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Comparative Instrumental Analysis of Siddha Raw Drug – *Manosilai* by two different Purification Techniques: A Novel approach on Procedure Optimization and Drug Standardization.

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Abstract

Purification is called by suddhi in the siddha system of traditional medicine. Each purification process has its own advantages and applications. Siddhars are an ancient pioneer of siddha medicine has clearly defined standard operating procedure for each purification process. This method renders enormous benefits to the formulations such as detoxification, minimizing adverse events, decreasing salts contents and synergizing the efficacy of the preparation. In the present investigation the drug manosilai comprises of arsenic and sulphur as major components subjected for purification by two different traditional methods using goat urine (MSGU) and butter milk (MSBM) followed by this the samples were analyzed by modern sophisticated analysis to conclude the change in nature of the manosilai subjected to two different purification methods. Results of the study shown that sample purified in butter milk appears brighter that the goat urine and the nature of sample remain same in both the cases. Physicochemical data's reveal that there is no significant difference in water soluble extractive values of sample subjected to both method of purification where as it was observed that there is mild decrease in alcohol soluble extractive value and loss on drying value in MSGU, similarly increase in total ash value observed in MSGU. FT-IR results shown the presence of functional group such as C-Br,N-N=O, –CO-NH and CHO are certainly unique for the sample MSBM which is not appeared in MSGU. Considering the results of SEM analysis of both the samples with respect to the morphology and size range it was observed that there is no significant difference in the size range of microparticles present in both the samples. Micro particles present in the sample MSBM is much categorized and segregated when compare to that of MSGU. XRD analysis report suggests that the basic elemental composition remains unaltered by both the purification process. It was concluded from the result of the investigation that out of two purification methods the sample purified by butter milk possess increased functional groups when compare to goat urine method. Further clinical data's has to be generated before arriving at the conclusion in clinical level with respect to the efficacy of the sample is concern.

Keywords: Purification, Siddha system, Manosilai, Physicochemical, FT-IR, XRD, SEM.

1. Introduction

Implication of standard protocols in the formulation and evaluation of siddha drugs are high difficult because of its complexity in nature and composition. Whereas there is a dire need of the proper universal analytical and formulation techniques which are mandate by the global regulatory requirement for exports and popularization of siddha drugs. Siddha system has well organized procedures for purification of raw as well as finished drugs even several centuries before. This novelty made siddha system of traditional medicine to become very unique among other traditional practices.

Regulatory authorities in both the producing and importing countries of traditional medicine becomes the matter of serious concern to come up with the efficacious and standard quality product [1]. 'Quality' can be taken as the denominator of any product for its acceptance [2]. Control refers to the function concerned with ensuring the production, right from the raw materials to the finished product, to accomplish the set standards.

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 [3]. Nevertheless, recently the inorganic arsenic is accepted as the first line chemotherapeutic agent against certain hematopoietic cancers in the allopathic medicine [4]. The issues related to public concerns should be settled down scientifically by pinpointing its physicochemical properties and its biological interactions [5].

Modern analytical techniques includes FTIR, SEM, XRD provides quick and justifiable evidence on nature and functional components of both herbal and herbomineral preparations. Fourier transform infrared (FTIR) spectroscopy is a reliable technique that allows rapid acquisition of a biochemical fingerprint of the sample under investigation, giving information on its main biomolecule content. Indeed, this spectroscopic tool is successfully applied not only to the characterization of the structural properties of isolated biomolecules, such as proteins, lipids, nucleic acids, and carbohydrates [6-11]. SEM is a microscopic technique which renders the information on particles size distribution, Surface morphology of the material including shape and arrangement. XRD provides evidence based data on character and nature of materials present in the test sample by comparing the diffraction pattern with that of the standard reference material. The aim of the present investigation is to examine the quality and standard of the siddha drug manosilai prepared by two different purification techniques and to provide documentary evidence on optimization of standard procedure for purification of the drug manosilai.

2. Materials and Methods

2.1. Procurement of Manosilai

Manosilai was purchased from a well reputed indigenous drug shop at Chennai. Goat's urine and butter milk was collected from anverthikanpet village, Vellure District, Tamil Nadu, India. All raw drugs were authenticated by the concerned authority prior to the formulation.

