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Research Article

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Modern Instrumental Approach towards Standardization and Comparative Analytical Evaluation of Novel siddha preparation Thurusu

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Abstract

Globally there was a paradigm shift towards traditional system of medicine were observed as it offers wide range of therapy for treating several diseases and disorders. It became paramount responsibility of the regulatory officials to lay down the guidelines for such traditional medicines. Siddha system of medicine is traditionally practiced in India since centuries back as it pioneers the art of healing which works behind the principles of fundamental elements. Metals and metalloids plays a vital role in siddha preparations as it was believed that metal has power of rejuvenation and alters the physiology during disease condition. At a same time it become essential for a physician to ensure the safety and standard of such metalloid based formulation like thurusu before prescribing the same for human. The main aim of the present investigation is to establish the monograph on functional group (FT-IR), SEM and XRD analysis of the unpurified thurusu (UPT) and purified thurusu (PT) a metalloid based novel siddha preparation. The results of FT-IR spectral analysis of UPT and PT reveals the presence of vibrational and intense absorption peaks corresponds to Cu2+, S-O,CuO and OH functional groups. SEM analysis reveals that the particle size of UPT and PT falls in the micro meter with average size ranges from 8.11 to 33.36 µm. The X-ray diffraction pattern of the sample UPT and PT confirms the elemental composition of the preparation which majorly comprises of copper sulfate. Hence it was concluded from the results of the present study that the test dug UPT and PT has biologically significant functional groups further the particles being micrometer in size may have repaid accesses to the target region by permeation through biological barriers.

Keywords: Siddha, Traditional system of medicine, Thurusu, Metalloid, FT-IR, SEM, XRD

1. Introduction

Since ancient time, Indian society depends on traditional medicinal systems practiced here. Introduction of allopathic drug during British era and neglecting Indian traditional medicine by British ruler are responsible for significant erosion of Indian traditional medicine. High scientific progress in allopathic medicine and modern facilities also resists the growth of traditional medicine. Still, about 70% rural populations of India are believed in traditional medicine for primary healthcare [1-2].

Siddha system of medicine is believed as a brilliant achievement and symbol of Tamil culture which originated in Southern parts of India. Siddha medicine invented from Dravidian culture and is grown in the time of Indus valley civilization. Chinese alchemy,

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Taoism, and Taoist Patrology are considered as a main source of inspiration for Siddha alchemy. It is believed that in ancient time, the system was developed by eighteen siddhar (a class of Tamil sages). Though Siddha system of medicine resembles with Ayurveda in many aspects it has own philosophy and concept, holistic approach, and lifestyle oriented measures [3-5].

Emerging trends on validation and standardization of siddha drugs using sophisticated analytical instruments has greater advantage of combatting the newer technology with traditional approach. Metals have higher chances of cumulating toxicity upon chronic usage for which the siddha practice have remarkable methodology of detoxification and purification procedures. Modern technology provides value added advantages of exploring the standards of several siddha preparations of metalloid origin.Siddhars are pioneer practitioners drafted a clear SOP before several centuries on formulation, standardization and validation of siddha preparation's.The regulation of traditional siddha formulation continues to present challenges to many countries regardless of the fact that an increased number of the population rely on this for their health care needs. The main aim of the present investigation is to establish the monograph on functional group (FT-IR), SEM and XRD analysis of the unpurified thurusu (UPT) and purified thurusu (PT) a metalloid based novel siddha preparation.

2. Materials and Methods

2.1. Source and authentication of raw drugs

The required ingredient is procured from a well reputed indigenous traditional Indian medicine drug shop from Chennai, Tamil Nadu, India and were authenticated by the concerned authorities before use.

2.2. Ingredients

- Thurusu - 500 gm
- Honey – 250 gm
- Ghee – 250 gm
- Whey water (Decanted milk water) – 800 ml (from 1000 ml of milk)

2.3.Purification Procedure [6]

Thurusu is triturated with honey and ghee and boiled in a crucible. Then soaked in decanted milk water for 3 days and dried.

