PHYSICO-CHEMICAL CHARACTERIZATION OF SIDDHA HERBAL FORMULATION NAANALKARUMBU CHOORANAM: AN APPROACH TO STANDARDIZATION

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ABSTRACT
Naanal Karumbu Chooranam is reputed drug mentioned in classical Siddha literature for the treatment of obesity. The main component of Naanal Karumbu Chooranam is the stem of Saccharum spontaneum. The aim of this study was carried out to standardize the drug Naanal Karumbu Chooranam by evaluating its Organoleptic characters, Physico chemical properties and modern scientific instruments like, SEM (Scanning Electron Microscope), Fourier Transform - Infra Red (FTIR) Spectroscopy. Organoleptic characters such as colour, odour, taste, particle size and Physico-Chemical Characters like ash Values, pH value, solubility were analyzed. The total ash value was found to be 6.68%/w/w, acid insoluble ash value is 1.29%/w/w and moisture content is 4.94%/w/w. The pH value is 5.53. The water soluble extractives and alcohol soluble extractives were found to be 23.83%/w/w and 17.27%/w/w. FT-IR spectroscopy showed the presence of functional groups like phenols, alcohols, amines, aliphatic amines, aromatic amines, amides and alkyl halides. These functional groups are key factors for Hypolipidemic activity. SEM analysis of the drug indicated the existence of nanoparticles. Nano particle sizes are 39.7nm, 79.4 nm and 107 nm.

KEYWORDS: Physico-chemical properties, ash values, FT-IR spectroscopy, SEM.

INTRODUCTION
Medicinal plants constitute an important part of the health care system for the prevention and treatment of various disorders, since the ancient civilization. Previous studies show that 30% of the marketed drugs contain active principles that were isolated from plants.[1] The World Health Organization defines herbal medicine as those containing plant parts or plant materials in raw state or processed form containing active principles.[2]

Standardization of herbal formulation is an essential in order to assess the quality of drugs. It is based on the concentration of their active principles, physical, chemical, Phyto-chemical and In-vitro, In-vivo parameters.[3] The standardization of the drugs is a key factor in assessing the quality control of the drugs to establish the medicine in a valuable mode. Standardization of drug is essential to exhibit conformation of its identity and determination of its purity, quality and quantity.[4]

Thus, The present study deals with standardization of Siddha single herbal formulation Naanal Karumbu Chooranam is mentioned in the classical Siddha literature for the treatment of obesity.[5] The term Chooranam (Powdered herbal preparation for internal use) is applied to the powder prepared by a single or combination of two or more herbal drugs.[6] Siddha is the oldest healing system of medicine and it has fundamental aspects for drug formulation. Major formulations used in Siddha are based on herbs. The medicinal herbs are utilized in the form of decoctions, infusions, tinctures and powders.[7]

There are no systemic protocols for standardization of Naanal Karumbu Chooranam, hence it was decided to evaluate the qualitative and quantitative analysis for Naanal Karumbu Chooranam scientifically to prevent its adulteration. For the standardization of this drug Organoleptic, Physico-chemical properties were carried out. Instrumental analysis like SEM (Scanning Electron Microscope), Fourier Transform-Infra Red (FTIR) Spectroscopy of the Naanal Karumbu Chooranam were also prepared to evaluate its quality.

MATERIALS AND METHODS
Selection of drug
Naanal Karumbu Chooranam was selected from Classical literature of Siddha.
Collection and authentication of the Plant material
Naanal Karumbu Chooranam consists of the stem of Saccharum spontaneum. For the preparation of Naanal Karumbu Chooranam, the stems of Saccharum spontaneum were collected from the river belt of Thamirabarani in Tirunelveli district at Tamilnadu. It was identified by the Botanist and authenticated by the Faculties and experts of PG Gunapadam Government Siddha Medical College, Palayamkottai, Thirunelveli and voucher specimen was deposited in the department of PG Gunapadam laboratory for future reference.

Purification of the drug
The stem of Naanal karumbu is washed away with the stream of running river water to remove stains, dust and send particles later it is dried inside room temperature to free from moisture in the stem.

Preparation of NaanalKarumbuChooranam
The stem of (Saccharum spontaneum) was cut into small pieces and dried under shade until complete evaporation of the moisture content. It was finely powdered by pulverizerand sieved through a white cotton cloth, filtered. Then the Chooranam was purified by steam boiling process as per the procedure mentioned in Siddha classical literature and kept in a cleanair tight container. It was labeled as Naanal Karumbu Chooranam (NKC).

