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Sophisticated Instrumental Evaluation of Novel Siddha Raw Drug -*Manosilai*: An Modern Approach towards Drug Standardization

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Abstract

In Siddha system of traditional medicine minerals and animal products are used as main drugs to treat various dreadful diseases. Standardization of Siddha preparations is of utmost important task in establishing the active components of the drug responsible for its biological activity. WHO has emphasized the need to ensure quality control of Indian Medicines including Siddha formulations by using modern techniques and by applying suitable parameters and standards. Siddha practitioners use several metallic preparations to treat diseases such as cancer, urolithiasis, kidney disorders and chronic liver diseases. One such novel drug is Manosilai comprises of arsenic and Sulphur as major components. The main aim of the present study is to purify the Manosilai and to analyses the same with modern sophisticated analytical instrumentation techniques. ICPOES results of the sample manosilai before and after purification reveals the presence of Mercury, Lead, Arsenic and Cadmium along with other trace elements. FT-IR analysis report of both unpurified and purified formulation confirm the presence of biologically significant functional group with characteristic IR absorption frequencies. Further the XRD patter of samples justifies the presence of arsenic sulfide and mercury being the major component of the manosilai. SEM analysis of the sample signifies that the mean particle size of the formulation ranges from 11.21 to 19.37 µm. Hence from the results of the present investigation it was clear that the manosilai purified in accordance with traditional siddha system confirms the stability and complies with the genuinity and standards as per AYUSH guidelines.

Keywords: Siddha system, Manosilai, Standardization, ICPOES, FT-IR, XRD, SEM, AYUSH guidelines.

1. Introduction

WHO has emphasized the need to ensure quality control of Indian Medicines including Siddha formulations by using modern techniques and by applying suitable parameters and standards [1]. It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication, with purity, safety, potency, and efficacy. As prescribed by the WHO, evaluations of physicochemical and phytochemical properties are essential to standardize the various Siddha formulations.

The continuing search for new drugs has seen researchers looking to the natural world for potential products. WHO estimates that about 80% of the population of developing countries relies on herbal medicines (HM) for their primary health care. They have been an important source of precursors and products used in a variety of industries, such as pharmaceuticals, food, cosmetics and agrochemicals. Traditional medicines (TM) are enjoying an upsurge in popularity because of their perceived low toxicity. Additional species are being gradually added to the MateriaMedica and the standards for purity and identification do not always keep pace with this expansion [2].

In ASU systems plants, minerals, and animal products are used as main drugs to cure various ailments [3]. Herbal medicine also called botanical medicine or phytomedicine refers to the use of plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease [4].

Through systematic standardization a formulator can profile the preparation with respect to the following (i) Physicochemical parameters (ii) Category of phytocomponents (iii) Nature of individual chemical component (iv) Structural and functional group analysis (v) Correlation of mechanism with respect to the functional group present in bioactive phytocomponents (vi) Drug stability (vii) Pharmacokinetic profiling (viii) Receptors on which the drug acts (ix) Chances of possible interaction. Because of the emerging knowledge in the field of drug standardization. Now siddha preparation which satisfy the quality and standard are being exported and it is in practice by other countries like Srilanka, USA and Indonesia.

In siddha system, Metallic preparations are used to cure many challenging diseases. Before preparation of medicine each drug must be purified to remove the impurities. The purification of metallic drug used in Siddha constitutes a step considered as crucial to ensure the quality and safety of medicines Advancement of sophisticated instrument method of analysis offers varying degree of improving the usage and exploration of Siddha preparations among the people of lower economic zone. Medicinal plants and other herbs collected from the cultivators often have chance of heavy metal traces like arsenic, mercury, lead and chromium in the preparation if not be properly purified. Upon continuous administration of such heavy metal containing preparations leads to lethal effects. Hence recent technology like AAS, ICPMS has advantage of detecting heavy metals even with PPM level. The use of modern analytical techniques is a must to characterize the drug, its interaction with specific body tissues/organs and to provide a molecular basis for the curative aspects. In the task of bringing into the mainstream, the unutilized potential of the ancient science of healing, a close interaction of practitioners of traditional Indian Systems of Medicine (ISM).Standardization of Siddha formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. Chemical and instrumental analyses are routinely used for analyzing synthetic drugs to confirm its authenticity. In the case of herbal drugs, however the scenario is different especially for metallic preparations like Manosilai. The main aim of the present study is to purify the Manosilai and to standardize the same with modern sophisticated analytical instrumentation techniques. Hence this documentary evidence will be made available as monograph for the benefit of the future researchers.

2. Materials and Methods

2.1. Source of raw drugs:

Manosilai was purchased from a well reputed indigenous drug shop at Chennai. Goat's urine was collected from Anverthikanpet village, Vellure District, Tamilnadu, India. Ulunthu (PhaseolusmungoLinn) was purchased from country grocery shop at Tambaram, Chennai, Tamilnadu, India. All raw drugs were authenticated Prof.P.Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India.

