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ORIGINAL ARTICLE

Comparative Analysis of Four Distinct Purification Methods of Sulphur as per Siddha with Contemporary Analytical Techniques

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Abstract

Background: Nonmetallic and mild toxic Sulphur is abundantly available in the world. *Kanthakam* is the name for Sulphur in Siddha Medicine. Sulphur is one of the unavoidable substances for medicine preparations with deferent purification methods. According to Siddha concept of Purification *(Shuddhi*) of drug not only a process of purification or detoxification, but also process to enhance potency and efficacy of the medicine.
Methods: The Unpurified Sulphur (S1) and Purified Sulphur Method I (S2), Method II (S3), Method III (S4) and Method IV(S5) Samples were employed for Comparative analysis of organoleptic character, Solubility profile, loss on drying, total and acid insoluble ash, alcohol and water soluble extractive, pH, Sulphur concentration, Microbial test, Aflatoxin assay by TLC, Test for basic and acid radicles, FTIR, and SEM
Results: finding gave considerable changes between unpurified Sulphur (S1) and purified samples. The purity percentage of Sulphur, were found increased from 8.97% w/w to 59.04% w/w and the particle size were reduces from 1247 d.nm to 468.0 d.nm in the S5 sample. FTIR show the functional group changes from C=C- alkene and C-I - Alkyl & Aryl Halides in to O-H-Alcohol, N-H- amine salt, C-H- alkane, C=C- alkene and C=O Esters & δ-lactone. Purified Sulphur samples show changes in Sulphur content, Particle size, functional group and solubility were within the safety limits as FDA permissible quantity. Heavy metals were not found in any samples.
Conclusion: According to comparative analysis, it is vital to select purifying methods for preparing medicines in accordance with the text.

Keywords: Sulphur, Purification, Analytical Techniques

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INTRODUCTION

The Siddha Medical system already structured and explained about fixed medicine for every aspect of human needs and diseases with the concern of availability of raw material, cost-effective, seasonal variations, geographical variations, condition of disease, state of patients and associated diseases as well as procedure of medicinal preparation, proper method for collection of raw materials and purification method ect Siddha Medicines (Drugs) are prepared from the raw materials of Plants, Metals, Minerals, Metal salt (*Pashanam)*, and Animal kingdoms[1]. Among the Metal salt, the *Kanthakam* (Sulphur) is one of the important and unavoidable materials for medicine preparations. All throughout the world sulfur is the fifth most common element on earth and the tenth most frequent element by mass [2]. This is used for the production of sulfuric acid for sulfate and phosphate fertilizers and chemical processes in addition to the medicine preparation. According to siddha, there are different types of *Kanthakam* mentioned in the texts, but the golden yellow colour *Nellikai Kanthakam* commonly used for medicine preparations, among the preparations, there are *Kanthaka parpam, Sarvavida thodangalin kulikai, Kanthaka chenthooram, Kanthaka Mathirai* and *Kanthaka thylum* etc. [3]. Medicine prepares with unpurified ingredients will cause the harmful effect to human [4] Before preparing medicine with Sulphur should be purified because of it has the ability to produce toxicity in different organs in the human. Browsing the literature show numerous purification methods of *Kanthakam* were mentioned clearly [1,3,4]. With siddha's spiritual insight, the safety level of sulphur after purification was ideal. Yet, the requirement for an evidence-based study of its purity in the modern scientific world creates interest in Comparative analysis of four distinct Purification methods with contemporary analytical techniques.

METHODS

This analysis compares differences between unpurified and purified samples about macroscopical changes, Physico chemical changes, functional groups through FTIR, and

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Surface with particle size with SEM

Collection and authentication of test raw drug

Raw Sulphur collected from retailed shop in Tirunelveli, Tamil Nadu, India and obtained authentication certificates from Animal and Mineral origin Drug Research Laboratory (AMDRL) of Siddha Central Research Institute Chennai, India by the method of Inductively Coupled Plasma- Optical Emission Spectroscopy (ICP- OES) is an analysis.