2.2. Purification of *Manosilai* using Butter milk

Manosilai was made into small pieces and kept soaked in to 175gms of fermented butter milk in a clay vessel. Followed by insulation and kindling frequently. Soon after incubation time the sample collected and washed until extracted in purified from.

2.3. Purification of *Manosilai* using Goat urine

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 [12].

2.4. Physicochemical Evaluation [13-14]

2.4.1. Percentage Loss on Drying

10gm of test drug was accurately weighed in evaporating dish .The sample was dried at 105^{o}C for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100

2.4.2. Determination of Total Ash

3 g of test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.4.3. Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Acid insoluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

Water Soluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.4.4. Determination of Alcohol Soluble Extractive

About 5 g of test sample will be macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Alcohol sol extract = Weight of Extract/ Wt of the Sample taken X 100

2.4.5. Determination of Water Soluble Extractive

About 5 g of the test sample will be macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.
Water soluble extract = Weight of Extract/ Wt of the Sample taken X 100

2.5. Fourier Transform – Infra Red Spectroscopy Study [15]

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. 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

2.7. XRD spectral Study [16]

The XRD spectrum of test sample was 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} 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.

3. Results

3.1. Results of nature and appearance of MSBM and MSGU

Organoleptic characteristic feature of MSBM reveals the presence of mild characteristic odor with very fine flow property and with yellowish orange in color. Whereas the color of MSGU appears light orange colored fine powder. The results were tabulated in Table 01.

Table 1: Organoleptic characteristic feature of MSBM and MSGU

Parameters	MSBM	MSGU
State	Solid	Solid
Appearance	Yellowish Orange	Light Orange
Nature	Very fine	Very fine
Odor	Mild	Very mild

3.2. Results of physiochemical analysis of MSBM and MSGU

The results obtained from the physicochemical evaluation of MSBM and MSGU revels that the loss on drying value of MSBM and MSGU was found to be 0.053 and 0.29 %w/w. The total ash value of MSBM and GU was 0.12 and 3.3% w/w. In which the acid insoluble ash value of MSBM (0.08 % w/w), MSGU (0 % w/w). The results of water soluble extractive of MSBM were 0.92 w/w and for MSGU it was 0.93 %w/w. Similarly the alcohol soluble extractive value of MSBM and GU was found to be 2.3 % and 0.55 %w/w. The results were tabulated in Table 02.

Table 2: Physicochemical Analytical data's of MSBM and MSGU

S.No	Parameter	MSBM	MSGU
1.	**Loss on Drying at 105 °C (%)**	0.053	0.29
2.	**Total Ash (%)**	0.127	3.3
3.	**Acid insoluble Ash (%)**	0.08	0.0
4.	**Alcohol Soluble Extractive (%)**	2.3	0.5
5.	**Water soluble Extractive (%)**	0.92	0.93

3.3.Results of FT-IR spectral analysis of MSBM and MSGU

The FT-IR absorption spectrum of the sample MSBM reveals the presence of strong intense peak at 3432 cm-1 may be due to –CO-NH group. Medium peaks at 2924 cm-1 may be due to presence of hydrogen bond on –OH group. Sharp intense peak at 2860, 1138, 603 cm-1is due to the presence of free CHO group. Absorption Peak at 559 cm-1 due to presence of C-Br. Strong intense peak at 747 cm-1 due to presence of hydrogen at benzyl group and peak at 895cm-1 due to presence of alkenes. Peaks at 2285 cm-1 due to presence of amino group and peak at 1448 cm-1 due to N-N=O group. As shown in spectrum Figure 1