2.1. Purification of raw drugs [5]

Raw drug was purified as mentioned in *Agathiyar Vaithiya Kaviyam.*

2.3. Purification of *Manosilai*

Manosilai was made into small pieces and make in to a bundle, the above bundle was boiled with goat's urine by using thula appliances and then the bundle was removed and kept in black gram boiled water followed by this bundle was opened and dried.

2.4. Analysis of Heavy metals by ICP-OES [6]

Accurately weighed quantity of about 25 mg of the sample was taken in in the Teflon container. To this 6 ml of concentrated HNO3 and 3 ml of concentrated HCL was added and the contents were allowed to react for approximately 5 minutes prior to sealing the material the sample was thoroughly filtered and the difference in weight was calculated. The sample was preferably stored in plastic container to prevent loss of elements by absorption and quantitatively determined by PE optima 5200 DV ICPOES vessels. Followed by the microwave digestion. The vessels were then heated to the required temperature. After digestion cooled and made upto a known volume in a standard flask with deionized water.

2.5. Fourier Transform – Infra Red Spectroscopy Study [7]

Fourier Transform – Infra Red Spectroscopy Study (FTIR) IR data acquired with FT-IR spectrometer FT/IR-4100 –Jascoasia portal. About 20 mg of the test sample 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.

2.6. XRD spectral Study [8]

The XRD spectrum of test sample were analyzed using Bruker discover D8 X ray diffractometer. Cu K Alpha radiation was used for recording the spectra. The range of diffraction angle 10-70^{o}operatinf at 30kV and 20 mA. The pattern was recorder from the angle 5 to 80 degree at a scanning rate of 3 degree/second. The XRD studies were carried out at IIT madras, Chennai, Tamil Nadu, India.

2.7. SEM Analysis

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross sections. The test sample powder was sputter coated with gold and viewed under SEM (FEI Quanta 200 FEG, Berlin, Germany) to determine the morphology at ×100,000 magnification and the particle size at ×200,000 magnification.

3. Results

3.1. Results of Heavy Metals Analysis by ICP-OES

ICP-OES estimation of the unpurified and purified Manosiali reveals the presence of heavy metals such as arsenic, lead, mercury, cadmium along with several other trace elements. The list of heavy metals and trace elements and its proportions were listed in table 1.

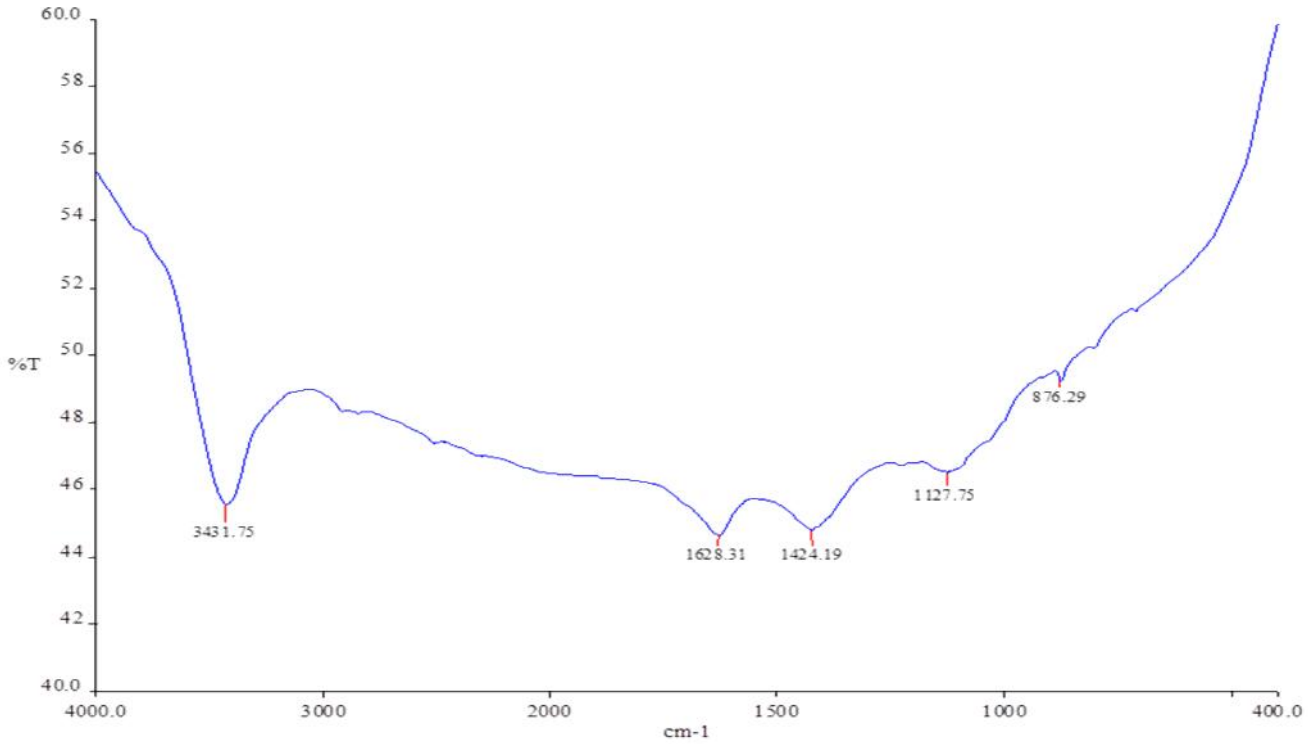
Table 1: Quantitative analysis by ICP-OES for both unpurified and purifiedraw drug Manosilai