Preparation of Purification process

Five samples were prepared for the comparative analysis respectively with the reference from the text book *Gunapadam – Thathu jeeva vaguppu* [1].

Sample I- S1, The Sulphur before purification, Sample II- S2, Purified Sulphur via Purification Process I which was the Sulphur melted and poured into milk this procedure was done only one time, Sample III- S3, Purified Sulphur via purification process II which was the Sulphur melted and poured into Plantain stem Juice this procedure was repeated ten times, Sample IV- S4, Purified Sulphur via Purification Process III which was the Sulphur has undergone the puda process with paste of *Lawsonia Inermis* and Cows curd repeated this procedure in seven times, Sample V- S5, Purified Sulphur via Purification Process IV which was the Sulphur melted and poured into milk and this procedure were repeated thirty times

Macroscopical changes

All samples were taken in a clean glass dish and check the color, odor, state, nature, touch/ consistency, flow property and appearance.

Physicochemical Evaluation

All five samples were undergoing the test of solubility profile, percentage of loss on drying, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive and pH.

Determination of Sulphur

To the estimation of Sulphur Carbon tetrachloride saturated with Bromine and Barium chloride – 10 per cent solution in water Solutions were used. Each g of the weight of precipitate is equivalent to 0.13734 g of Sulphur and determined the Sulphur concentration.

Microbial test

This carry out with pour plate method; all samples were separately dilute with chloroform. Incubate the plates of *Soybean–Casein Digest Agar* at 30 to 35 for 3 to 5 days and the plates of *Sabouraud Dextrose Agar* at 20 to 25 for 5 to 7 days. Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC. Take the arithmetic mean per culture medium of the counts, and calculate the number of cfu per g or per ml of product.

Aflatoxin assay by TLC

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly, the test samples were placed and allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

Fourier transform infrared spectroscopy- FTIR

FTIR study was done in the Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA Deemed University, NH67, Tirumalaisamudram, Thanjavur. The solid samples analyzed with KBr or Nujol mull method. The S1, S2, S3, S4 and S5 samples were grounded using an agate motor and pestle to give a very fine powder. The fine powder sample was mixed with about 100 mg dried potassium bromide salt. The mixture was then pressed under hydraulic press using a die to yield a transparent disc (measure about 13 mm diameter and 0.3 mm in thickness) through which the beam of spectrometer passed separately. The transformation of the interferogram into a spectrum is carried out mathematically with a dedicated online computer. The analyzed between MIR 450-4500 cm^{-1}, resolution 1.0cm^{-1} scan range in the Fourier-transform infrared (FTIR) spectrometer (Spectrum100, Perkin Elmer, USA).

Scanning Electron Microscope pictures - SEM

SEM was done at the Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA Deemed University, NH67, Tirumalaisamudram, Thanjavur to evaluate grain size, particle size distributions, material homogeneity and intermetallic distributions. The surface morphology of the drugs was studied qualitatively using a cold field emission scanning electron microscope (SEM, JSM6701F, Jeol, Japan). The samples were separately mounted on a brass stub and sputter coated with platinum and introduced into the specimen chamber and imaging was carried out at an acceleration voltage of 3 kV. Test resolution: 1.2 nm gold particle separation on a carbon substrate, Magnification: From a minimum of 12X to greater than 1,00,000 X, the horizontal line in the right corner of the micrograph corresponds to micro in length would be given. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the particles was calculated.

RESULTS

Macroscopic alterations, solubility profiles, percentage of loss after drying, total ash, acid-insoluble ash, alcohol-soluble extractive, water-soluble extractive, pH, Sulphur levels, and the presence of microorganisms and aflatoxins are all shown in Table 1 as significant variations across samples.

FTIR spectra

According to table 2, significant changes of IR- wave length shown, and compared functional group and its property (Figure 1)

SEM

The Scanning Electron Microscope pictures show the results from 1k.x Magnification, 5k.x Magnification, 10k.x Magnification and 500x Magnification. The Cluster View Magnification taken and analysis with Categorised View Magnification and reveals the Size of particle, Clumsiness of the particle and Character of external surface of particle. The following pictures explain separately about the sample individually (figure 2).