PHARMACOGNOSTIC STUDIES

Organoleptic evaluation
Organoleptic evaluation was used for identification of sensory characteristics like colour, odour, taste and texture etc. NKC was evaluated by its Organoleptic characters such as colour, odour, taste, texture, particle size and other morphology according to the procedures described in the text.

Physicochemical evaluation
Physicochemical analysis of Naanal Karumbu Chooranam had been done according to the text. Determination of total ash, water insoluble ash, acid insoluble ash and alcohol soluble extractive, water soluble extractive, Loss on drying were studied by the typical manner and documented.

Preparation of extract for basic and acidic radical studies
The drug NKC was subjected for qualitative analyses of cations and anions based on the texts mentioned.

Instrumental analysis

SEM (Scanning Electron Microscope)
To assess the size of the particle, surface topography SEM analysis was carried out using S-3400n SEM-Hitachi at a magnification range of 12 X to 1,00,000X at Anna University, Chennai. The drug was fixed 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 drug builds up the resultant image. The micrographs got sufficient statistics about the structure of the drug.

FTIR (Fourier Transform - Infra red Spectroscopy)
Identification of functional groups present in NKC was determined by FT-IR Spectrometer Scan Range MIR 400-4000 cm-1 at a resolution of 1.0 cm-1 and the procedure was carried out at Anna University, Chennai. For sampling technique, KBr pellet technique was adopted. IR interacts with the sample drug and the bonds between atoms in the molecule stretch, bends and absorbs infrared energy and creates the infrared spectrum. The computer display showed spectrum of graphs with peaks and the results were printed on the graphs.

RESULTS

Physicochemicalparameters
Naanal Karumbu Chooranam (NKC) shows straw yellow in colour with sweet in taste and odourless. It was a fine powder in nature and completely passed through sieve no. 88 which are shown below in the Table no.1.

Tab. no. 1 Organolepticparameters of Naanal Karumbu Chooranam

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Straw Yellow</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Odour less</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Sweet</td>
</tr>
<tr>
<td>4</td>
<td>Consistency</td>
<td>Fine powder</td>
</tr>
<tr>
<td>5</td>
<td>Particle size</td>
<td>Completely passes through sieve No 88</td>
</tr>
</tbody>
</table>

Physicochemical parameters
Physicochemical evaluation of Naanal Karumbu Chooranam was done and the results were tabulated in Table 2. The pH of the drug is 5.53. Total ash value was (6.68% w/w), Acid insoluble ash value (1.29% w/w), Water soluble extractive (23.83% w/w) Alcohol soluble Extractive (17.27% w/w), Loss on drying at 105°C (4.94% w/w).
Table. No.2. Physicochemical parameters of Naanal Karumbu Chooranam

<table>
<thead>
<tr>
<th>S No</th>
<th>Physicochemical parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>pH</strong></td>
<td>5.53</td>
</tr>
<tr>
<td>2</td>
<td>Total ash</td>
<td>6.68% w/w</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>1.29% w/w</td>
</tr>
<tr>
<td>4</td>
<td>Water Soluble extractive</td>
<td>23.83% w/w</td>
</tr>
<tr>
<td>5</td>
<td>Alcohol Soluble extractive</td>
<td>17.27% w/w</td>
</tr>
<tr>
<td>6</td>
<td>Loss on Drying at 105°C</td>
<td>4.94% w/w</td>
</tr>
</tbody>
</table>

Results of acidic and basic radical studies
The result of Acid-Basic radical studies showed (Table no.4) that Naanal Karumbu Chooranam contains Sulphate, Chloride, Calcium, ferrous iron and starch.

Table no.4 Results of acidic and basic radical studies

<table>
<thead>
<tr>
<th>Acidic and basic radicals</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for Calcium</td>
<td>+</td>
</tr>
<tr>
<td>Test for Chloride</td>
<td>+</td>
</tr>
<tr>
<td>Test For Sulphate</td>
<td>+</td>
</tr>
<tr>
<td>Test for ferrous iron</td>
<td>+</td>
</tr>
<tr>
<td>Test for Starch</td>
<td>+</td>
</tr>
</tbody>
</table>

Instrumental Analysis
SEM: (Scanning Electron Microscope)
The SEM picture (Fig.No.1) under 1.90 KX resolutions and the examining area of 800x800μm² surface are taken for the samples. Particles (particle size) are ranging from 39.7nm, 79.4 nm and 107 nm in NKC.