S.No	Elements	Wave Length in nm	ManosilaiBefore Purification In mg\L(PPM)	Manosilai After Purification In mg\L(PPM)
1	Arsenic	As 188.979	9207	7279
2	Calcium	Ca 315.807	188.7	157.5
3	Cadmium	Cd 228.802	122.8	100.8
4	Copper	Cu 327.393	0.9610	0.9430
5	Mercury	Hg 253.652	4.708	2.7404
6	Magnesium	Mg 285.213	3.208	2.862
7	Sodium	Na 589.592	4.762	3.172
8	Nickel	Ni 231.604	0.014	0.002
9	Lead	Pb 220.353	0.614	0.763
10	Phosphorus	P 213.617	0.276	0.314
11	Sulphur	S 180.731	264.8	182.8
12	Potassium	K 766.490	2.200	0.48
13	Cobalt	C0228.616	0.042	0.014
14	Iron	Fe 238.204	13.62	11.87
15	Selenium	Sc 196.026	0.622	7.699
16	Chromium	Cr 267.716	0.005	0.016

3.2. Results of FT-IR analysis of Manosilai before and after Purification

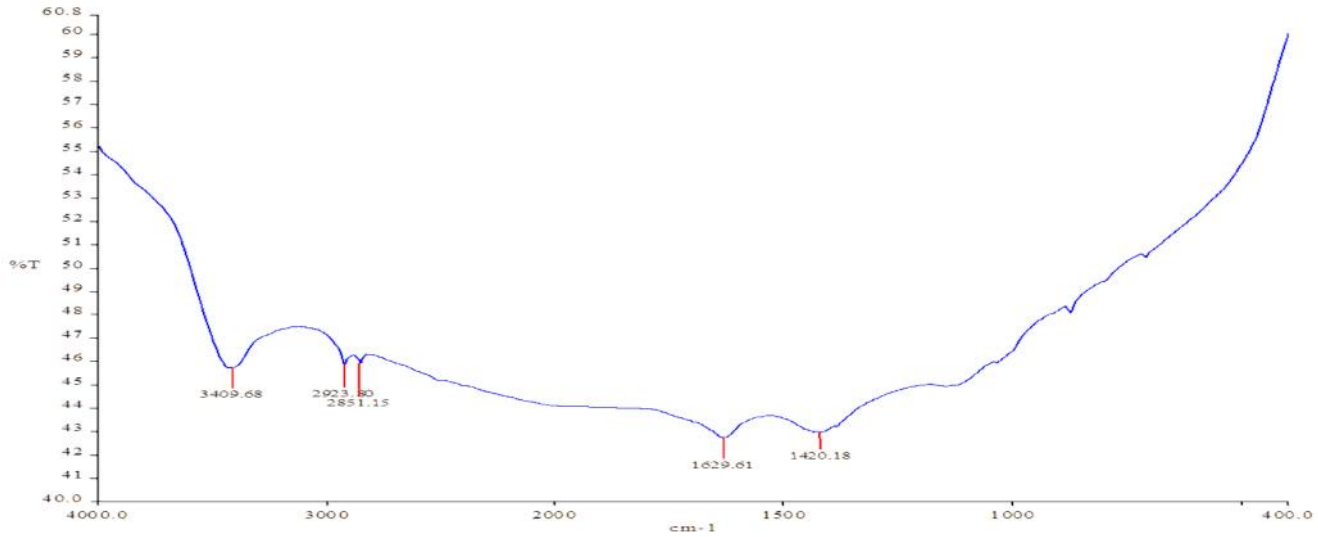
The FT-IR absorption spectrum of the sample Mansolai before purification reveals that the IR absorption peak at 1127.75cm-1 may be due to the presence of =S. Wide predominant peak at 3431.75cm-1 due to free O-H vibration. Medium intensity peak at 1424.19 cm-1 and 876.29 due to presence of S=O functional group stretching. IR absorption peak at 1628.31 cm-1 due to NH2 scissoring. Absorption peak at 1127cm-1 due to presence of C-O stretching. As represented in figure 1.