Table 1. Comparison of Macroscopical, Physicochemical, Sulphur content, Microbial and aflatoxins among sample

Description	*Kanthakam* - S1	*Kanthakam* - S2	*Kanthakam*- S3	*Kanthakam*- S4	*Kanthakam* - S5
Colour					
Odor	Pungent odor	Characteristic	Characteristic	Pungent	Mild Pungent
State	Solid	Solid	Solid	Solid	Solid
Nature	Fine	Moderately Coarse	Fine	Very fine	Moderately Fine
Touch / Consistency	Soft	Soft	Soft	Soft	Slightly coarse
Flow Property	Moderately free flowing	Free flowing	Moderately free flowing	Free flowing	Free flowing
Appearance	Yellowish	Brownish	Pale Brownish	Whitish yellow	Blackish brown
With Chloroform	Soluble	Soluble	Soluble	Soluble	Soluble
with Ethanol	Insoluble	Soluble	Soluble	Soluble	Partially Soluble
With Water	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
With Ethyl acetate	Insoluble	Soluble	Partially Soluble	Partially Soluble	Soluble
With Dimethylsulfoxide	Insoluble	Insoluble	Partially Soluble	Partially Soluble	Insoluble
Loss on Drying at 105 °C (%)	0.2 ± 0.17	0.36 ± 0.057	0.16 ± 0.05	0.62 ± 0.03	0.82 ± 0.05
Total Ash(%)	0.18 ± 0.12	0.53 ± 0.05	1.19 ± 0.14	13.07 ± 2.54	20.57 ± 0.50
Acid insoluble Ash (%)	0 ± 0	0 ± 0	0 ± 0	0.069 ± 0.025	0.14 ± 0.01
Alcohol Soluble Extractive(%)	0 ± 0	0 ± 0	0 ± 0	0.015 ± 0.002	0.027 ± 0.004
Water soluble Extractive (%)	0 ± 0	0.026 ± 0.002	0.34 ± 0.055	0 ± 0	0 ± 0
pH	5	5	7	7	9
Content of Sulphur API of India(Formulation),Part-II,Vol-I,p246	8.97% w/w	4.52% w/w	7.08% w/w	7.13% w/w	59.04% w/w
Presence of Total viable bactrial count	+ ve $6x10^3$cfu/mg	- ve -	+ ve $2x10^3$CFU/mg	+ ve $3x10^3$CFUmg	+ ve $4x10^3$CFU/mg
E. COLI	- ve	- ve	- ve	- ve	- ve
Presence of total fungal	+ ve $2x10^3$cfu/mg	+ ve $1x10^3$CFU/mg	- ve -	+ ve $6x10^2$CFUmg	- ve -
Aflatoxin B1&B2	NDA	NDA	NDA	NDA	NDA
Aflatoxin G1 & G2	NDA	NDA	NDA	NDA	NDA

Table 2. IR spectra wavelength and it's functional group

NO	Frequency	Absorption (cm^{-1})	Functional group/compound class					Name of group	Functional group
			S1	S2	S3	S4	S5		
1.	3428.52	3550-3200			√			Strong O-H stretching-	Alcohol
2.	3428.10						√		
3.	2922.43	3000-2800			√			Strong N-H stretching-	Amine salt
4.	2921.08						√		
5.	2851.74	3000-2840			√			Medium C-H stretching-	Alkane
6.	2851.15						√		
7.	1742.23	1750-1735					√	Strong C=O stretching	Esters & δ-lactone

Table 2. Continued

NO	Frequency	Absorption (cm^{-1})	Functional group/compound class					Name of group	Functional group
			S1	S2	S3	S4	S5		
8.	1633.96				√			Medium C=C stretching	Cyclic alkene
9.	1633.94					√			
10	1633.14	1650-1566		√					
11	1623.11		√						
12	1160.63	1400-1000					√	Strong C-F stretching,	Fluoro compound
		1250-1020						Medium, C-N stretching,	Amine
13	1020.47				√				
14	465.66				√			C-I stretch	Alkyl & Aryl Halides
15	465.43			√					
16	465.37	<600				√			
17	465.20		√						

