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**Research Article**


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# Qualitative and Quantitative analysis of Seenthil Chooranam

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## Abstract

The drug Seenthil Chooranam was prepared with care as per the literature evidence. In Ingredients of Seenthil Chooranam were Seenthil, karisalai, poonagam. The drug was analysed for its physico chemical properties and contents by using qualitative biochemical analysis and modern techniques such as inductively couped plasma-optical Emission spectorometry. Analysis reveals the presence of calcium, starch, ferrous iron, Tannic acid, Unsaturared compound, reducing sugar, amino acid in Seenthil chooranam By scanning electrom microscope (SEM) the sizes of the particles were analysed. The particle size is about 1-3 Micron.

**Keywords:** Seenthil Chooranam, Physico- chemical, FTIR, HR-SEM, ICP-OES.

## Introduction

Siddha system is one of the oldest system of medicine in India. The word siddha comes from the word 'siddha'. Which means an object to attain perfection or heavenly bliss. Siddha generally refers to 'Attama Siddhi' that is the eight super natural powers those who attained or achieved the above said powers are know as 'siddhars' siddhars were saintly figures. There were eighteen important siddhars in older days and they developed this system of medicine.

### Chemistry in siddha system

In siddha system, chemistry had been found well developed into science auxiliary to medicine and alchemy.

The siddhars were aware of several chemical operation divided into several process such as

1. Calcinations
2. Sublimation
3. Distillation
4. Fusion
5. Seperation
6. Fermentation
7. Exallation etc.

## Aim and objective

The aim of this study is evaluate the Qualitative and Quantitative analysis, Physicochemical analysis of Seenthil Chooranam.

## Objective

To collect raw materials and purification of the raw materials based on the relevant literature.

To analyse the ingredients present in the drugs before and after purification.

To prepare Seenthil Chooranam as per tecutal method.

To evaluate the physiochemical analysis of Seenthil Chooranam

## Materials and Methods

### Qualitative and Quantitative analysis

### Physicochemical analysis

| | | |
|---|---|---|
| Sample Description | : | Seenthil Chooranam |
| Equipment used | : | Atomic Absorption Spectrometer (AAS) |

#### Colour:

About 50g of **Seenthil Chooranam** was taken in a clean glass beaker and tested for its colour by viewing again a water opaque background under direct sunlight.

#### pH:

The pH of **Seenthil Chooranam** was estimated as per the method prescribed in Indian Standard (IS) – 6940 (1982). One gram of the **Seenthil Chooranam** was taken into a 100ml graduated cylinder containing about 50ml of water and filled up to the mark with water. The cylinder was stopped and shaken vigorously for two minutes and the suspension was allowed to settle for an hour at $25^0$ to $27^{0.}$ About 25ml of the clear aqueous solution was transferred into a 50ml breaker and tested for **pH** using DIGISUN digital **pH** meter (DIGISUN Electronics, Hyderabad, India)

#### Determination of Ash Value:

Weighed accurately 2 grams of **Seenthil Chooranam** in tarred platinum or silica dish and incinerate at a temperature not be exceeding $450^0$C until free from carbon, cooled and weighed. Calculate the percentage of ash with reference to the air dried drug.

#### Water Soluble Ash:

To the gooch crucible containing to the total ash, added 25ml of water and boiled for 5 minutes. Collected the insoluble matter in a sintered glass crucible or on ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature nor exceeding $450^0$ C subtract the weight of the insoluble matter from the weight of the ash the difference of the weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug.

#### Acid Insoluble Ash:

Boiled the ash 5 minutes with 25ml of 1:1 dil HCL. Collect the insoluble matter in gooch crucible on an ash less filter paper wash with hot water and ignite. Cooled in a desiccators and weighted calculated the percentage of acid insoluble ash with reference to the air dried drug.

#### Loss on Drying:

Five grams of Seenthil chooranam is heated in a hot oven at $105^0$C to constant weight and the percentage of loss of weight has calculated there from.