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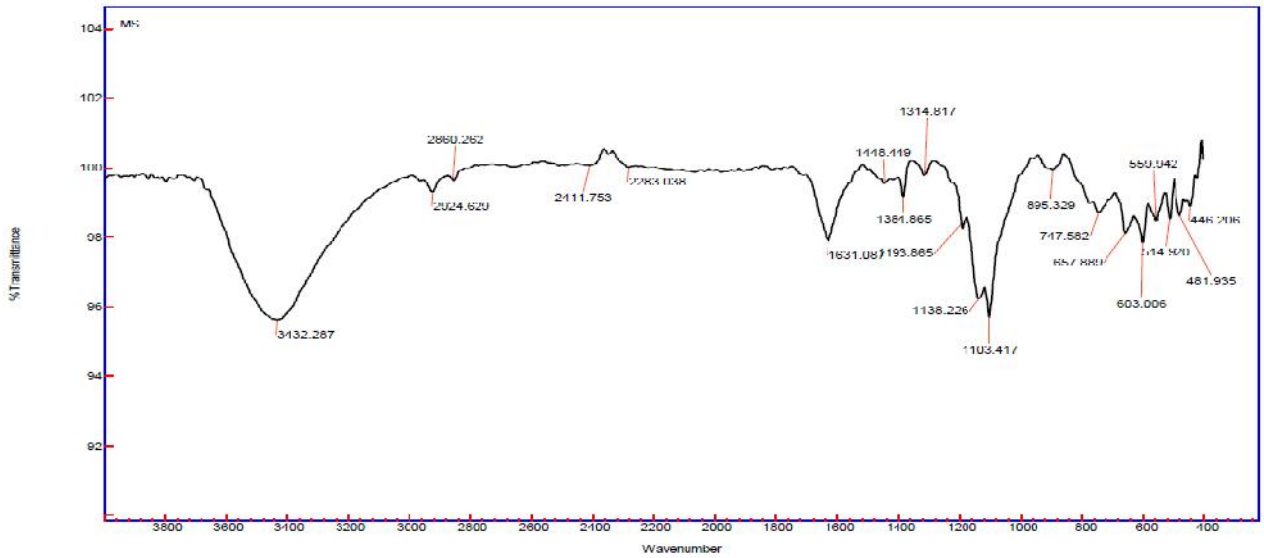

Figure 1: FT-IR absorption frequencies of Organic Functional Groups in MSBM

The FT-IR absorption spectrum of the sample MSGU reveals that the IR absorption peak at 1420 cm-1 may be due to the presence of S=O. Wide predominant peak at 3409 cm-1 due to presence of primary amine Sharp absorption peak 2851 cm-1 due to C-H and wide intense peak at 1629cm-1 due to presence of NH2 functional group. IR absorption peak at 2923 cm-1 due to O-H overlapping. As shown in spectrum Figure 2.

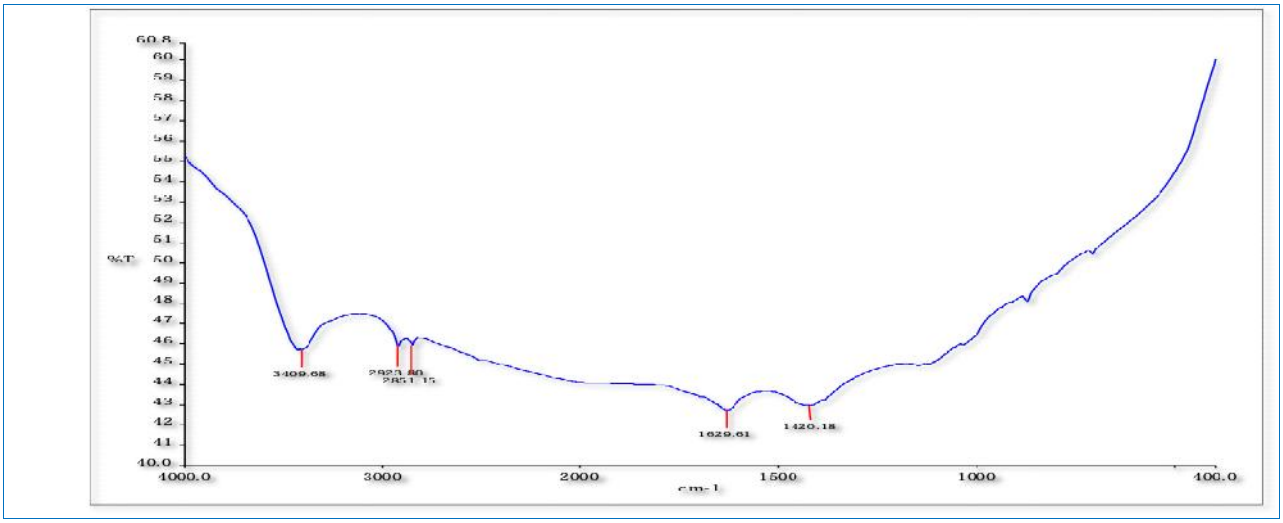

Figure 2: FT-IR absorption frequencies of Organic Functional Groups in MSGU

FT-IR spectral data's of the samples signifies the nature and presence of active functional group in both the samples. Presence of hydroxyl group appears common in both the sample and this group seems mandate for most of the biological activity of the manosilai.