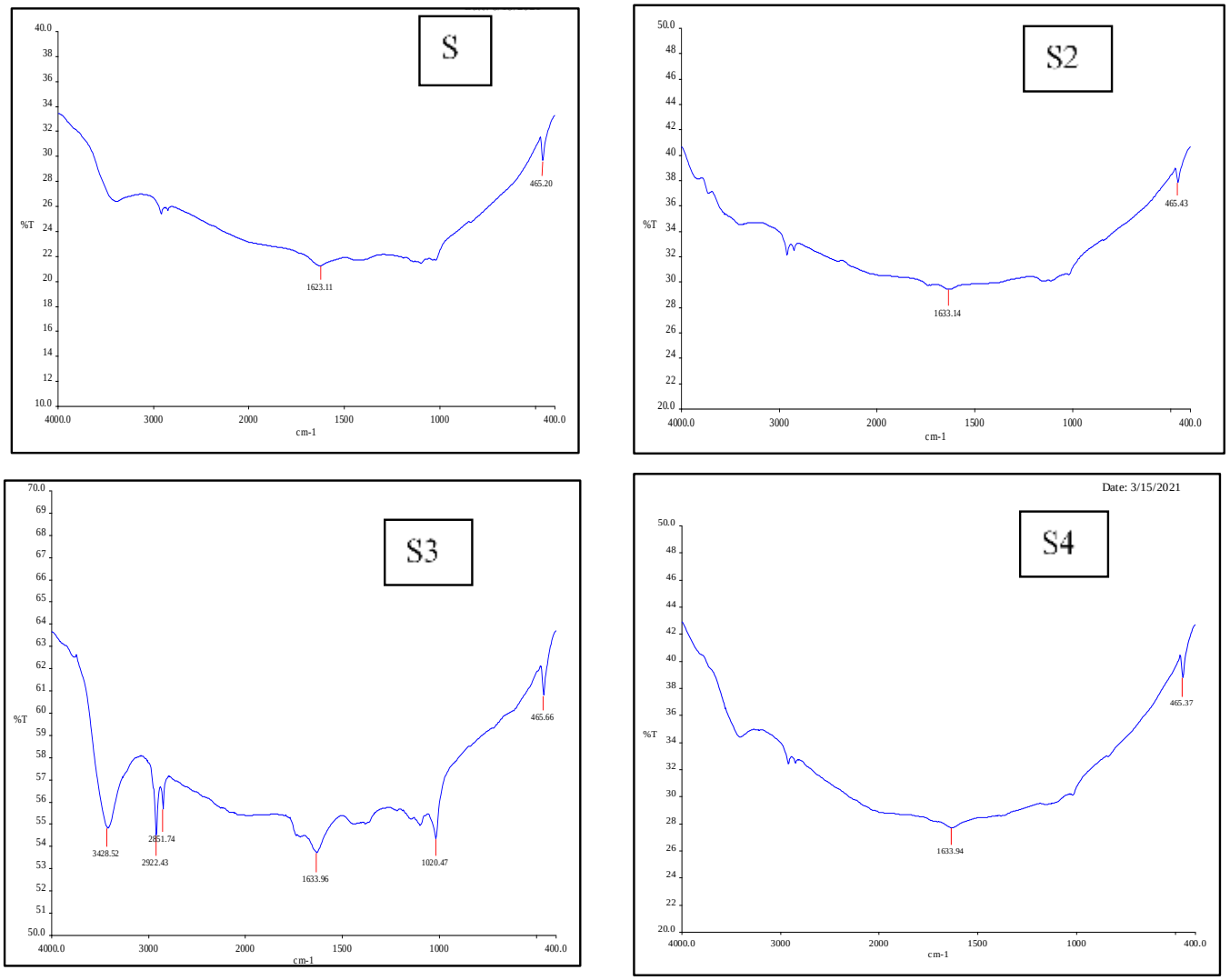

Figure 1. FITR – IR spectra graph comparison among sample

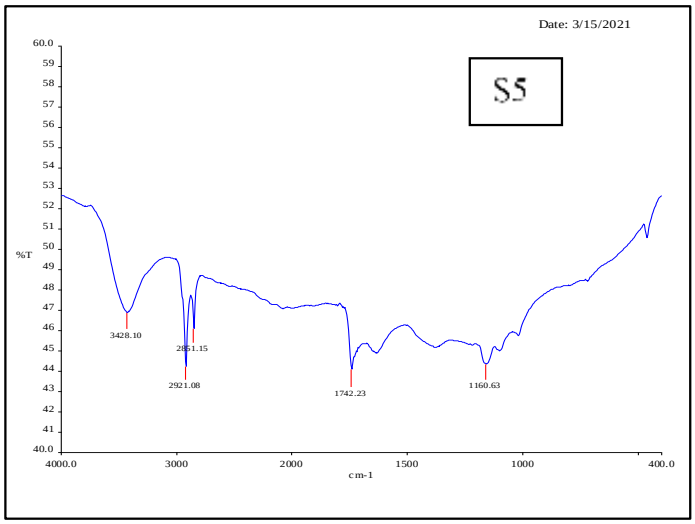

Figure 1. Continued

DISCUSSION

The drug description in the purified samples S2, S3, S4, and S5 and the raw sample S1 both underwent significant alterations, according to the physicochemical analysis. Sulfur is yellow when it is raw; it turns brownish in S2, pale brownish in S3, and eventually blackish brown in S5 samples after going through S4 processing. Due to the use of too much heat during melting, the S2 and S5 samples change to a brownish color.

After purification, the S1 smell is pungent, and in samples S2, S3, S4, and S5, it changes to characteristic, pungent, and mildly pungent, respectively. Drug condition remained unchanged. S1 & S3, which were fine in nature, S2, which was moderately coarse in S1, very fine in S4 and fairly coarse in S5. S1, S2, S3, S4, and S5 were all considered gentle in terms of touch and consistency, whereas S5 only slightly turned coarse. While S2, S4, and S5 had free flowing properties, S1 and S3 had properties with a moderately free flow.

Solubility Profile reported as: The all samples were soluble in Chloroform [5] and Insoluble in water [6]. The S1 insoluble in the Solvent Ethanol, S2, S3 and S4 Soluble in ethanol but S5 partially soluble in ethanol. S1 insoluble in ethyl acetate, S2 and S5 soluble but S3 and S4 partially only soluble. S1, S2 and S5 insoluble in Dimethyl sulfoxide, but S3 and S4 soluble in DMSO (Dimethyl Sulfoxide). Chloroform best solvent for all sample, even after purification water is not suitable solvent for Sulphur samples. After purification considerable changes in the solubility in the solvent Ethanol and Ethyl acetate. Water content reduce the solubility of S_8 while increasing temperature increases the solubility. At higher temperatures aniline is expected to react with S_8, all other solvents are indifferent [7].