2.4. Fourier Transform – Infra Red Spectroscopy Study [7-8]

Fourier Transform – Infra Red Spectroscopy Study (FTIR) IR data acquired with Spectrum one: FT-IR Spectrometer with scan range of MIR 450-4000 cm-1. About 20 mg of the test sample SPK was taken on a micro spatula and grounded well with required quantity of KBr salt. Sample admixed with KBr with trituration aided by mortar and pestle until to get a uniform fine powder of sample- KBr mixture. Further mixture was loaded in pellet die and subjected to 5000-10,000 psi in pelletizer. Resulting pellet was placed in FTIR sample holder and expose to IR radiation to get the spectra.

2.5. Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDX) [9-10]

The study was conducted in a very fine powder of the drug and the sample was quick frozen in liquid nitrogen. The sample was mounted rigidly on a specimen holder called specimen stub. The mounted sample was placed inside the microscope's vacuum column evaporator through an air tight door. On expelling air from the air pump, a beam of electrons passed from an electron gun. This beam travelled through a series of magnetic lenses designed to focus the electrons. The focused beam moved back across the mounted sample row by row by a set of scanning coils. As the electron beam hit each spot on the sample, secondary electrons are backscattered from its surface. A detector counts these electrons and sends the signals to an amplifier. The final image was built up from the no of electrons emitted from each spot on the sample. The micrographs obtained give sufficient data about the topography of the subjected sample. Model- SEM-Hitachi with the scan range of 3400n and resolution of 1.2 nm gold particle separation on a carbon substrate .Magnification from a min of 12 x to greater than 1, 00,000 X

2.6. XRD spectral Study

The XRD spectrum of test drug was Bruker discover D8 X ray diffractometer. Cu K Alpha radiation was used for recording the spectra. The range of diffraction angle 10-70^{o} operating at 30kV and 20 mA. The pattern was recorder from the angle 5 to 80 degree at a scanning rate of 3 degree/second [11].

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3. Results

3.1. Result Analysis of FT-IR spectrum of UPT and PT

FT-IR spectrum of UPT reveals the Infrared absorption pattern of CuO stretching was observed in the region of 617.83cm−1 .Broad Intense absorption peak at 3353 cm−1 corresponds to O-H stretching .Wide absorption peak at 1095cm-1 due to stretching vibration mode of S-O group. Intense absorption peak at 875 cm-1 may be due to vibrational mode of metal ion. Cu2+ and also may be observed at Cu-O-H vibration. Absorbance at 1645cm-1 due to O-H shift and its corresponding stretching. Wide short intense peak at 1144 cm-1 may be due to presence of C=S thiocarbonyl. As represented in figure 1.