3.4. Results of SEM analysis of MSBM and MSGU

The morphology of the MSBM sample subjected to SEM analysis reveals the presence of numerous micro particles with the size range of 16.06 µm to 30.70 µm. Similarly the morphology of the MSGU sample subjected to SEM analysis reveals the presence of micro particle with the size range of 8.4 µm to 21.3 µm. As shown in Figure 3 and 4.

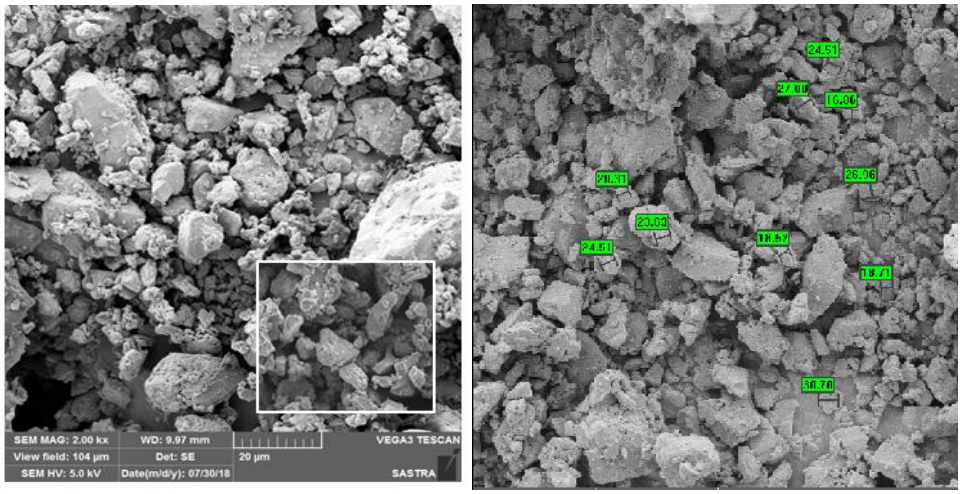

Figure 3: SEM image of MSBM on clustered and categorized view

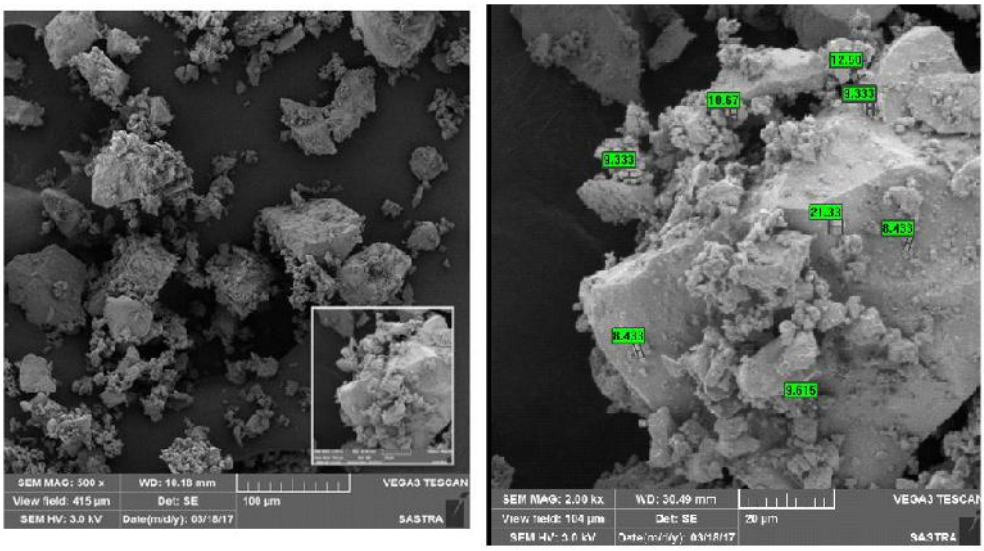

Figure 4: SEM image of MSGU on clustered and categorized view

3.5. XRD interpretation of MSBM and MSGU

The X-ray diffraction pattern of the prepared sample MSBM reveals the presence of major peak with 2-Theta value of 35.57 with the relative intensity of 29.9% corresponds to Arsenic. Major peak observed in test sample with 2-theta values of 29.20 and their corresponding intensities 34.4% matching with the material sulphur. As shown in Figure 5.

