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STANDARDISATION OF NOVEL SIDDHA FORMULATION SEENAKARA PARPAM

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ABSTRACT
Siddha system of medicine is a renowned holistic system of traditional medicine emphasizing curative and preventive measures. The medicines used in Siddha system are of plant origin, metals, minerals and animal products which is broadly classified into 32 types of internal and external medicines. Parpam is one among the type of internal medicines which is a nano sized formulation with a life expectancy of 100 years. *Seenakara Parpam* is a classical siddha formulation used in the treatment of Kalladaippu (Renal Calculi) and Neeradaippu (Obstruction of urethral passage). In present scientific scenario, standardisation of Siddha medicines has become mandatory to establish its safety, efficacy and to find out the active components present in it. The aim of this study is to standardise the nano formulation *Seenakara Parpam* (SKP) as per the siddha literature using sophisticated techniques like Elemental Analysis (AAS), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray powder diffraction (XRD) and Scanning Electron Microscope (SEM). For more validity, three batches of SKP is prepared and analysed. On elemental analysis by AAS shows the presence of lead and mercury that too within the WHO prescribed limit. FTIR shows the presence of following functional groups viz.., C-H, N-H, O-H, C-N. SEM with EDAX proves presence of nano sized particles with minimum size of 42.1nm and highest of 184.7 nm.

KEYWORDS: Seenakara Parpam, Siddha Formulation, Standardisation, Sophisticated techniques.

INTRODUCTION

Siddha is one of the most ancient systems of health care prevalent in India and presently getting popularity in developed countries also. Siddha system of medicine attains greater importance in recent days as most of the novel siddha preparation considered to be a valuable lead for the treatment of various infectious, non-infectious and other metabolic disorders in mankind. Gaining popularity mandate the drug to be standardized to compete in the global market.

Standardization is a process of elucidating the nature and class of formulation to ensure its purity and genuinity through standard guideline as established by AYUSH. Standardization renders some valuable information to the researcher about the Class and Nature of individual components present within the formulation, Limit of elements including heavy metals, Knowledge about genuinity of the raw materials used, Physicochemical property of the finished product, Sterility of the products, Detail on trace of pesticides and toxins if any, Particle size determination and stability of the product, Structure of the individual compounds, Detail on functional group, nature of ring and active side chain, Toxicity nature of the drug.

Siddha practitioners are using traditional medicine made of herbs and organic minerals that counteracts the crystal growth formation and also increases the urine output and also restores the calcium ion hemostasis in living biological system. One such noble formulation is Seenakara parpam (SKP) derived from Purified Seenakaaram (Alum/Alumen), Vediuppu (Potassium nitrate / salt petre) and Karchunna Neer (Quick Lime solution). It is indicated for the treatment of Kalladaippu (Renal Calculi) and Neeradaippu (Obstruction of urethral passage).

This study aims at standardizing the novel siddha formulation *Seenakara Parpam* using the sophisticated techniques like Elemental Analysis (AAS), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray powder diffraction (XRD) and Scanning Electron Microscope (SEM). To make this study more valid, three batches of Seenakara Parpam is prepared and analysed.

MATERIALS AND METHODS

Details regarding the samples

The ingredient of Seenakara Parpam includes Seenakaram (Alum), Vediuppu (Potassium Nitrate) and Karsunna Neer (Lime water). The raw drugs were procured from market sample at Govindhasamy chetty store, Chennai. The minerals were authenticated by Dept of Geology, Univeristy of Madras, Guindy campus, Chennai. The samples were prepared at the Gunapadam Laboratory, National Institute of Siddha, Chennai. Three batches of Seenakara parpam viz SKP 1, SKP 2, SKP 3.., was prepared for a valid standardisation.