### Phytochemical analysis

#### Test for Alkaloids

To 3 ml of the extract in a test tube, 1 ml of 1% HCl was added. The mixture was heated for 20 minutes cooled and filtered. About 2 drops of Mayer's reagent was added to 1 ml of the filtrate. A creamy precipitate indicates the presence of alkaloids.

#### Test for Saponins

##### Frothing test:

2 ml of the extract was vigorously shaken in a test tube for 2 minutes and observe for frothing.

#### Test for Flavonoids

##### Lead acetate test:

Ten mg of extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

### Test for Terpenoids

To 5 ml of the extract, 2 ml of chloroform was added and mixed, 3 ml of concentrated $H_2SO_4$ was carefully added to form layers and a reddish brown coloration of the interface was formed, indicating the presence of terpenoids.

### Test for Phenols and Tannins

#### Lead acetate test:

Ten mg of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

### Test for Carbohydrates

#### Fehling's test:

Five ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.

### Test for Protein & amino acids

#### Biuret test:

To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

### Ninhydrin test:

About 0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

### Lipid test

- Add a **few drops** of the liquid food sample to a dry test tube.
- Add 2 cm3 **ethanol** and shake it thoroughly
- Add 2 cm3 of **deionized water**.
- Make **observations**.

## Transform – Infra Red Spectroscopy Perkin Elmer – Spectrum one

### Introduction

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy.

In IR spectroscopy, the resonance absorption in made possible by the change in dipole moment accompanying the vibrational transition. The infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

### Principle

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecurlar and electronic structure of the substance and with intra – and inter molecular interactions.

FT-IR spectroscopy is used primarily for qualitative and quantitative analysis of organic compounds, and also for determining the chemical structure of inorganic materials. The region between 500-4000 wave number is referred to as the finger print region. Absorption bands in this region are generally due to intra molecular phenomena and are highly specific for each material. The specificity if these bands allow computerized data searches to be performed against reference libraries to identify a material.

## Table of Characteristic IR Absorptions

| Frequency, cm-1 | Bond | Functional group |
|---|---|---|
| 3640 - 3610 (s, sh) | O-H stretch | Free hydroxyl alcohols phenols |
| 3500 - 3200 (s,b) | O-H stretch, H – bonded | Alcohols, phenols |
| 3400 – 3250 (m) | N – H stretch | Primary, secondary, amines, amides |
| 3300 – 2500 (m) | O – H stretch | Carboxylic acids |
| 3330 - 3270 (n, s) | –C (triple bond) C – H : C - H stretch | Alkynes (terminal) |
| 3100 – 3000 (s) | C – H stretch | Aromatics |
| 3100 – 3000 (m) | = C – H stretch | Alkenes |
| 3000 – 2850 (m) | C – H stretch | Alkenes |
| 2830 – 2695 (m) | H – C = 0; C –H stretch | Aldehydes |
| 2260 - 2210 (v) | C (triple bond) N stretch | Nitriles |
| 2260 – 2100 (w) | C (triple bond) C- stretch | Alkynes |
| 1760 – 1665 (s) | C = 0 stretch | Carbonyls (general) |
| 1760 – 1690 (s) | C = 0 stretch | Carboxylic acids |
| 1750- 1735 (s) | C = 0 stretch | Esters, saturated aliphatic |
| 1740 – 1720 (s) | C = 0 stretch | Aldehydes, saturated aliphatic |
| 1730 – 1715 (a) | C = 0 stretch | Alpha, beta – unsaturated esters |
| 1715 (s) | C = 0 stretch | Ketones, saturated aliphatic |
| 1710 – 1665 (s) | C = 0 stretch | Alpha, beta – unsaturat aldehydes, ketones |
| 1680 – 1640 (m) | -C = C - | Alkenes |
| 1650 – 1580 (m) | N – H bend | Primary amines |
| 1600 – 1585 (m) | C-C stretch (in – ring) | Aromatics |
| 1550 – 1475 (s) | N – 0 asymmetric stretch | Nitro compounds |
| 1500 – 1400 (m) | C –C stretch (in – ring) | Aromatics |
| 1470 – 1450 (m) | N – 0 asymmetric stretch | Nitro compounds |
| 1370 – 1350 (m) | C – H bend | Alkanes |
| 1360 – 1290 (m) | C – H rock | Alkanes |
| 1335 – 1250 (s) | C – N stretch | Aromatic amines |
| 1320 – 1000 (s) | C – 0 stretch | Alcohols, carboxylic acids, esters, ethers |
| 1300 – 1150 (m) | C – H wag ( - CH2X) | Alkyl halides |
| 1250 – 1020 (m) | C – N stretch | Aliphatic amines |
| 1000 – 650 (s) | =C – H bend | Alkynes |
| 950 – 910 (m) | O – H bend | Carboxylic acids |
| 910 – 665 (s, b) | N – H wag | Primary, secondary amines |
| 900 – 675 (s) | C – H “oop” | Aromatics |
| 850 – 550 (m) | C – CI stretch | Alkyl halides |
| 725 – 720 (m) | - C (triple bond) C-H : C- H bend | Alkynes |
| 690 – 515 (m) | C - Br stretch | Alkyl halides |