Loss on Drying give the meaning of the term “moisture” in this circumstance. Moisture refers to all matter within a sample which can be vaporized, and thus includes not just water but fats, volatile solvents, and alcohols. Moisture is one factor responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability and quality drugs. After Loss on drying at 105°C (%) analysis S1 0.2±0.17 changes in to 0.36 ± 0.057, 0.16 ± 0.05, 0.62 ± 0.03 and 0.82 ± 0.05 respectively in S2, S3, S4 and S5. After purification the moisture content were increased it due to purification methodology, there's no a limit for loss on drying. But it is preferred to be < 1%. On the other hand, moisture content is necessary while formulating a solid dosage form [8].

Ash values are helpful in determining the quality and purity of crude drugs. Main objective of ashing is removal of all traces of organic matter (Organic matter refers to the large source of carbon based compounds). On incineration, crude drugs normally leave an ash consisting of carbonates, Phosphate and silicates of sodium, potassium, calcium and magnesium. Total Ash (%) value increased in all sample after purification. S1 value is -0.18 ± 0.12 and S2, S3. S4. and S5 values respectively 0.53 ± 0.05, 1.19 ± 0.14, 13.07 ± 2.54 and 20.57 ± 0.50.In the sample inorganic content present more than before purification and comparatively high in S5 . Acid insoluble ash value is 0.069 ± 0.025 and 0.14 ± 0.01 respectively in the sample of S4 and S5. Result shows that small amount of inorganic compound is insoluble in acid. Acid insoluble ash can enhance the amount of component absorbed in the gastrointestinal channel with oral administration [9].