Elemental analysis – Atomic Absorption Spectroscopy

Elemental analysis of sample SKP1, 2 and 3 were carried out with atomic absorption spectroscopy technique. Model of instrument used for analysis was thermo fisher M series, 650902 V1.27. Basic heavy metal profiling such as lead, cadmium, mercury and arsenic was analyzed using AAS technique. Wave length ranges from 180 to 260 nm. Slit width of 0.5mm with carrier gas used was argon and acetylene of flow rate of 1.1 L/min. Flame vapour technique was adopted for the study.

Fourier Transform – Infra Red Spectroscopy Study

The Perkine Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FT IR Spectra of Seenakara parpam in Potassium Bromide (KBr) matrix with scan rate of 5 scan per minute at the resolution 4cm-1 in the wave number region 450-4000cm-1. The samples were grounded to fine powder using agate motor and pestle and the mixed with KBr. They were then Pelletized by applying pressure to prepare the specimen (the size of specimen about 13 mm diameter and 0.3 mm in thickness) to recorded the FT-IR Spectra under Standard conditions. FT- IR Spectra were used to determine the presence of the functional groups and bands in the seenakara parpam.

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy

SEM with X-ray spectroscopy (EDX) was used to access the surface morphology of the particles present within the sample SKP and further EDX data will be utilized to study the percentage proportion of potassium, carbon, alum and sulphur present in the varying batch samples of SKP1, 2 and 3. Energy of electrons used of bombardment is 0.5 – 30 kV. Surface topographic features on the outermost surface (< 5 nm) is achieved by using a primary electron beam with an energy of < 1 kV.

XRD spectral Study

The XRD analysis of SKP was carried out using Bruker discover D8 X ray diffractometer. Cu K Alpha radiation was used for recording the spectra 60. 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.

RESULTS AND DISCUSSIONS

Elemental analysis – Atomic Absorption Spectroscopy

Table 1: Elemental Analysis of SKP 1, SKP 2 and SKP 3 by AAS Technique.

ELEMENTS	SKP 1	SKP 2	SKP 3	Permissible limits
Lead	0.935 ppm	0.553 ppm	0.612 ppm	10 ppm
Cadmuim	BDL	BDL	BDL	0.3 ppm
Mercury	0.024 ppm	0.010 ppm	0.031 ppm	1 ppm
Arsenic	BDL	BDL	BDL	3 ppm

BDL – Below Detective Level

The results of heavy metal analysis by AAS technique reveals that the heavy metals such as lead and mercury present in the formulations are within the WHO prescribed limit. The results of heavy metal analysis were tabulated in Table 1.

Fourier Transform – Infra Red Spectroscopy Study

IR spectrum of all the three samples of SKP belongs to various batches showing sharp intense peak at 1116 cm^{-1} corresponds to C-N stretching. Similarly another sharp peak was observed in the region of 3480 – 3451 cm^{-1} shows the presence of usual O-H bending. Peak emerging in the region of 618 cm^{-1} corresponds to C- H deformation and presence of less intense peak in the range of 679 - 662 cm^{-1} corresponds to N-H wagging Peaks emerging in the range of 1635- 1641 cm^{-1} shows the C=C variations. FT-IR spectral data's of sample SKP1, SKP2 and SKP 3were represented in Figure 1 to 3. FT-IR results of all three samples of SKP having more or less closely similar results.