M = medium, w = weak, s=strong, n = narrow, b = broad, sh = sharp

### Sampling techniques:

There are a variety of techniques for sample preparation depending on the physical form of the sample to be analyzed.

| | | |
|---|---|---|
| Solid | : | KBr or Nujol mull method |
| Liquid | : | CsI / TIBr Cells |
| Gas | : | Gas Cells |

### Experimental Procedure: Done at SAIF, IIT Madras, Chennai – 36KBr Method

- The Sample was grounded using – an agate mortar and pestle to give a very fine powder.
- The finely powder sample was mixed with about 100 mg dried KBr salt.
- The mixture was then pressed under hydraulic press using a dye to yield a transparent disc (measure about 13 mm diameter and 0.3mm in thickness), through which the beam of spectrometer passed.

### HR SEM-Methodology:

An SEM is essentially a high magnification microscope, which used a focused scanned collection beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways

Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.

Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.

Ionized atoms can relax by electron shell-to-shell transitions. Which lead to either X-ray emission or Auger electron ejection. The X-rays emitted are characteristic of the elements in the top few urn of the sample.

### Sample Preparation:

Sample preparation can be minimal or elaborate for SEM analysis depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a **Seenthil Chooranam** that will fit into the SEM chamber. And it should be analyzed.

### Inductively Coupled Plasma Optical Emission-Spectrometry (ICP – OES)

### ICP OES Methodology

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry, When plasma energy is given to an analysis sample from outside, the component elements (atoms) is excited. When the excited atoms return to low energy position, emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the ray's intensity.

To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas in ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation –emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

### Sample preparation:

Solids cannot be analyzed directly. Such samples should be made into clear aqueous medium quantitatively. When acids are used to prepare solutions care should be taken. The concentration of the acids in the final provided solution should not be more than 2% v/v. highly acidic and organic solutions cannot be analyzed. As a guide line weigh exactly, around 200mg of substance and dissolve in 5mL of 5% of water or aquaregia or whatever acid to make 100mL of final solution. Make proper dilutions, if necessary. Free HF should not present in the final solution to be aspirated.

Ideal concentration is around 100 ppm of the element of interest.

Total dissolved solids should be not more than 0.2% w/v in the final solution.

Very dilute solution may not give reliable results. Each element has a detection limit.

A minimum solution volume of 25 ml is necessary for analysis.

In ICP intensity of light emitted when the sample "sprayed or aspirated into an argon plasma" is measured at different wavelengths. The intensity of light at a given wavelength will be proportional to a particular elemental ion concentration. The intensity is calibrated with known standard concentration. For accurate quantitative results It is necessary to simulate the sample matrix condition with that of the standard. Each element generally will have many emission lines and the sensitivity is different for each of this wave length. When more than one element is present it is quite common that some emission lines interfere due to overlapping.