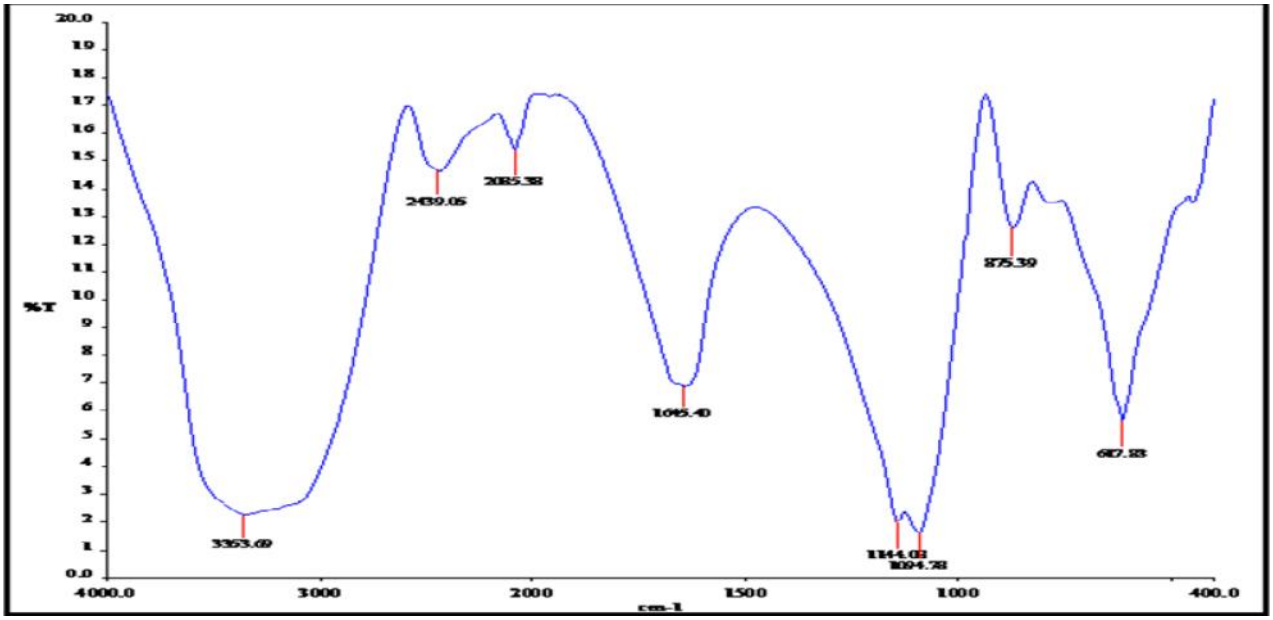

Figure 1: FTIR Spectrum of the sample UPT

FT-IR spectrum of PT reveals the infrared absorption pattern of CuO stretching was observed in the region of 618.05cm−1 .Intense absorption peak at 2850 and 2920 cm−1 corresponds to O-H stretching .Wide short intense peak at 1105.58 cm-1 and 1150.84 cm-1 may be due to presence of C=S thiocarbonyl. Absorbance peak at721.33cm-1 may be due to presence of S-OR esters.Absorbance peak at1465 cm-1 may be due to C-H bending vibration. Absorbance peak at 1743cm-1 may be due to C=O functional group oscillation. As represented in figure 2.

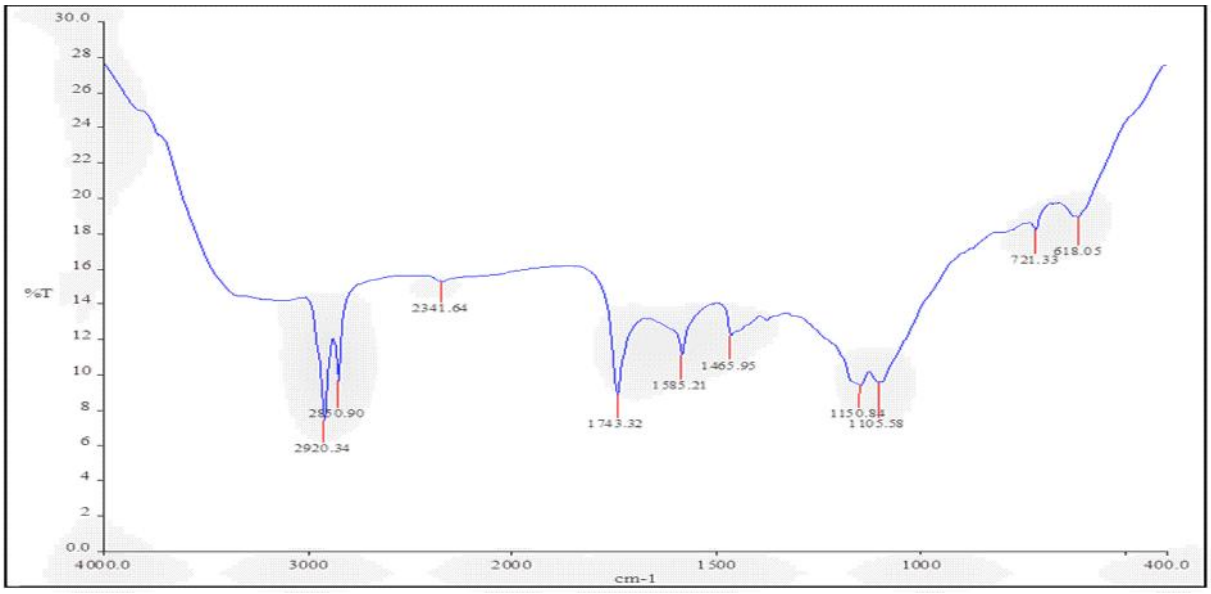

Figure 2: FTIR Spectrum of the sample PT

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3.2. Results of SEM image Analysis of UPT and PT

The results of SEM analysis reveals that the particle size of UPT and PT falls in the micro meter range which justifies the better penetrability of the drug on the biological membrane. The average particle size of UPT ranges from 8.11 to 26.57µm. Similarly the average particle size of PT ranges from 9.3 to 33.36µm. As represented in figure 3 and 4.