Figure 1: FT-IR absorption frequencies of Organic Functional Groups raw drug Manosilai before Purification



The FT-IR absorption spectrum of the sample Mansolai after purification reveals that the IR absorption peak at 1420.18 cm-1 due to presence of S=O functional group stretching. Vibrational peak at 3409.68 cm-1 maybe due to the presence of primary amine. Sharp absorption peak at 2851 cm-1 due to presence of CH stretching. Wide intense peak at 1629.61 cm-1 may be due to NH2 scissoring .IR absorbance peak at 2923.80 cm-1 due to O-H overlapping. As represented in figure 2.

Figure 2: FT-IR absorption frequencies of Organic Functional Groups raw drug Manosilai after Purification



3.3. Results of XRD analysis of Manosilai before and after Purification

The X-ray diffraction pattern of the of the prepared formulation AsS (Before purification) reveals the presence of major peak with 2- Theta value of 28.12 which exactly matches to the ICDD (International Centre for Diffraction Data) 71- 2434. ICDD 71- 2434 corresponds to the crystalline pattern of Arsenic Sulfide (AsS). Hence the reference matching material was conformed as Arsenic Sulfide (AsS)

Major peaks observed in Test SampleAsS (Before) with 2-theta values of 28.12 and their corresponding intensities were 1370.The major peak observed in the reference matching material was 28.10 with the intensity value of 415. The XRD pattern of the test sample AsS (Before purification) exactly matches with the reference material AsS, which justifies the presence of stable and purified AsS in the formulation. From the result of the present XRD analysis it was concluded that the elemental composition of sample AsS (Before) confirms the presence of AsS at its stable state. Further Mercury being the major component of the sample AsS (Before purification).

The X-ray diffraction pattern of the material present in the prepared formulation was 28.10 with the intensity value of 415. The XRD pattern of the test sample AsS (After purification) exactly matches with the reference material AsS, which justifies the presence of stable and purified AsS in the formulation. From the result of the present XRD analysis it was concluded that the elemental composition of sample AsS (After purification) confirms the presence of AsS at its stable state. Further Mercury being the major component of the sample AsS (After purification)

Figure 3: Diffractogram showing peaks of crystalline phase of Manosilai before Purification

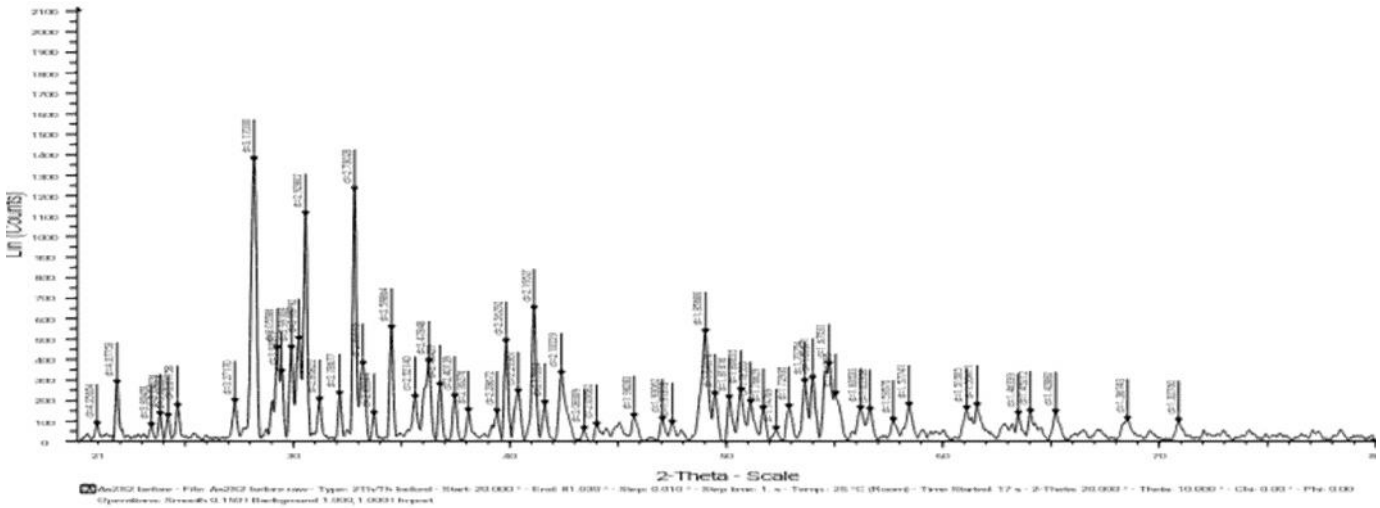


Figure 4: Diffractogram showing peaks of crystalline phase of Manosilai after Purification