It is preferable to use plastic containers for sample handling and preserving samples for **ICP-OES** analysis. Glass containers can give problems especially when analyzing certain metal ions at low concentration.

The samples of **Seenthil Chooranam** was prepared.

## Results and Discussion

### Qualitative and quantitative analysis

**Table -1 Colour characters of Seenthil Chooranam**

| No | Nature of drug | Nature of colour |
|---|---|---|
| 1 | Seenthil chooranam | Brown colour |

**Table 2— Physicochemical analysis of samples of Seenthil Chooranam [Values are mean of three determinations ±SEM]**

| Parameters | Total ash | Values |
|---|---|---|
| Ash value | Water soluble ash<br>Acid insoluble ash | 6.70±0.058<br>2.90±0.003 |
| Extractive value | Ethanol soluble extractive value<br>Water soluble extractive value | 9.10±0.500<br>10.20±0.500 |
| Loss on drying | Loss on drying at 70°C | 9.03±0.500 |

SEM- singularity **expansion** method

**Table-3 Particle size and pH of Seenthil Chooranam**

| S.No | Parameters | Values obtained |
|---|---|---|
| 1 | Particle size by SEM | 1-3 µm |
| 2 | pH | 6.48 |

**Phytochemical analysis**

| S.No | Test | Observation | Result |
|---|---|---|---|
| 1. | **Test for alkaloids** | No characteristic change was observed | Absence of alkaloid |
| 2. | **Test for flavonoids** Lead acetate test | No characteristic change was observed | Absence of flavanoids |
| 3. | **Test for saponins** | Froathing occurs | **Presence of saponins** |
| 4. | **Test for protein** Biuret reaction | No characteristic change was observed | Absence of protein |
| 5. | **Test for amino acids** Ninhydrin test | No characteristic change was observed | Absence of Aminoacid |
| 6. | **Test for terpenoid** | Reddish brown coloration | **Presence of terpenoid** |
| 7. | **Test for carbohydrate** Fehlin's test | No characteristic change was observed | Absence of carbohydrate |
| 8. | **Test for lipid** | Yellow coloration | **Presence of lipid** |
| 9. | **Test for phenol** Lead acetate test | No characteristic change was observed | Absence of phenol |

The extract prepared from the given sample **Seenthil Chooranam** shows the presence of **Saponin, Terpenoid**, **lipid.**

## Bio-chemical analysis of Seenthil chooranam

### Preparation of the extract:

5gms of the drug was weighed accurately and placed in a 250ml clean beaker. Then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is make up to 100ml with distilled water. This fluid is taken for analysis.

## Qualitative analysis

| S.No | Experiment | Observation | Inference |
|---|---|---|---|
| 1. | **Test for calcium** 2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% ammonium oxalate solution | A white precipitate is formed | Indicates the presence of calcium |
| 2. | **Test for sulphate** 2ml of the extract is added to 5% barium chloride solution. | No white precipitate is formed | Absence of sulphate |
| 3. | **Test for chloride** The extract is treated with silver nitrate solution | No white precipitate is formed | Absence of chloride |
| 4. | **Test for carbonate** The substance is treated with concentrated hcl. | No brisk effervessence is formed | Absence of carbonate |
| 5. | **Test for starch** The extract is added with weak iodine solution | Blue colour is formed | Indicates the presence of starch |
| 6. | **Test for ferric iron** The extract is acidified with glacial acetic acid and potassium ferro cyanide. | no blue colour is formed | Absence of ferric iron |