FT-IR Spectrum of Sample SKP 1

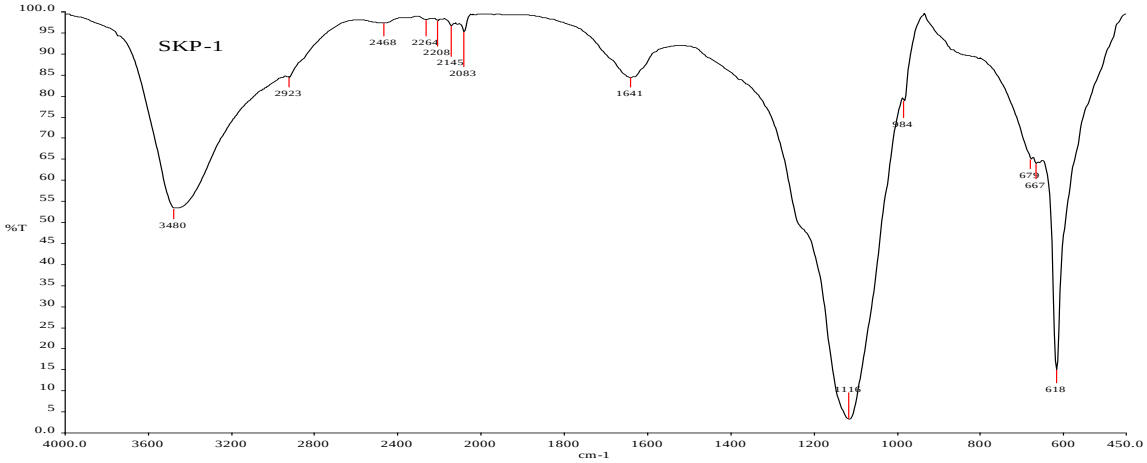

Figure 1: FT-IR Spectrum of Sample SKP 1.

FT-IR Spectrum of Sample SKP 2

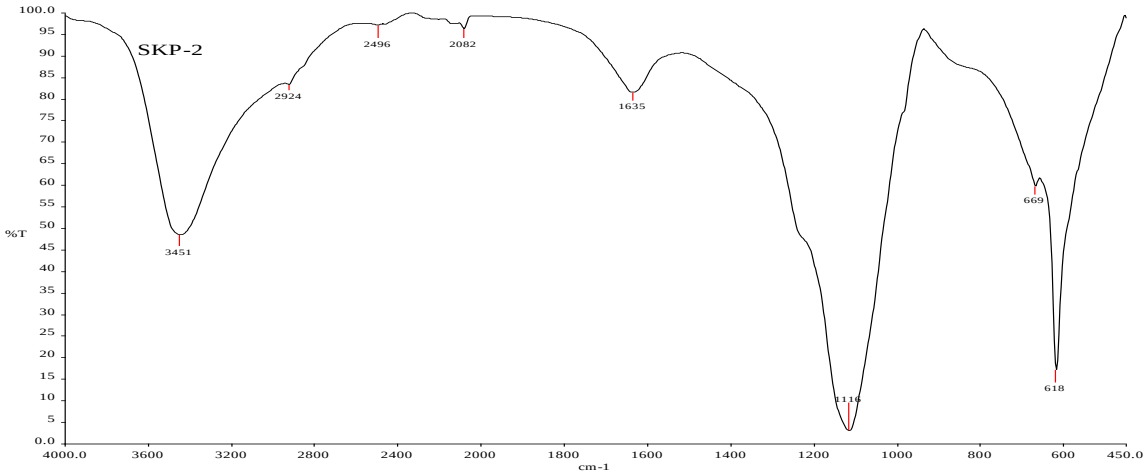

Figure 2: FT-IR Spectrum of Sample SKP 2.