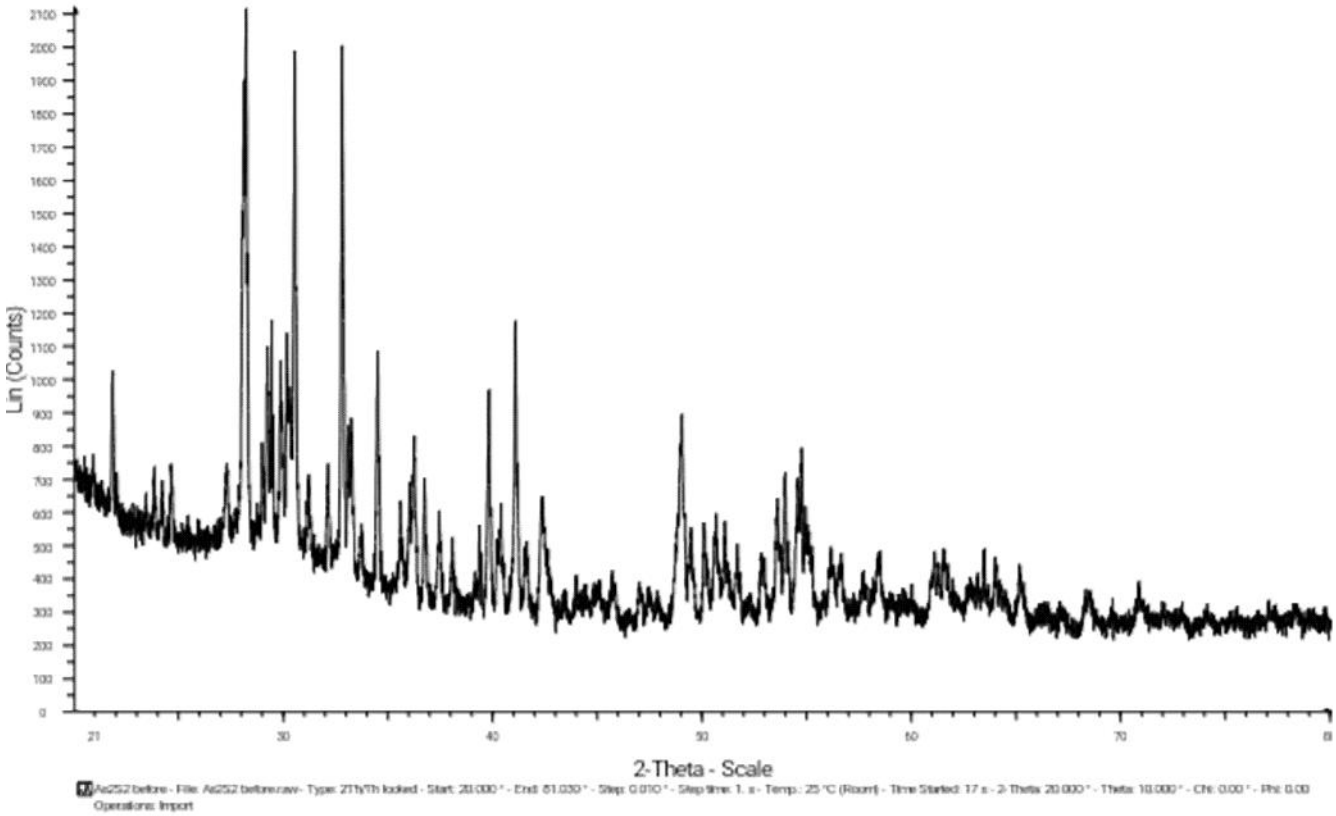


Figure 5: XRD pattern of Test Sample AsS before Purification