| | | | |
|---|---|---|---|
| 7. | **Test of ferrous iron**<br>The extract is treated with concentrated nitric acid and ammonium thio cyanate solution | Blood red colour is formed | **Indicates the presence of ferrous iron** |
| 8. | **Test for phosphate**<br>The extract is treated with ammonium molybdate and concentrated nitric acid | No yellow precipitate is formed | Absence of phosphate |
| 9. | **Test for albumin**<br>The extract is treated with esbach's reagent | No yellow precipitate is formed | Absence of albumin |
| 10. | **Test for tannic acid**<br>The extract is treated with ferric chloride. | Blue black precipitate is formed | Indicates the presence of tannic acid |
| 11. | **Test for unsaturation**<br>Potassium permanganate solution is added to the extract | It gets decolourised. | Indicates the presence of unsaturated compound |
| 12. | **Test for the reducing sugar**<br>5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 mts and add 8-10 drops of the extract and again boil it for 2 mts. | Colour change occurs. | Indicates the presence of reducing sugar |
| 13. | **Test for amino acid**<br>One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninnhydrin is sprayed over the same and dried it well. | Violet colour is formed | Indicates the presence of amino acid |
| 14. | **Test for zinc**<br>The extract is treated with potassium ferrocyanide. | No white precipitate is formed | Absence of zinc. |

## Inference:

Analysis reveals the presence of calcium, starch, ferrous iron, Tannic acid, Unsaturared compound, reducing sugar, amino acid in **Seenthil chooranam.**

**Sophisticated Analytical Instrument Facility IITM, Chennai-36**

**Perkin Elmer Optima 5300 DV ICP-OES**

| Sample ID | Elements Symbol Wavelength (nm) | Concentration |
|---|---|---|
| | (wt:0.23227g) | |
| As 188.979 | BDL | |
| Ca 315.807 | 17.390 mg/L | |
| Cd 228.802 | BDL | |
| Cu 327.393 | BDL | |
| Fe 238.204 | 08.346 mg/L | |
| Hg 253.652 | BDL | |
| K 766.491 | 13.811 mg/L | |
| Na 589.592 | 24.310 mg/L | |
| Ni 231.604 | BDL | |
| Pb 220.353 | BDL | |
| P 213.617 | 96.341 mg/L | |

BDL- Below detection limit

**Seenthil chooranam**

**Table of characteristic IR Absorptions**

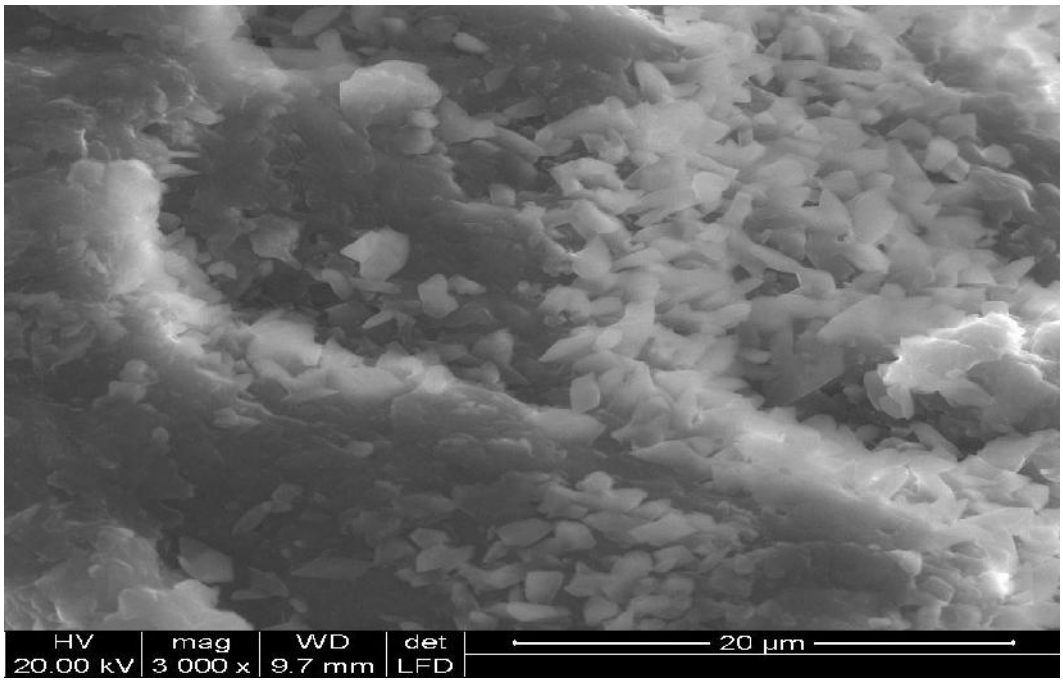
| Frequency, cm-1 | Functional Group |
|---|---|
| 3640-3610 (s, sh) | - |
| 3500-3200 (s,b) | + |
| 3400-3250(m) | - |
| 3330-3270 (m) | - |
| 3100-3000 (n,s) | - |
| 3100-3000 (s) | - |
| 3000-2850 (m) | + |
| 2830-2695 (m) | - |
| 2260-2210 (m) | + |
| 2260-2100 (v) | - |
| 1760-1665 (w) | - |
| 1760-1690 (s) | - |
| 1750-1725 (s) | - |
| 1740-1720 (s) | - |
| 1730-1715 (s) | - |
| 1715 (s) | - |
| 1710-1665 (s) | - |
| 1680-1640 (s) | - |
| 1650-1580(m) | - |