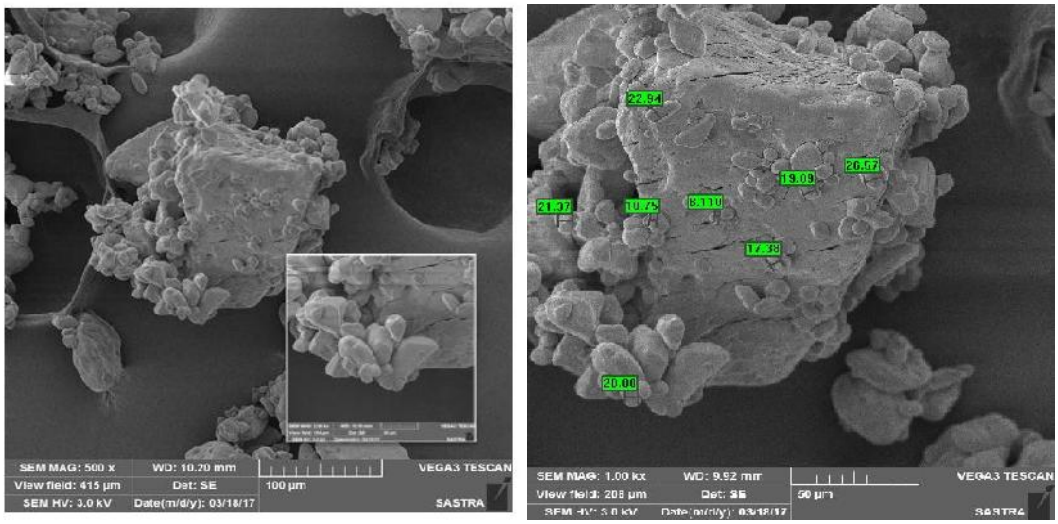

Figure 3: SEM analysis of the sample UPT

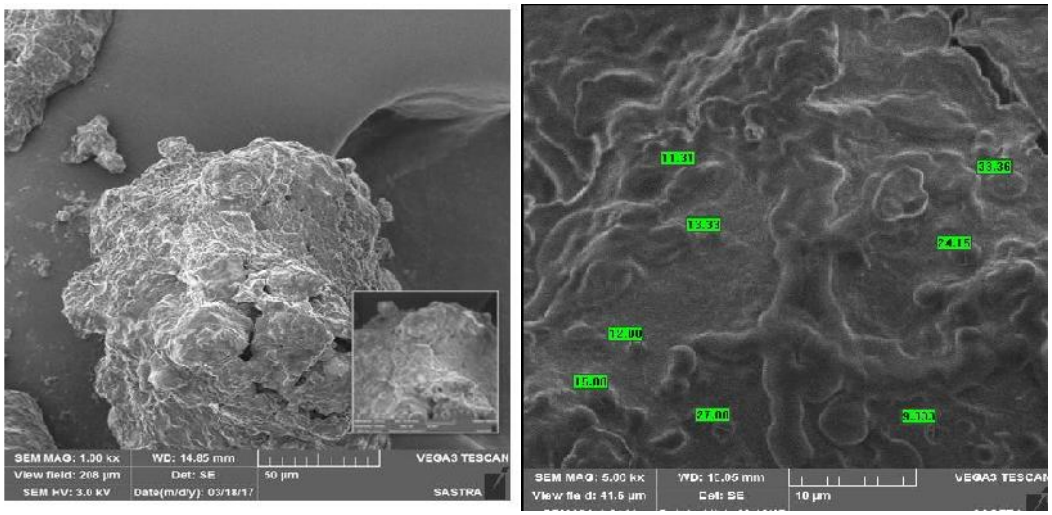

Figure 4: SEM analysis of the sample PT

3.2. Results of XRD spectral analysis of UPT and PT

The X-ray diffraction pattern of the sample UPT reveals the presence of major peak with 2- Theta value of 22.29 which exactly matches to the ICDD (International Centre for Diffraction Data)86-2456 and 72-0090. ICDD86-2456 corresponds to the crystalline pattern of Copper (I)Sulfate.

