drug DLD.
The above SEM studies of microscopic resolution of 1.00kx and examining surface area of 800x800µm², showed objects of sizes ranging from 119nm to 166nm.

DISCUSSION

The physical parameters revealed that the Dhasalavana Dhravagam is a transparent, odourless, volatile matter 0.907% specific gravity 1.214 pH 5.7 which is an acidic medium, lots of protons are present. Therefore, greater amount of acidic drug is unionized. Thus in acidic medium acidic drug is present more in unionized form, which increases its absorption. This is why acidic drugs are well absorbed from the stomach.[8]

The acid-basic radical analysis showed the presence of Calcium, zinc, magnesium. Calcium plays a vital role in treating PCOS and it is proved by many studies. Calcium plays an important role in egg maturation and stimulates folliculogenesis in the ovaries. Vitamin D is essential for promoting calcium absorption from various foods and supplements in the intestines. In addition, vitamin D deficiency is one of the main reason for PCOS related problems includes infertility, obesity and insulin resistance.

PCOS can be found simply with zinc deficiency. Zinc deficiency causes hair loss, impaired sugar metabolism, dandruff, acne, migraine headaches, menstrual irregularities, ovarian cysts, and infertility.[9] Zinc can exert beneficial effects on two common conditions associated with PCOS: acne and obesity. Recent research suggested that zinc may be helpful in healing acne. Patients with Zinc deficiency has shown increase in the level of circulating leptin. Insufficient leptin levels are believed to be the primary cause of food cravings and overeating, both of which may contribute to obesity associated with PCOS.

Many women with PCOS exhibit symptoms of insulin resistance and metabolic disorder, increases the risk factor for cardiovascular disorders and major problems like diabetes and stroke. Magnesium deficiency is often related with diabetes and some research indicates that a dietary supplement of the mineral may improve insulin sensitivity, and PCOS.[10]

FTIR analysis is done to know the functional groups of the bio molecules, to elucidate the structure and to confirm the active molecules responsible for the therapeutic effect of the test drug Dhasalavana dhravagam. The study revealed the presence of phenolic group of compounds which exert antioxidant activity and also responsible for chemo preventive properties like anti-cancer, anti-mutagenic, anti-inflammatory activity and induce apoptosis.
which decreases the cancer risks. The antioxidant potential of phenols, especially polyphenols scavenge the free radicals produced by oxidative stress, lipid peroxidation may damage the cells leading to cancer, neurodegenerative diseases, enzyme inhibition leading to many diseases.\cite{11}

SEM analysis of *Dhasalavana dhravagam* facilitates to find out the morphology of the particles which depicts the size and shape of the particles. The size of the particles was 119nm, 122nm, 150nm, 159nm, 166nm.

1. Microparticles are defined as particulate dispersion of the particles with a size in the range of 100-1000 nm in diameter.\cite{12}
2. Size and surface of microparticles can be easily manipulated to achieve both active and passive drug targeting.\cite{13}
3. They control and sustain 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 bio-availability and reduced side effects.\cite{14}

**CONCLUSION**

The above physico chemical, acid basic radical and instrumental analysis proved the drug *Dhasalavana Dhravagam* is superior in treating PCOS. This drug is safe and can be easily administered and cost effective. Moreover the drug stimulates folliculogenesis process effectively, hence it is concluded that *Dhasalavana Dhravagam* is mentioned in Siddha classical literature is safe and efficacious drug for PCOS. Further pre-clinical and clinical studies will validate the trial drug *Dhasalavana Dhravagam* for therapeutic purpose.

**ACKNOWLEDGEMENT**

The author is thankful to the Principal, Govt. Siddha Medical College, Chennai, H.O.D and Assistant Professor of Mechanical Engineering and Chemistry Department at Anna University, Chennai, for their valuable support and guidance helped lot in the completion of the study by the way of standardization of the drug and in carrying out instrumental analysis respectively.

**REFERENCES**