| | |
|---|---|
| 1600-1585 (m) | - |
| 1550-1475 (m) | - |
| 1500-1400 (s) | + |
| 1470-1450 (m) | - |
| 1370-1350 (m) | + |
| 1360-1290 (m) | + |
| 1335-1250 (m) | + |
| 1320-1000 (s) | - |
| 1300-1150 (s) | + |
| 1250-1020 (m) | + |
| 1000-650 (m) | + |
| 950-910 (s) | + |
| 910-665 (m) | - |
| 900-675 (s,b) | + |
| 850-550 (s) | + |
| 725-720 (m) | - |
| 690-515 (m) | + |

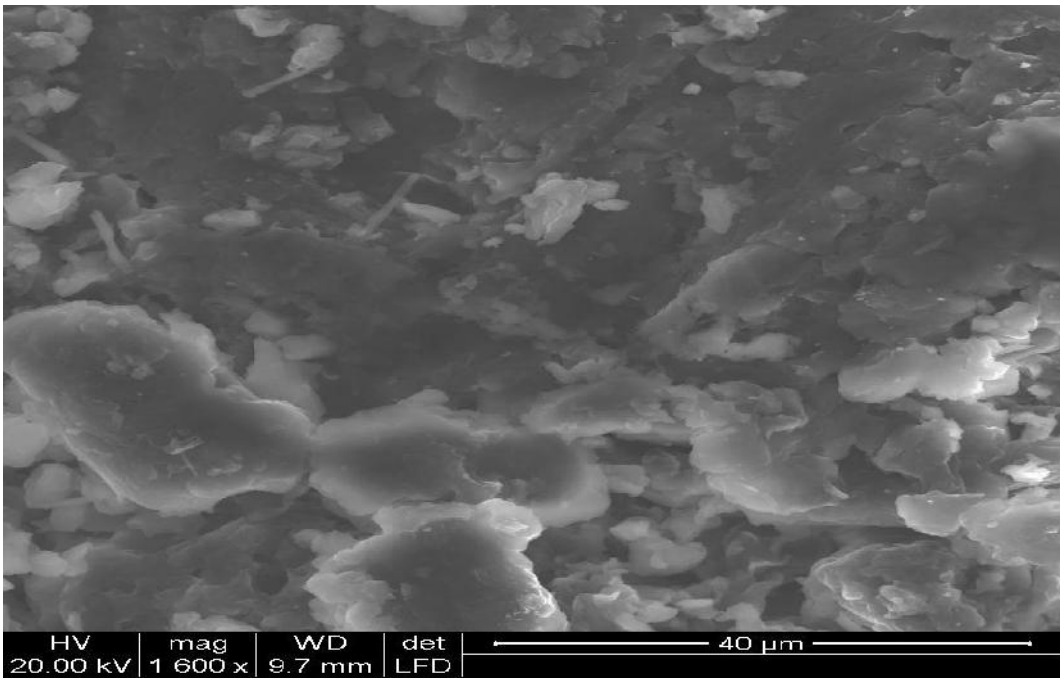
m= medium, w= weak, s=strong, n=narrow, b=broad, sh=sharp