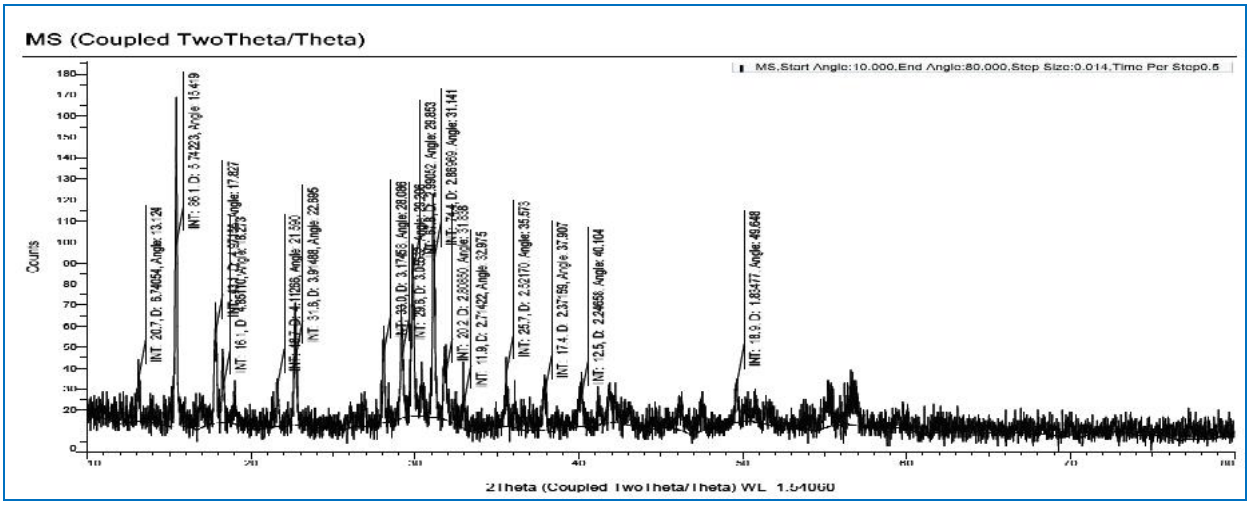

Figure 5: XRD pattern of Sample MSBM

The X-ray diffraction pattern of the of the prepared formulation MSGU reveals the presence of major peaks with 2-theta values of 28.12 and their corresponding intensities were 1316.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 exactly matches with the reference material AsS, which justifies the presence of stable and purified AsS. As shown in Figure 6.