Alcohol Soluble Extractive (%) is 0.015 ± 0.002 and 0.027 ± 0.004 in respective sample of S4 and S5 thus alcohol extraction recommended for S4 and S5. Water extraction only suitable for S2 and S3(0.026 ± 0.002 and 0.34 ± 0.055) there is no alcohol and water-soluble extractive value for S1. Here after purification only the Sulphur produce water and alcohol soluble content [10]

The pH of sample also shows the considerable changes.

pH indicates the level of H+ ions, where low pH indicates too many H+ ions and high pH indicates too many OH- ions. S1 and S2 have the pH of 5(Acidic), S3 and S4 show the pH of 7(Neutral) and S5 have the pH of 9(Alkaline). Strongly acidic and alkaline nature of the drug cause abnormal effects to the body and poorly absorbed from GIT. All samples show the pH range near to Neutral pH therefore easily absorbed from the GIT and safe [11].

In consideration with the Determination of Sulphur in each sample, Sulphur levels show different change in before and after or during purification of deferent method of purification. S1 possess the 8.97% w/w Sulphur, but in the sample S2, S3 and S4 reveals the reduction of Sulphur content during respected purification method. Rather significant increase of

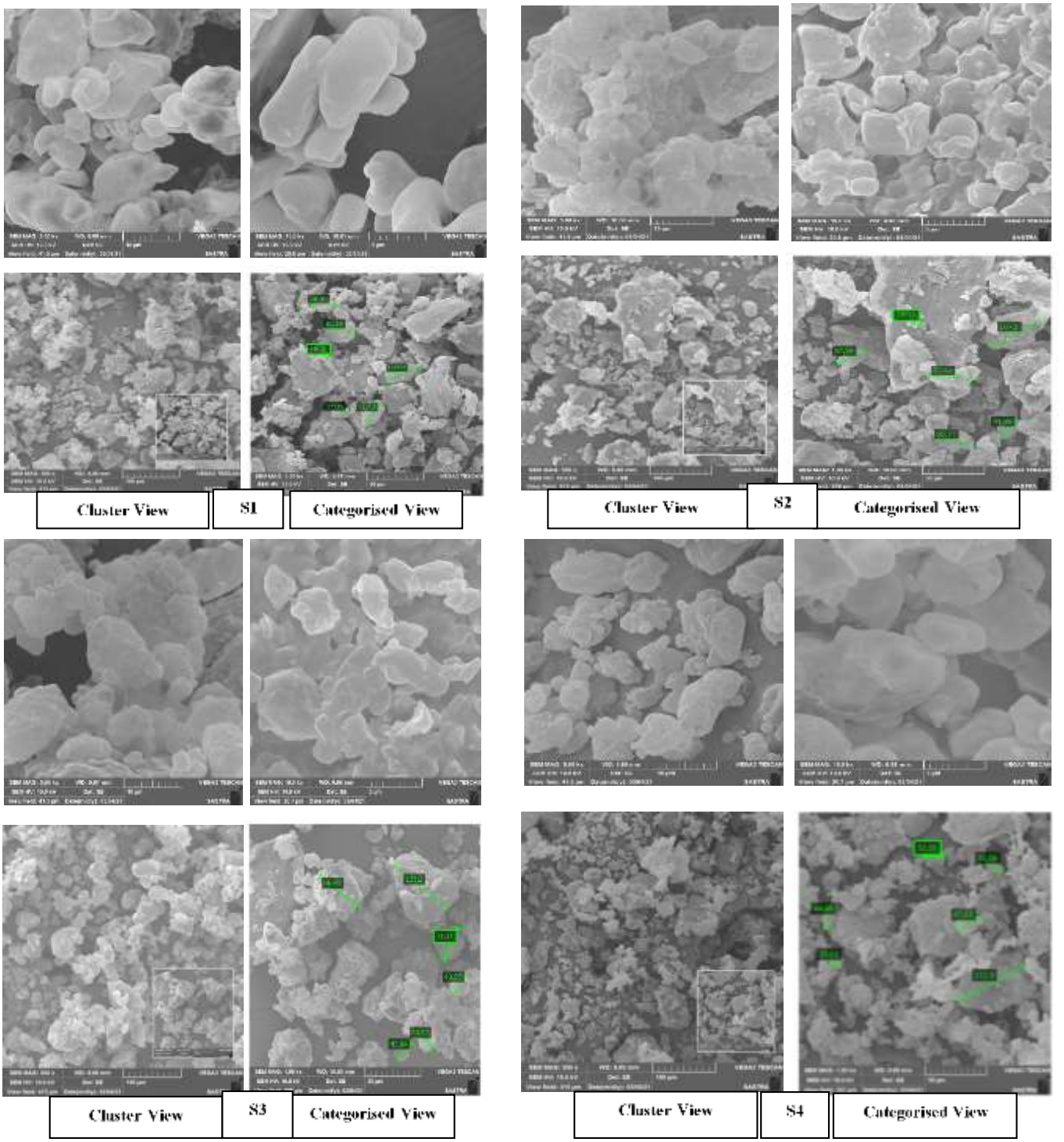

Figure 2. SME image comparison among sample