FT-IR Spectrum of Sample SKP 3

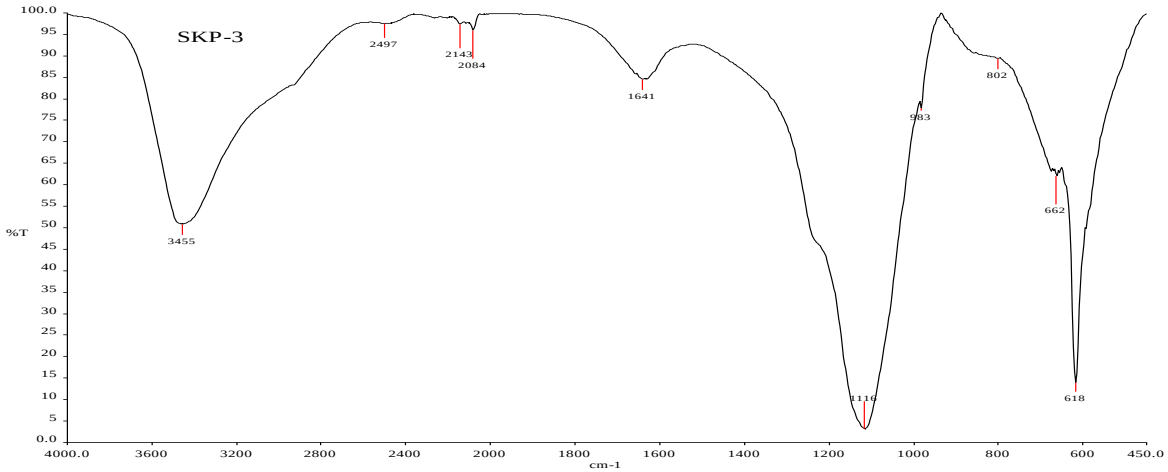

Figure 3: FT-IR Spectrum of Sample SKP 3.

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy

The results of SEM analysis of samples SKP1,2 and 3 illustrates that the sample SKP1 reveals the presence of nanoparticle in the size range of 42.1 to 92.4 nm, similarly the sample SKP2 with particle size distribution ranges from 82.3nm to 128.9 nm. Sample SKP3 has nano particle in the size range of 93.4nm to 184.7 nm. Overall observation clarifies that the lowest size of nano particle found in analysis is 42.1 and the highest will be 184.7 nm. The SEM analysis of all three samples SKP1, SKP2 and SKP 3were represented in Figure 4 to 6.

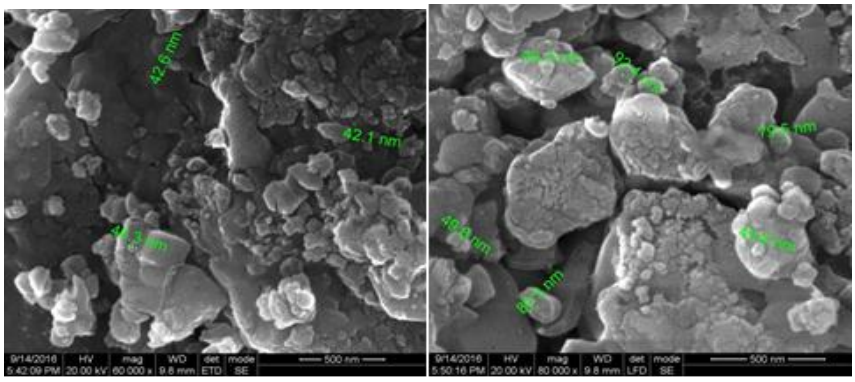

Figure 4: SEM images of Sample SKP 1.

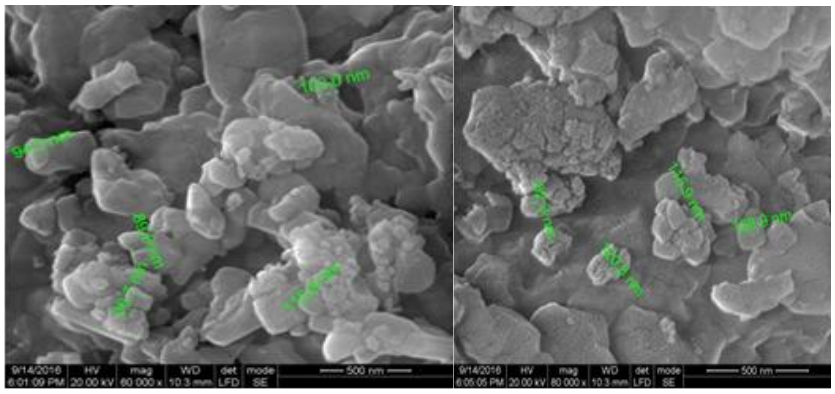

Figure 5: SEM images of Sample SKP 2.