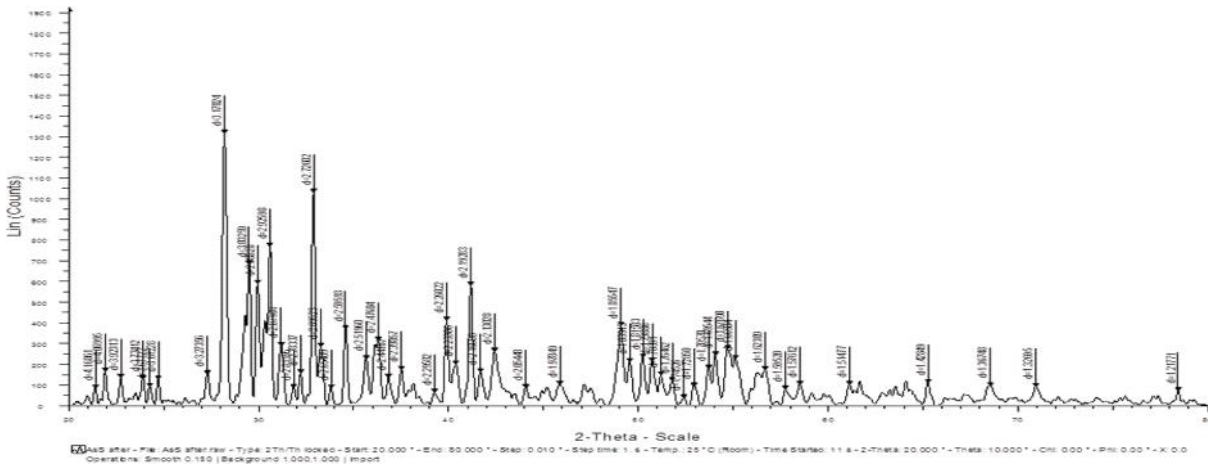

Figure 6: XRD pattern of Sample MSGU

4. Discussion

India is among the important mega biodiversity centers of the world .This diversity coupled with a rich heritage of traditional knowledge (TK) has made India home to several important health care systems viz., *Ayurveda*, *Siddha* and *Unani*. Whilst there is increasing demand for Indian TM globally, it is only for products validated by modern scientific research. Indian exports of around $100 million are relatively low compared to Chinese figures of $3 billion. Report by Group-II of Task Force on "Pharmaceuticals and Knowledge Based Industries" 1999 states that Indian exports of herbal products are low due to several factors, quality being most important [17]. To be a strong player in global herbal market, it is critical that India develops appropriate quality control systems for standardization of raw materials and finished products and to strengthen the regulatory mechanism.

Purification is the mode and method to alleviate the impurities, improvise the therapeutic property and nullifies the toxicity of the preparation. Siddha system has several methods of drug purification of which it is mandatory to optimize the current methods of purification to retain the stability and efficacy of the drug. Traditional purification methods still exist in the siddha system due to its methodological way of drug processing without compromising the nature and quality of the drug. It was evident that many siddha formulations have minerals, herbs which are known to have chemical components and biological activities. The most important of these bioactive constituents of siddha drugs are minerals and alts. Functional groups like, NH, OH,CHO, C-Br etc offers greater therapeutic responses Several analytical techniques have been developed for determining the active functional group such as FT-IR, gas chromatographic (GC) [18], mass spectrometry [19], thin layer chromatography [20], UV spectrometry [21], and high performance liquid chromatography (HPLC) [22].

FT-IR spectral data's of the samples signifies the nature and presence of active functional group in both the samples. Presence of hydroxyl group appears common in both the sample and this group seems mandate for most of the biological activity of the manosilai. Presence of S=O and amino group seems unique for the sample MSGU where as it certainly not appeared in other sample. Presence of C-Br,N-N=O, –CO-NH and CHO are certainly unique for the sample MSBM which is not appeared in MSGU. On arriving at the conclusion the sample MSBM has more number of functional groups when compare to MSGU.

Nano formulation rules the world in recent times. It was observed from the present investigation that both the MSBM and MSGU possess increased level of micro particles which may aids in drug absorption and penetration through biological barriers. Results of SEM analysis of both the samples MSBM and MSGU with respect to the morphology and size range it was observed that there is no significant difference in the size range of micro particles present in both the samples. It was further observed that the particle present in the sample MSBM is much categorized and segregated when compare to that of MSGU

X-Ray diffraction technique's becomes a valuable tool for analysis siddha preparation which comprises of minerals and metals. This modern analytical technique provides the details on elemental diffraction pattern of the sample under investigation. From the result of the present XRD analysis it was concluded that the elemental composition of sample MSGU confirms the presence of AsS at its stable state. Further form the pattern of MSBM it was clouded that Arsenic and sulphur may be the key ingredient present in the sample MS. Hence it was concluded that the basic elemental composition remains unaltered by both the purification process.

5. Conclusion

Development of newer techniques and optimization of existing purification methodologies often go hand in hand in globalization of siddha traditional medicines. By comparing the results obtained from the present investigation it was concluded that the siddha drug manosilai purified using butter milk may possess increased functional groups evident by FTIR and also has sequential increase in micro particle distribution justified by SEM. Further study has to be carried out on comparing the efficacy of both the samples before subjecting the optimized protocol for clinical application.

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