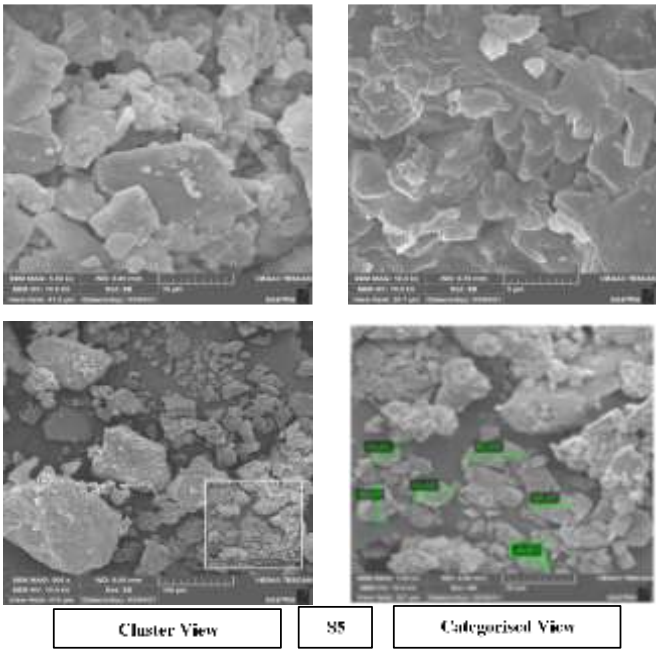

Figure 2. Continued.

Sulphur content in S5 59.04% w/w which indicates the higher purity of Sulphur.

In consideration of Microbial Counts, The Sulphur undergoes number of biological transformations in nature carried out exclusively by microorganisms. The transformations of inorganic Sulphur compounds in nature have been formalized in the so-called Sulphur cycle [12]. The microorganisms that participate in Sulphur cycle are physiologically diverse and comprise both heterotrophic and autotrophic organisms [13] And fungi also have the ability to play a significant role in oxidation of Sulphur present in soils although the rates of oxidation achieved by thiobacilli are high in the in-vitro conditions. In vivo S-oxidation rates by fungi similar to those of heterotrophic bacteria [14] but less than those obtained for thiobacilli also recorded. Heterotrophic S-oxidation is also an important process in soils under certain circumstances [15]. S1 reveals the presence of Bacteria and fungus $6x10^3$cfu/mg and $2x10^3$cfu/mg, during the S2 the fungus only presents, then in S3 and S5 method of purification eliminate the total fungus from the sample and bacterial count were reduced from purified sample. In the S4 bacterial count were reduced but the amount fungal count increased.