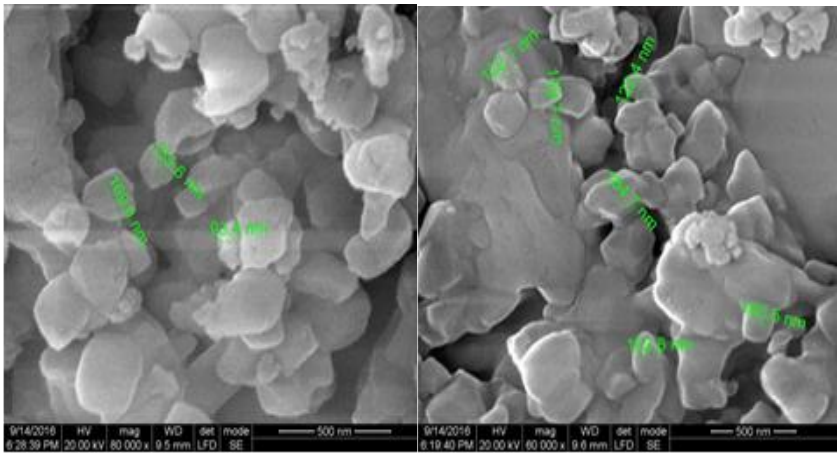

Figure 6: SEM images of Sample SKP 3.

Weight percentage of carbon present in the range of 26.65 to 36.08 % with respect to the outermost cell K shell excitation. Oxygen with percentage range of 27.03 to 50.03 and aluminium with 5.40 to 8.18%. Similarly sulphur with 10.44 to 13.33 % and potassium with 18.28 to 26.91 %. The EDX analysis of all three samples SKP1, SKP2 and SKP 3were represented in Table 2 and Figure 7 to 9.

Table 2: Atomic Weight Percentage of sample SKP in EDX analysis.

Elements	SKP 1 units in Wt %	SKP 2 units in Wt %	SKP 3 units in Wt %
CK	36.08	26.65	26.70
OK	27.03	30.03	27.67
AlK	8.17	6.31	5.40
SK	10.44	12.93	13.33
KK	18.28	24.08	26.91

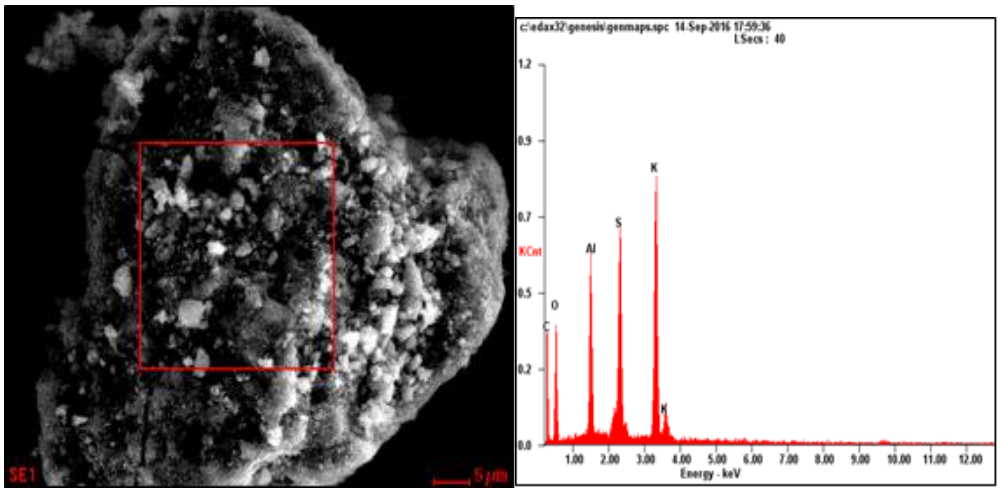

Figure 7: EDX images of Sample SKP 1.