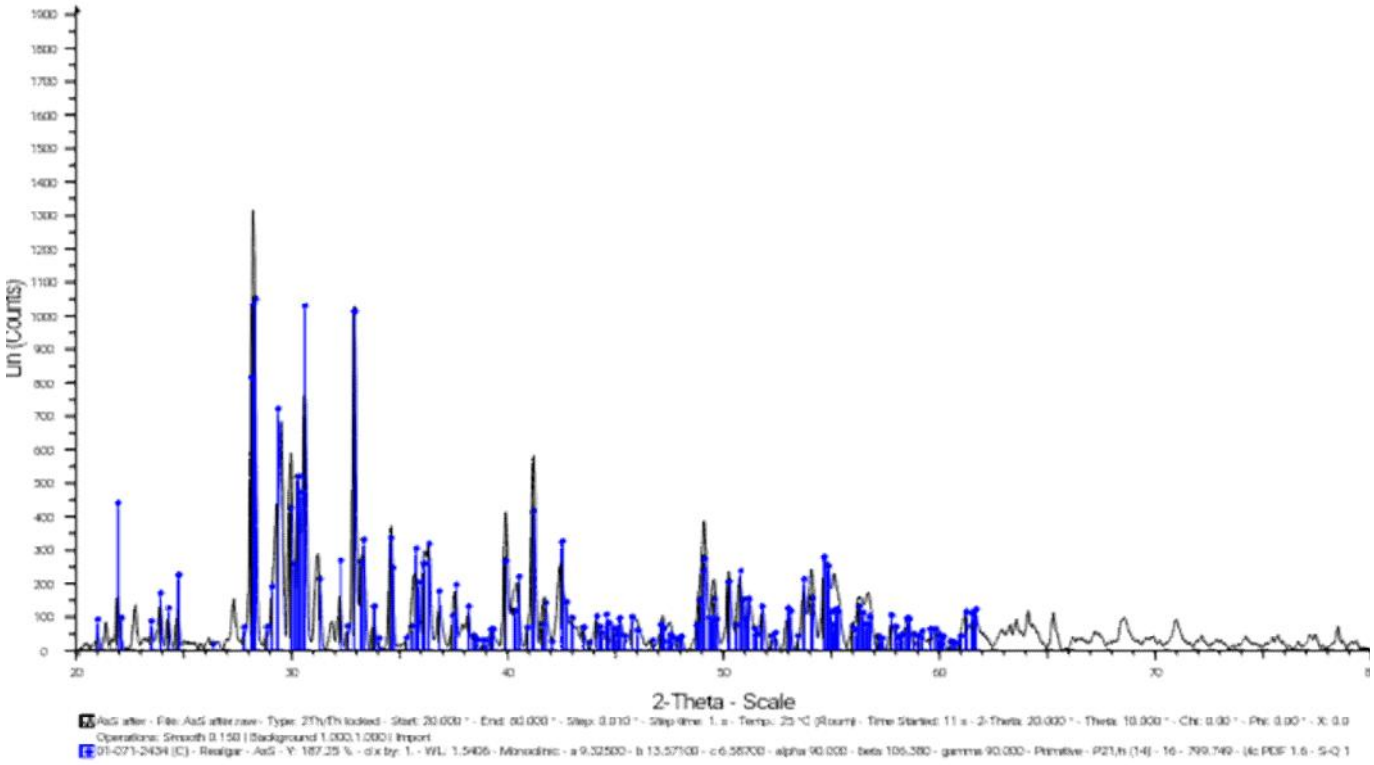
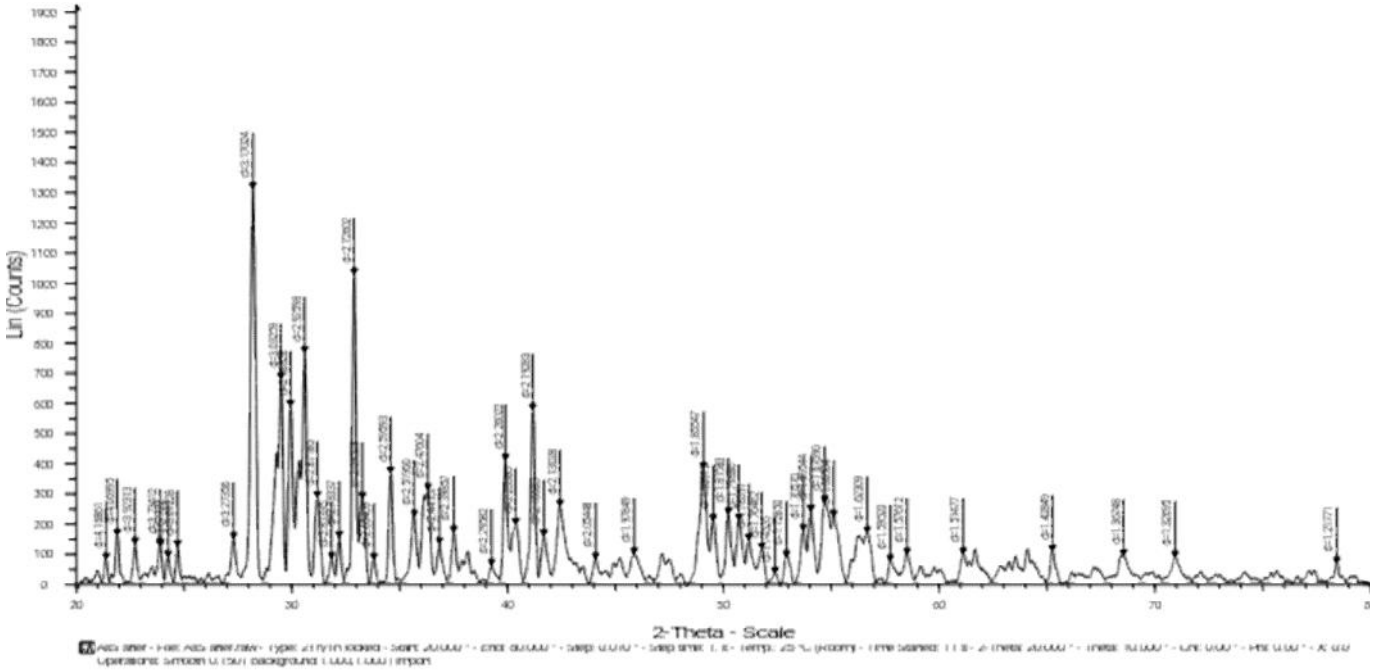