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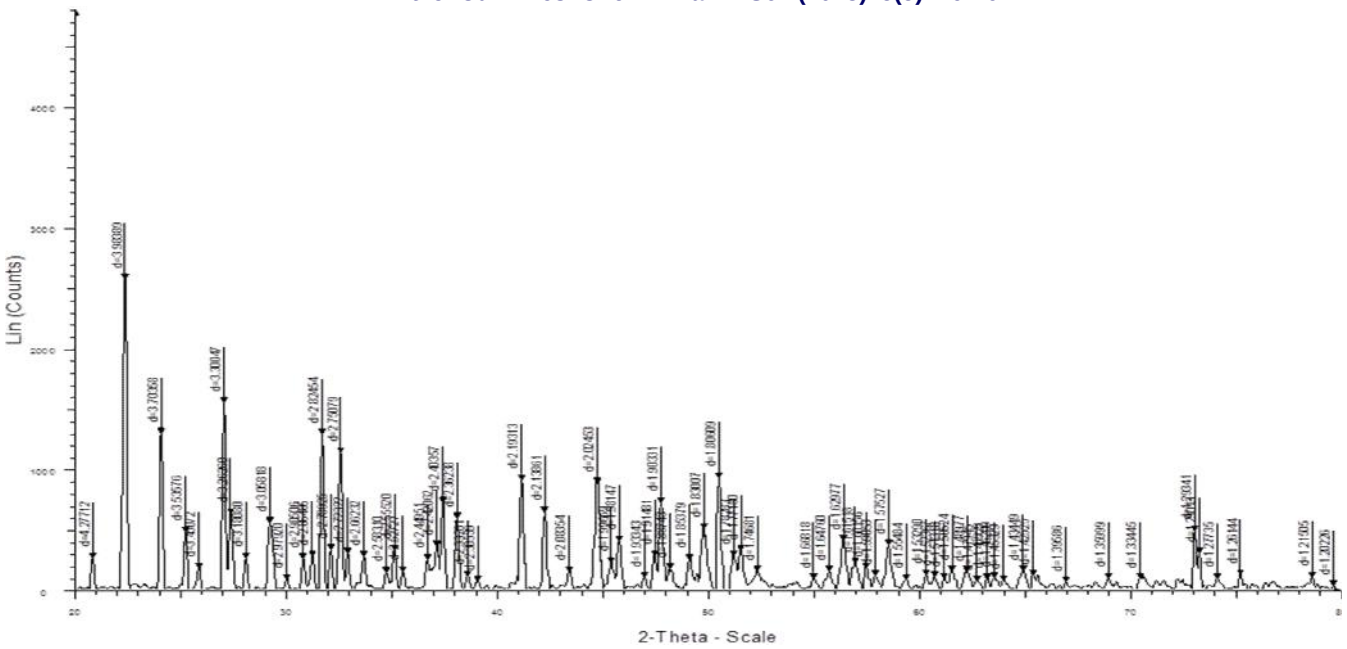

Figure 5: XRD Spectrum of the Sample UPT

ICDD72-0090 corresponds to the crystalline pattern of Copper (II) Sulfate. Major peaks observed in test sample U P T with 2-theta values of 22.92 and their corresponding intensities were 2584. The major peak observed in the reference matching material Cu_2SO_4 was 28.05 with the intensity value of999and in$CuSo_4$ it was 25.07with intensity value of 999. The XRD pattern of the test sample U P T matches with the reference materials such as Copper(I)Sulfate and Copper(II) Sulfate which justifies the presence of stable and purified nature of above mentioned compounds in the forming redients or impurities in the test sample. The datas were tabulated in table 1 and represented in figure 5.