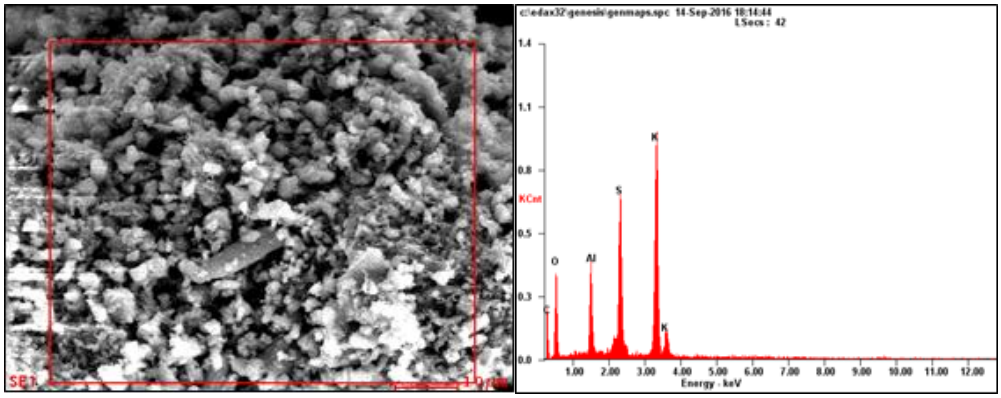

Figure 8: EDX images of Sample SKP 2.

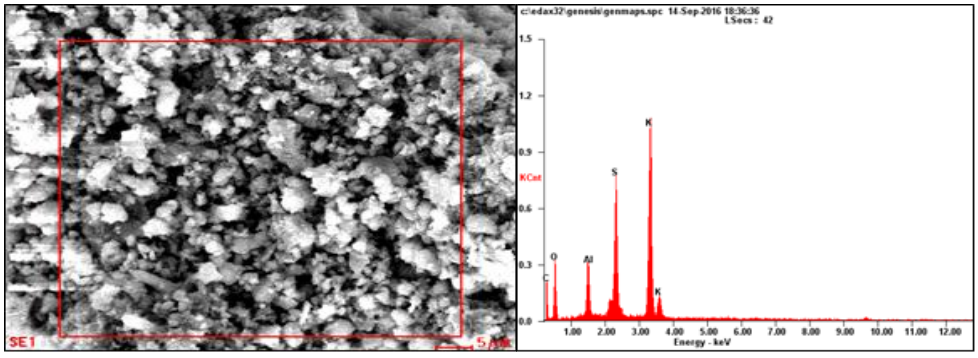

Figure 9: EDX images of Sample SKP 3.

XRD spectral Study

X-ray diffraction (XRD) patterns are obtaining for Nanoparticles with different precursor of aluminum, potassium nitrite and lime stone. All the diffraction peaks were found to be polycrystalline in nature of nano particle with planes (222), (101), (511), (440), (103), (200), (114), (211) and (116) respectively. The XDR peaks are indexed into diffraction pattern of one cubic and tetragonal structure, observed with JCPDS cord no. 01-1156 and 65-267. The XRD spectrum illustrated that

the diffraction peaks are slight shift to the higher direction was detected at different concentrations of precursor. Hence it indicates that the as-deposited thin films are well crystalline nature at different value of composite. From the favorite (103) reflection peaks are estimated full width at half maximum (FWHM) values of the nanoparticle. The XRD analysis report of all three samples SKP1, SKP2 and SKP 3were represented in Figure 10.