Figure 6: XRD pattern of Test Sample AsS after Purification



3.4. Results of SEM analysis of Manosilai before and after Purification

SEM analysis of the sample before purification reveals the present of clustered and isolated particle with the average size range of 19.37 ± 7.24 µm. Similarly, the average particle size of the purified sample shows the presence of particle with the average size range of 11.21 ± 4.30 µm.

Figure 6: SEM image of Manosilai before Purification

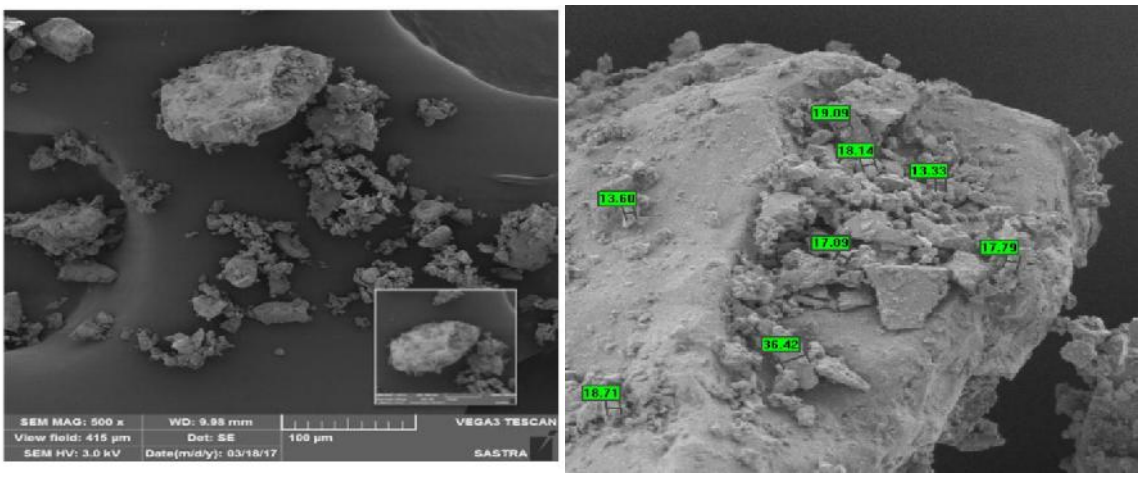
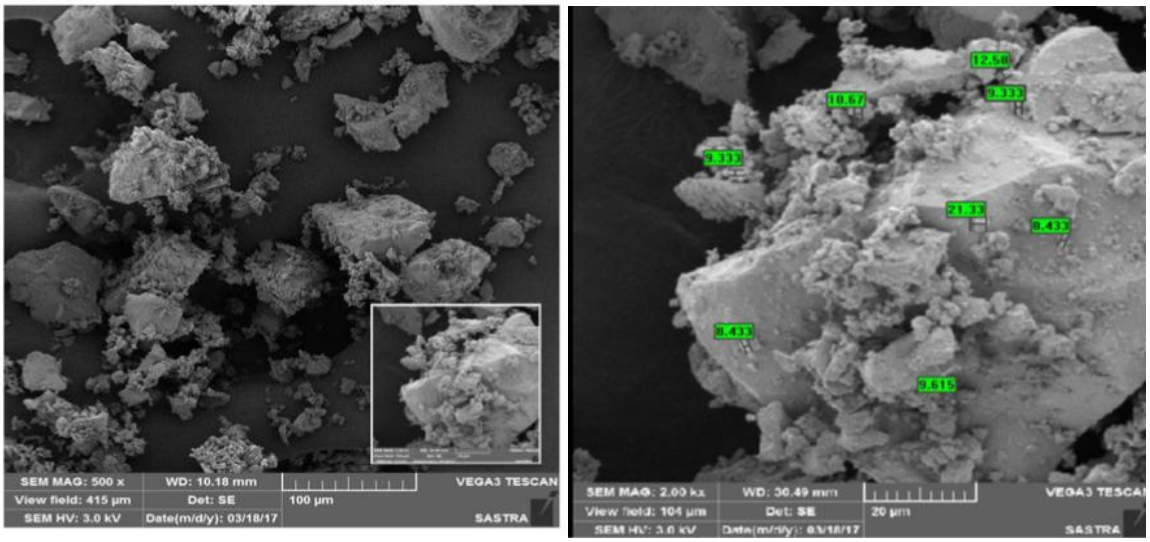


Figure 7: SEM image of Manosilai after Purification



4. Discussion

Standardization encourages marketing opportunism for siddha formulation [10]. It enables drug companies to claim exclusive patents for processed herbs. Standardization of herbal formulation in terms of raw materials, manufacturing practices and composition is important to ensure quality and optimum level of active principles for their bio potency. Identification of major and unique compounds as markers and development of analytical methodologies for monitoring them are the key steps involved in marker based standardization [11].