Table 1: XRD peak analysis table of sample UPT

Left Angle	Right Angle	Left Int.	Right Int.	**Obs. Max**	d (Obs. Max)	Max Int.	Net Height	**FWHM**	Chord Mid.	I. Breadth	Gravity C.	d (Gravity C.)	Raw Area	Net Area
2-Theta °	2-Theta °	Cps	Cps	**2-Theta °**	Angstrom	Cps	Cps	**2-Theta °**	2-Theta °	2-Theta °	2-Theta °	Angstrom	Cps x 2-Theta °	Cps x 2-Theta °
22.110	22.440	465	465	**22.294**	3.98444	2587	2121	**0.185**	22.291	0.185	22.288	3.98546	546.6	392.9

The X-ray diffraction pattern of the of the prepared sample PT reveals the presence of major peak with 2-Theta value of 36.67 which exactly matches to the ICDD (International Centre for Diffraction Data)86-2456 and 72-0090.ICDD86-2456 corresponds to the crystalline pattern of Cu2SO4-Copper(I)Sulfate. Major peaks observed in test sample P T with 2-theta values of 36.67 and their corresponding intensities were 3214.

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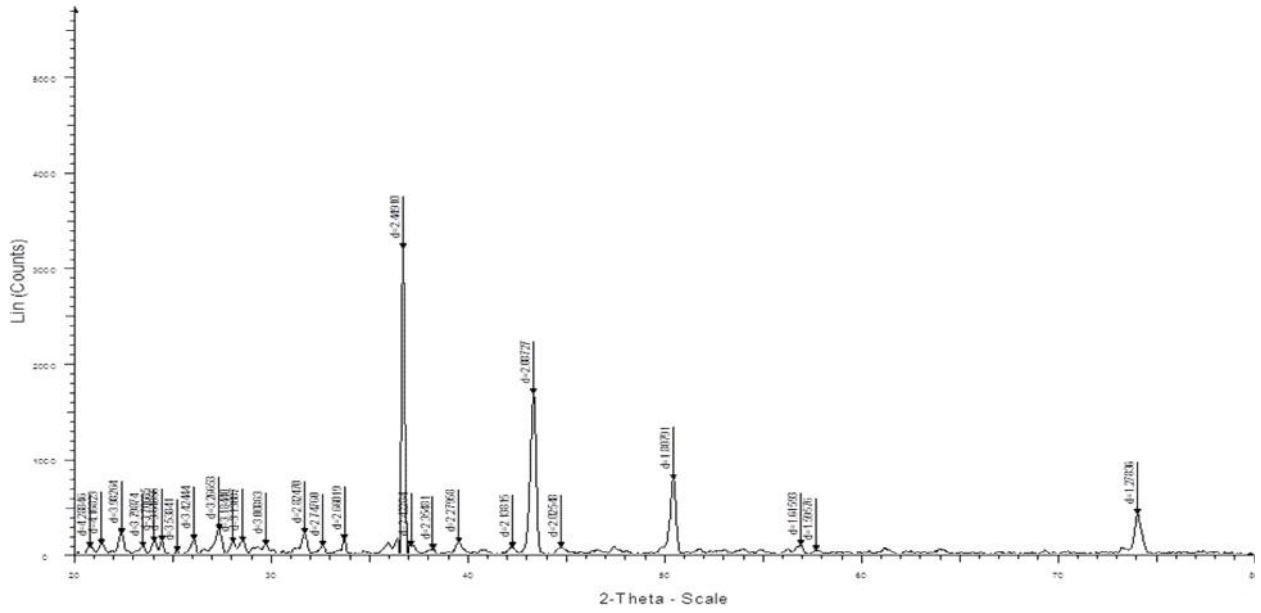

Figure 6: XRD Spectrum of the Sample UPT

The major peak observed in the reference matching material Cu_2SO_4 was28.05 with the intensity value of 999.The XRD pattern of the test sample P T matches with the reference materials Copper(I)Sulfate which justifies the presence of stable and purified nature of above mentioned compounds in the form ingredient in the test sample. The data's were tabulated in table 2 and represented in figure 6.