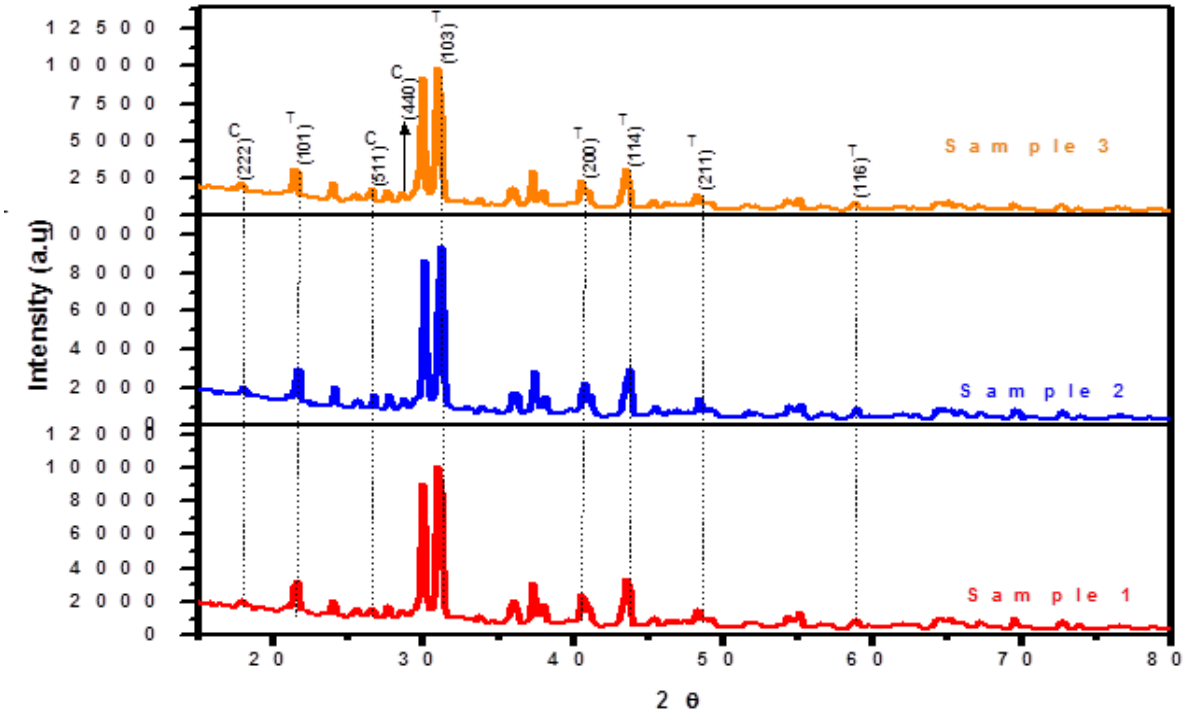

Figure 10: XRD Spectrum of Sample SKP 1, SKP 2 and SKP 3.

DISCUSSIONS

Atomic absorption spectroscopy Analysis

Prevalence of heavy metals in siddha preparations has been documented by several researchers. Heavy metal accumulation in biological system impairs the function of vital metabolic organs mainly kidney and liver. Other than these heavy metals are known for causing inflammatory disorders in brain, heart, liver and kidney. Recent evidence also suggests that heavy metal renders allergic and immunogenic response to the animals and humans. Polluted soil becomes the ultimate source of leaching heavy metals in to the raw materials and also to the finished product. Thus it becomes a part of the quality regulatory process that formulation should pass the limit test for heavy metals and proves the content of elements should be within the permissible limit. The results of heavy metal analysis by AAS technique reveals that the heavy metals such as lead and mercury present in the formulations are within the WHO prescribed limit.

Fourier Transform – Infra Red Spectroscopy analysis

FT-IR holds greater importance as it accounts for elaborating the detail on significance of functional group to the researchers. The presence of organic compound, inorganic and their core structural activity with respect to the functional group shall be identified with FT-IR spectral analysis. In the presence of electromagnetic radiation each electron undergo spin-spin coupling and it emerges in the form of stretching and bending vibrations. IR spectrum of all the three samples of SKP belongs to various batches showing sharp intense peak at 1116 cm^{-1} corresponds to C-N stretching. Similarly another sharp peak was observed in the region of 3480 – 3451 cm^{-1} shows the presence of usual O-H bending.