The usage of arsenicals in Indian system of medicine has a very long history of treating various diseases including gonorrhea, epilepsy, syphilis, asthma, psoriasis, chronic fever, cancer, tuberculosis and other respiratory diseases [12]. Metallic preparations offered many advantages over plant by virtue of their stability over a long period, lower doses, easy storability and sustained availability.

XRD analysis is one of the important technique by which compounds of material and free metals etc can be detected. So, in this scientific era it is very essential to determine changes in the material during the process of purification (before and after). By this, one can say authentically the transformation of material in to a compound or orally administrable form [13]. The data's obtained from the XRD analysis of the sample before and after purification confirms the presence of Arsenic Sulfide (AsS) and Mercury (Hg) and it was further justified with the diffraction pattern of reference matching material.

Siddha system of medicine is always peculiar due to the use of metals and minerals in their preparations. The metal/ mineral drugs are treated with herbs which are given as parpam and chenduram. Through process of purification and processing, the metals are becoming biocompatible. In the herbo-mineral preparations, the metals are transforming into very potent drugs [14,15].

Fourier transform infrared spectroscopy is a physicochemical analytical technique which provides a clear picture of the metabolic composition of leaves at a given time [16]. It is possible to detect the minor changes in the primary and secondary metabolites in leaves by observing the IR spectra. FTIR is employed to elucidate the structure of unknown composition and the intensity of absorption spectra associated with molecular composition or content of respective chemical functional groups [17]. In the present investigation the FT-IT analysis of the sample before and after purification reveals the presence of S=O, C-O, NH2 and O-H functional group.

SEMs have a variety of applications in a number of scientific and pharmaceuticals related fields, especially for characterizations of metallic preparations it is highly beneficial. SEMs can be as essential research tool in fields such as life science, biology, gemology, medical and forensic science, metallurgy. Advantages of a Scanning Electron Microscope include its wide-array of applications, the detailed three-dimensional and topographical imaging and the versatile information with respect to particle size and topography garnered from different detectors. SEM also provides useful information such as crystalline structure, chemical composition and morphology of the specimen under investigation [18].

Scanning Electron Microscopy (SEM) is a powerful method for the investigation of surface structures of mollicutes. This technique provides a large depth of field, which means, the area of the sample that can be viewed in focus at the same time is actually quite large [19]. SEM has also the advantage that the range of magnification is relatively wide allowing the investigator to easily focus in on an area of interest on a specimen that was initially scanned at a lower magnification. Furthermore, the three-dimensional appearing images may be more appealing to the human eye than the two-dimensional images obtained with a transmission electron microscope [20]. Therefore, an investigator may find it easier to interpret SEM images. Finally, the number of steps involved for preparing specimens for SEM investigation is lower and thus the entire process is less time consuming than the preparation of samples for investigation with a transmission electron microscope. In the present investigation SEM analysis of the sample before purification reveals the present of clustered and isolated particle with the average size range of 19.37 ± 7.24 µm. Similarly, the average particle size of the purified sample shows the presence of particle with the average size range of 11.21 ± 4.30 µm.

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 herbal medicines (THM). The consumption of THM contributes to the intake of both essential and non-essential trace elements by the human body [21]. ICPOES results of the sample manosilai before and after purification reveals the presence of Mercury, Lead, Arsenic and Cadmium along with other trace elements.

5. Conclusion

In conclusion the present research work had generated an evidence based data with respect to the nature of metals present in the formulation manosilai and also the X-Ray diffraction pattern of the metals with respect to the standard reference materials, which confirm the genuinity and stability of the formulation. ICP-OES analysis confirms the presence of heavy metals along with trace elements at varying proportion before and after purification. Further the level of elements presents in the formulation had reduced significantly after purification.

FT-IT analysis of the sample before and after purification reveals the presence of S=O, C-O, NH2 and O-H functional group. SEM analysis of the sample before purification reveals the presence of clustered and isolated particle with the average size range of 19.37 ± 7.24 µm. Similarly, the average particle size of the purified sample shows the presence of particle with the average size range of 11.21 ± 4.30 µm. Hence it was concluded that the present research work purification process of manosilai confirms the stability and genuinity as prescribed in the siddha formularies.

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