Table 2: XRD peak analysis table of sample UPT

Left Angle	Right Angle	Left Int.	Right Int.	**Obs. Max**	d (Obs. Max)	Max Int.	Net Height	**FWHM**	Chord Mid.	I. Breadth	Gravity C.	d (Gravity C.)	Raw Area	Net Area
2-Theta °	2-Theta °	Cps	Cps	**2-Theta °**	Angstrom	Cps	Cps	**2-Theta °**	2-Theta °	2-Theta °	2-Theta °	Angstrom	Cps x 2-Theta °	Cps x 2-Theta °
36.530	36.810	530	530	**36.671**	2.44867	3224	2693	**0.167**	36.666	0.161	36.666	2.44895	582.6	433.9

4. Discussion

Trace elements have both a curative and a preventative role in combating diseases. Trace elements, for example the metals selenium, zinc and copper, are essential to maintain the metabolism of the human body. However, non-essential metals such as cadmium and chromium lead to adverse effects, even though they are only present in trace amounts. Elements, in one form or another play an important role in the field of medicine, including the trace elements present in traditional preparations. The consumption of such traditional formulations contributes to the intake of both essential and non-essential trace elements by the human body [12].

IR is a technique based on the vibrations of the atoms of a molecule. The wavelength of IR covers wide range from 0.76 to 1000 μm. This wide IR region is divided into three regions, respectively named near-IR (0.76–2.5μm), mid-IR (2.5–25μm), and far-IR (25–1000μm), among which the mid-IR is widely used for structure identi-fication. The peak position can be represented with wavelength λ (μm) or wavenum-berσ (cm^{-1}). Wavenumber is the more commonly used form and calculated by the following equation.Two necessary conditions must be satisfied to produce an IR spectrum. One is that the energy of electromagnetic radiation should be equal to the energy difference of two vibrational states. Another is that the dipole moment must change during the molecular vibration [13-14].

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In the present investigation FT-IR spectrum of UPT reveals the Infrared absorption pattern of CuO stretching was observed in the region of 617.83cm−1 .Broad Intense absorption peak at 3353 cm−1 corresponds to O-H stretching .Wide absorption peak at 1095cm-1 due to stretching vibration mode of S-O group. FT-IR spectrum of PT reveals the infrared absorption pattern of CuO stretching was observed in the region of 618.05cm−1 .Intense absorption peak at 2850 and 2920 cm−1 corresponds to O-H stretching .Wide short intense peak at 1105.58 cm-1 and 1150.84 cm-1 may be due to presence of C=S thiocarbonyl. Presence of hydroxyl group is an added advantage for the formulation as it was evident through literature that hydroxyl group has capable of forming bond with core amino acid residue of the several biologically significant enzymes.

SEM analysis considerable most significant advancement in the field of analytical research in predicating the structural morphology and particle analysis of the samples. Particle size of the formulations plays very essential role in determining the bioavailability and pharmacological activity of the drugs. It was proven that decrease in size of the particle has increased access in crossing biological membrane of the body. The results of SEM analysis reveals that the particle size of UPT and PT falls in the micro meter .The average particle size of UPT ranges from 8.11 to 26.57µm. Similarly the average particle size of PT ranges from 9.3 to 33.36µm.

X-ray diffraction (XRD) is a powerful nondestructive technique for characterizing crystalline materials. It provides information on nature, structures, phases, preferred crystal orientations (texture), and other structural parameters, such as average grain size, crystallinity, strain, and crystal defects. X-ray diffraction peaks are produced by constructive interference of a monochromatic beam of X-rays scattered at specific angles from each set of lattice planes in a sample [15]. From the result of the present XRD analysis it was concluded that the elemental compositionoftestsample UPT and PT comprises of the copper sulfate present as the purified ingredient.

5. Conclusion

Siddha system of medicine pioneering in emphasize the biological activity of the various metals and metalloids with respect to the etiology and pathophysiology of various dread full disease emerging in humans and animals. Siddha pharmacopoeia established in recent times have imposed more on standardization aspect of the formulation. Starting from preparatory phase to storage each and individual step involved in formulating siddha preparation has its own quality check evaluations. It is concluded from the results of the present investigation that the siddha drugThurusu has biologically significant functional groups further the particles being micrometer in size may have repaid accesses to the target region by permeation through biological barriers.

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