Aflatoxigenic fungi produce four major aflatoxins: B1, B2, G1 and, G2 plus two additional metabolic products, M1 and M2, that are of significance as direct contaminants of foods and feeds [16]. Aflatoxin Not Detected in sample S1, S2, S3, S4 and S5. Therefore, all the purification methods were suitable for medicine purification and free from aflatoxin

Based on the peak reference obtained in FTIR of Samples, the presence of functional groups in S1,S2 and S4 were cyclic alkene(C=C stretching) and Alkyl & Aryl Halides(C-I stretch), S3 possess the functional groups of Alcohol(O-H stretching), amine salt(N-H stretching), alkane(C-H stretching), cyclic alkene(C=C stretching), amine(C-N stretching) and Alkyl & Aryl Halides(C-I stretch) and S5 have the functional groups of Alcohol(O-H stretching), amine salt(N-H stretching), alkane(C-H stretching), Ester δ-lactone,γ: (stretching) & fluoro compound (C-F stretching). These functional groups were responsible for the therapeutic effects. The presence of deferent organic groups after purification through FTIR confirm role of material which used to purify the Sulphur in enhancement of pharmaceutical activity for Sulphur [17].

A number of neurotropic agents contain a conjugated alkene (C=C) group incorporated in an iminostilbene or dibenzosuberene ring system. Examples include the anticonvulsant and antidepressants [18]. Halogenes (C-I) (Bromine, Iodine and Chlorine) are acute sedative and Anticonvulsant. Alcohol(O-H) act as vasodilator, antiseptic, disinfectant and antidote these changes happen after purification only. The amine salt(N-H) act as antiseptic, antiviral, hypolipidemia and anti-inflammatory. Then the Ester(C=O) which posse the. Anti-histamine activity.

According to the SEM image 1.00kx, 5.00kx,10.0kx and 500X, the morphological character of surface smooth in S1 sample, but it will change in to rough. And the Size of the particle was reduced in all four samples after purification. The rough surface samples have high adhesive property with the other element or plasma protine as well as smaller particle size also easily absorbed by the body. the vital role of surface modification in improving the performance of nano-adsorbents was clearly observed [19]. The shapes of particles may be determined by the intrinsic crystal habit of the material, or by the influence of the environment around their creation, such as the inhibition of crystal growth on certain faces by coating additives, the shape of emulsion droplets and micelles in the precursor preparation, or the shape of pores in a surrounding solid matrix [20].

LIMITATION

Numerus Sulphur Purification methods were mentioned in varies siddha texts, but select mostly followed purification methods for this study, while melt the Sulphur limitations of melting temperature not mentioned. Purification methodologies should be standardize based on indication.

CONCLUSION

According to the present comparative analysis, the results of samples gave considerable changes before and after purification process. As well as the comparison among the samples also show significant differences. During the purification process the purity percentage of Sulphur, was found increased from 8.97% w/w to 59.04% w/w and the particle size was reduces from 1247 d.nm to 468.0 d.nm in the S5 sample only the FTIR show the functional group also change from C=C- alkene and C-I - Alkyl & Aryl Halides to O-H-Alcohol, N-H- amine salt, C-H- alkane, C=C- alkene and C=O Esters & δ-lactone.

In the present study the detoxification of Sulphur with deferent methods shows the changes specific for each method about Sulphur content, Particle size, functional group, solubility profile and other present elements Yet the values were within the safety limits as prescribed by FDA

permissible quantity. The heavy metals were not found in any samples.

fingerprint region in FTIR spectrum indicate the functional group of S1, S2 and S4 were same which possess C=C and C-I But functional group of S3 changed or added new groups such as O-H, N-H, C-H, C-N as well as the S5 also show the new functional group such as O-H-, N-H, C-H and C=O.

From the above analysis can conclude that each and every detoxifying method as per Siddha text specific for particular prepared medicine and particular indications. Therefore, various detoxification methods are mentioned. The methodology should be selected for medicine preparation which was mentioned by Siddhars with their spiritual knowledge.

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