Peak emerging in the region of 618 cm^{-1} corresponds to C- H deformation and presence of less intense peak in the range of 679 - 662 cm^{-1} corresponds to N-H wagging Peaks emerging in the range of 1635- 1641 cm-1 shows the C=C variations. FT-IR results of all three samples of SKP having more or less closely similar results. From the data of the FT-IR analysis it was confirmed that all the three samples of SKP did not varying much with respect to its functional group.

SEM/EDX analysis

SEM analysis provided evidence based data on the structural morphology of the nano particle with in the formulation. Further the average particle size distribution of the drug renders information on the biological significance of the nano particles present with in the drug. The results of SEM analysis of samples SKP1,2 and 3 illustrates that the sample SKP1 reveals the presence of nanoparticle in the size range of 42.1 to 92.4 nm, similarly the sample SKP 2 with particle size distribution ranges from 82.3nm to 128.9 nm. Sample SKP3 has nano particle in the size range of 93.4nm to 184.7 nm. Overall observation clarifies that the lowest

size of nano particle found in analysis is 42.1 and the highest will be 184.7 nm. Recent days nano particles have rich biological application in the treatment of cancer, neuronal inflammation and metabolic disorder like diabetes.

Results of SEM analysis clearly reflects all three samples of SKP has uniform distribution of nano particles with average nano size range .The presence of smallest nano particle within the formulation could definitely attributes to the wider biological action against stipulated disease target.

EDX works behind the principle of electron collision. Each ion has a unique tendency of reflecting the high energy electrons. The characteristic spectrum obtained by the migration of electrons from the outer most shell and its valence score depicts the individuality of the EDX analysis. Weight percentage of carbon present in the range of 26.65 to 36.08 % with respect to the outermost cell K shell excitation. Oxygen with percentage range of 27.03 to 50.03 and aluminium with 5.40 to 8.18%. Similarly sulphur with 10.44 to 13.33 % and potassium with 18.28 to 26.91 % .From the result of EDX analysis it was observed that SKP 1, 2 and 3 has no much variations with respect to the atomic and weight percentage.

XRD spectral Analysis

X-ray diffraction analysis provides greater information on mineral content of the formulation, system generated signals on diffraction peak fraction corresponds to the concentration of the minerals correlated good justification for quantification of mineral content. X-ray diffraction (XRD) patterns are obtaining for Nanoparticles with different precursor of aluminum, potassium nitrite and lime stone. All the diffraction peaks were found to be polycrystalline in nature of nanoparicle with planes (222), (101), (511), (440), (103), (200), (114), (211) and (116) respectively.

The XDR peaks are indexed into diffraction pattern of one cubic and tetragonal structure, observed with JCPDS cord no. 01-1156 and 65-267. The XRD spectrum illustrated that the diffraction peaks are slight shift to the higher direction was detected at different concentrations of precursor. Hence it indicates that the as-deposited thin films are well crystalline nature at different value of composite.

From the favourite (103) reflection peaks are estimated full width at half maximum (FWHM) values of the nano particle. The XRD spectrum illustrated that the diffraction peaks are slight shift to the higher direction was detected at different concentrations of precursor. Hence it indicates that the as-deposited thin films are well crystalline nature at different value of composite.

CONCLUSION

The present investigations were carried out to standardise and characterize the siddha formulation Seenakara parpam. It shows that the

- Presence of biologically active functional groups like C-N, N-H and O-H renders promising efficacy to the drugs against urolithiasis.
- Further AAS analysis of sample shown that the presences of heavy metals are very low and below the prescribed limit.
- Uniform distribution of nano particles may enhance the bio-availability and therapeutic property of the formulation.
- Characteristic pattern of diffraction peaks observed at XRD analysis reveals the presence of polycrystalline nano particle with unique planes.
- Standardization and analytical evaluation studies shows that the formulation SKP is of high standard and has good stable nano particles with active functional groups and devoid of toxic trace metals. Hence forth the biological activity of the drug may also be due to the presence of bio-active nano particle and other significant functional group present within the formulation.

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