![SEM picture showing nanoparticles](image)

Naanal Karumbu Chooranam shows the presence of alcohols, phenols, alkanes, alkenes amines, aliphatic amines, aromatic amines, alkyl halides and carboxylic acids, ester, ether as functional groups.

![Fig.No.1. showing the graph of FTIR spectroscopy](image)

Fourier Transform – Infra Red (FTIR) Spectroscopy
The test drug was identified to have 12peaks. The results of Table no.5 and Figure no.2 demonstrated that the drug

Table. No.5. Results of FTIR analysis of Naanal Karumbu Chooranam

<table>
<thead>
<tr>
<th>Absorption peak cm⁻¹</th>
<th>Stretch</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3402</td>
<td>O-H stretch</td>
<td>Alcohols, Phenols</td>
</tr>
<tr>
<td>2923.7</td>
<td>=C-H stretch</td>
<td>Alkanes</td>
</tr>
<tr>
<td>2113.9</td>
<td>-C≡C- stretch</td>
<td>Alkynes</td>
</tr>
<tr>
<td>1645.5</td>
<td>C-C≡C symmetric stretch</td>
<td>Alkenes</td>
</tr>
<tr>
<td>1252.9</td>
<td>C-N stretch</td>
<td>Aromatic amines</td>
</tr>
</tbody>
</table>
DISCUSSION

The field of the herbal drugs and formulations are enormous and there is still lot to explore on the subject of standardization of these. So, while developing an herbal formulation it is must to have all the related knowledge of that particular drug including all its organoleptic characters to pharmacological action to its standardization in respect to various parameters via various techniques.17

Ash values

Ash values may be effective parameter to assess the degree of purity of a given drug. Total ash value of plant material indicates the amount of minerals and other earthy materials present.18 The total inorganic content (Calcium, Chloride, Sulphate and Iron, etc.,) present in the drug is measured via Total ash value and its value is 6.68% for NKC. Acid insoluble ash value of the NKC represents the quantity of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. It is 1.29% for NKC.

Extractive Values

The percentage of soluble matters present in the drug is established by the values of water extractive and alcohol extractive. Based on the extractive value suitable solvent can be selected.

Loss on Drying

Loss on Drying at 105°C indicates that only 4.94% of water and volatile components have been lost when 1g of NKC kept at 105°C. This moisture content helped to prevent deprivation of efficacy and degeneration19 of NKC. High moisture content can adversely affect the active principles of the drug. It may possibly get early infection of the drug. Thus low moisture content could get maximum stability and long shelf life.

The size of the particle is reduced to micro and nano particles which pass through sieve no: 8820 so that the drug is easily assimilable in GIT.

Acidic and basic radicals study

Calcium is binding with and preventing the absorption of dietary fat. Chondroitin Sulphate has lowered serum cholesterol levels and it reduced the risk of heart attacks.

SEM (Scanning Electron Microscope) analysis

SEM analysis of Naanal Karumbu Chooranam exposed the presence of nano particles of size 39.7nm, 79.4 nm and 107 nm and the particles were homogenously distributed and has smooth surface. Hence the drug NKC will have chance for smooth flowing within the digestive system without any irritation.

Nanoparticles demonstrate exclusive physico-chemical characters such as ultra small size, greater surface area per weight than larger particle and high reactivity. The existence of nano and near nano particles in NKC facilitates to aid absorption by enhancing bio-availability of the drug.

FTIR (Fourier Transform – Infra Red (FTIR) Spectroscopy) analysis

FTIR analysis is done to know the functional groups of the bio molecules, to elucidate the structure and to confirm the active compounds responsible for the therapeutic effect of the drug. NKC showed the association of functional groups and 12 effective peaks were attained between 4000 cm\(^{-1}\) to 400 cm\(^{-1}\).

CONCLUSION

In the present study it is concluded that the Organoleptic characters and Physico-chemical parameters such as the total ash value 6.68%, acid insoluble ash value is 1.29% w/w and moisture content is 4.94% w/w. The pH value is 5.53. The water soluble extractives and alcohol soluble extractives were found to be 23.83% w/w and 17.27% w/w. can be capably used for standardization of herbal formulation. The sophisticated analysis of instruments like FT-IR spectroscopy exposed the existence of functional groups like alcohols, phenols, amines, aromatic amines, aliphatic amines, amides and alkyl halides are primarily responsible for hypolipidemic activity. SEM analysis of the drug revealed the presence of nano particles. These nano particle sizes of Naanal Karumbu Chooranam permit rapid absorption. Thus results from all these standardization results it would be concluded that the NKC has better quality assessment and its safety and efficacy was also exposed.

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