## Result

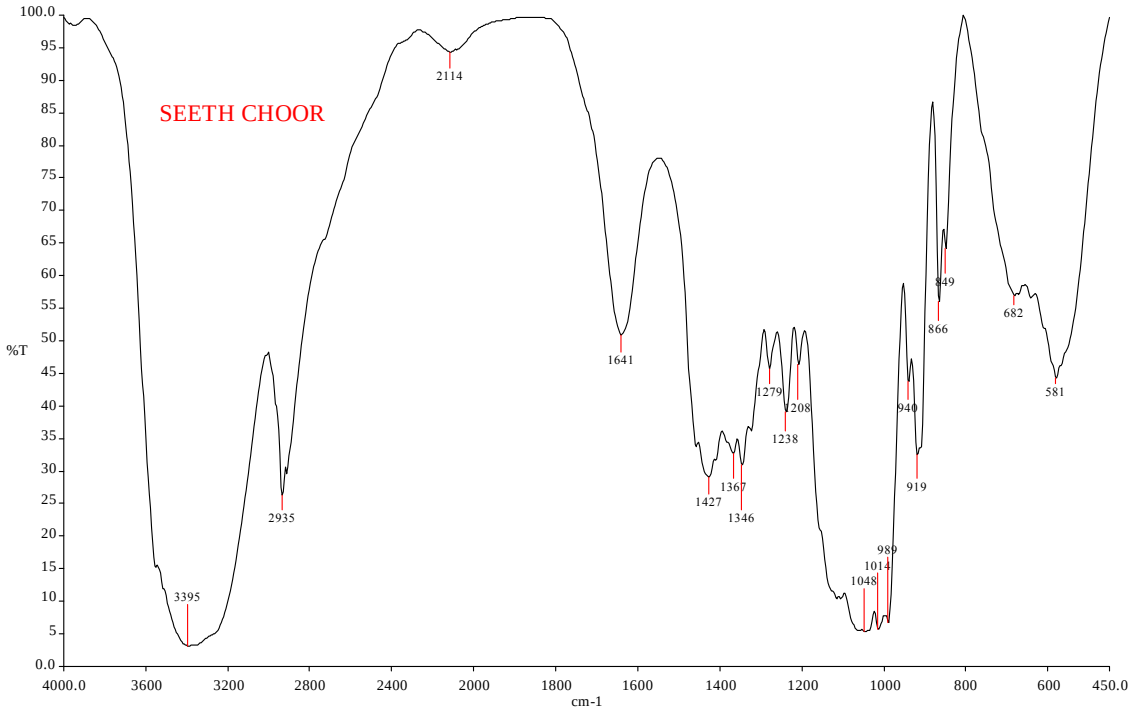
**FTIR data:** Seenthil chooranam contains **alcohols, phenols, Alkenes, Nitriles, Aromatics, alkanes, aromatic amines, alkyl halides, Aliphatic amines, alkynes, carboxylic acid.**




The picture shows that the particles are stabilize, have irregular morphology, **Seenthil chooranam** has the particle size of **20 µm.**




The picture shows that the particles are stabilize, have irregular morphology, **Seenthil chooranam** has the particle size of **40 µm.**




**Characteristic IR Absorptions**

## Discussion

The present study is aimed to find out the qualitative and quantitative analysis of "Seenthil Chooranam".

The ICP-OES analysis revealed that heavy metals like arsenic, cadmium, mercury and lead are in below detection limit.

By scanning electron microscope (SEM) the sizes of the particles were found to be in the range of 1 to 3 Micron.

Biochemical analysis shows the presence of calcium, starch, ferrous iron, tannic acid, unsaturared compound, reducing sugar, amino acid.

Phytochemical evaluation of the drug reveals the presence of alcohols, phenols, Alkenes, Nitrioes, Aromatics, Alkanes, Aromatic amines, Alkyl halides, Aliphatic amines, Alkynes Carhoxylic acid.

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