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QUALITY ASSESSMENT AND SYSTEMATIC COMPARATIVE ANALYSIS OF SIDDHA COMPONENT THALAGAM BY FTIR, SEM AND XRF TECHNIQUES

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ABSTRACT

Biological system requires varying amounts of heavy metals such as Iron, cobalt, copper, arsenic manganese, molybdenum, and zinc at trace level. Metals mediates wide range of enzymatic and biochemical pathways which are essential for fundamental functionality. Siddha system of medicine has versatile metal based preparation which are indicated for treating broad range of diseases and disorders. As per principles of siddha metals and minerals acts more vigorously on the neural system, hence pioneering in treating neruo disorders since several centuries. Thalagam known by its name arsenic trisulphide commonly used as an ingredient in several preparations for claiming its therapeutic potential. But still there is no proper documentary evidence on proving the structural and functional changes that occurs before and after purification of thalagam. The main objective of the present study is to carry out systematic comparative analysis of unpurified (T1) and purified (T2 & T3) arsenic trisulphide using modern instrumental techniques such as FT-IR, SEM and XRF analysis. It was observed from the FT-IR spectra analysis of the purified and purified thalagam that presence of aliphatic ketones and esters are more in unpurified form than in purified samples, similarly purified form of thalagam reveals the presence of C-Br and C-I stretching which are potentially active functional group. It was observed from the SEM analysis of the purified and purified thalagam that the average particle size of the purified form was high when compare to that of the un purified form. Results of the XRF analysis it was observed that the elemental composition is greatly reduced in the purified form when compare to that of the unpurified form. By comparing the elemental analysis of sample T2 and T3 purified form the sample T3 has very minimal trace of metals and further its devoid of the following functional groups such as SiO2, BaO, MOO3, Fe2O3, Al2O3, Cao and ZnO when compare to that of the T2 purified form. It was concluded from the results of the present study that the purification process enhances the bioactive functional group and minimizes the elemental traces which justifies the safety of the preparation.

KEY WORDS: ***Arsenic trisulphide. FT-IR, SEM, XRF analysis, Bioactive functional group, Elemental traces***

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1. Introduction

WHO has declared that the purpose of quality control is to ensure quality of the products by adhering to appropriate specifications and standards. Information on appropriate standards can be found in official pharmacopoeias, monographs, handbooks, etc. In choosing analytical methods, the availability, robustness and validity of the methods must be considered and if such advanced methods are used, a full validation for each test would be necessary [1].

Traditional medicine is defined as an amalgamation of knowledge, skill, and practices based on theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used for therapeutic, restorative, prevention, diagnosis and maintenance of physical and mental health [2]. These systems are recognized globally for complementing disease prevention, treatment and generic health maintenance [3]. There is widespread use of traditional medicine across developing countries (across Asia, Africa and Latin America) with rapidly emerging markets in North America and Europe [4]. However, despite increasing national and international attention, the formal health systems, particularly in resource-poor settings, are yet to harness its true potential. Recognizing such intricacies, the 67th World Health Assembly resolution on traditional medicine has been instrumental in the development of updated WHO traditional medicine strategy with objectives to harness its contribution and promote effective use [5].

Arsenic is a traditional medicine, is an effective treatment for acute pro-myelocytic leukemia (APL) [6,7]. Previous studies have demonstrated that As2O3 has an inhibitory effect on the proliferation of various types of cancer, including APL, gastric carcinoma and breast cancer [8,9].Since arsenic has been widely used for therapy still now there is no proper documentary evidence proving its elemental nature before and after purification, hence the main objective of the present study is to carry out systematic comparative analysis of unpurified (T1) and purified (T2 & T3) arsenic trisulphide using modern instrumental techniques such as FT-IR, SEM and XRF analysis.

2.Materials and Methods

2.1. Fourier Transform – Infra Red Spectroscopy Study [10]

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.

2.2. SEM Analysis [11,12]

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

2.3. XRF spectral Study

The physical principle of the XRF is based on the excitation by an X-ray photon, a core-shell electron from the specific atom is ejected as a photoelectron. The formed core-hole is then filled by a neighbouring higher energy orbital electron, which results in emission of an X-ray fluorescence photon. The energy of the emitted photon is equal to the difference in binding energies of the two shells involved in the transition [13,14]. Since the binding energy is varied with the nuclear charge, each element has a unique photon energy, i.e., characteristic fingerprint X-ray fluorescence, which enables the multi-element analysis.

3.Results

3.1. Results of FT-IR spectral analysis of Unpurified and purified Thalagam

The FT-IR absorption spectrum of the unpurified sample reveals the presence of intense peak 1482.61 cm-1due to C=O, symmetric bending, peak at 412.79, 1512.61 and 1582.60 cm-1 due to presence of Al-O stretching, N-O stretching and C=C stretching, cyclic alkene. Peak at 1711.15 cm-1, 1756.86 cm-1 due to

presence of aliphatic ketones and esters. As shown in Table 1 and spectrum Figure 1.

Table 1: Peak Table analysis of Unpurified Thalagam – T1

S.No	Peak	Intensity	Corr. Intensity	Base(H)	Base(L)	Area	Corr.Area	Comment
1	412.79	93.35	3.11	419.93	399.93	105.981	37.376	Al-O stretching
2	622.76	92.76	0.14	625.61	617.04	60.929	0.537	C=O
3	714.17	92.19	0.38	718.45	679.89	275.032	8.889	C-H bending, monosubstitued
4	1466.90	85.93	1.69	1474.04	1456.90	228.504	17.416	C-H bending, methylene group
5	1482.61	85.31	1.68	1488.33	1474.04	199.460	14.368	C=O , symmetric bending
6	1512.61	86.13	2.15	1519.75	1508.32	148.530	13.139	N-O stretching
7	1571.17	85.12	0.24	1574.03	1568.31	84.275	0.675	C=C stretching, cyclic alkene
8	1582.60	84.07	0.49	1585.45	1576.88	132.412	1.727	C=C stretching, cyclic alkene
9	1641.16	83.95	1.63	1645.45	1636.87	130.451	6.854	C=C stretching, α,β unsaturated ketone
10	1691.15	83.38	2.39	1698.29	1684.01	220.727	17.529	C=O stretching, conjugate aldehyde
11	1711.15	83.14	2.05	1716.86	1698.29	290.228	18.827	C=O stretching, aliphatic ketone
12	1724.00	82.45	0.82	1726.86	1716.86	165.636	3.192	C=O stretching, aliphatic ketone
13	1756.86	82.38	0.61	1759.71	1748.29	194.383	2.715	C=O stretching, Esters
14	1838.27	81.22	0.85	1845.41	1828.27	314.417	7.495	C-H bending

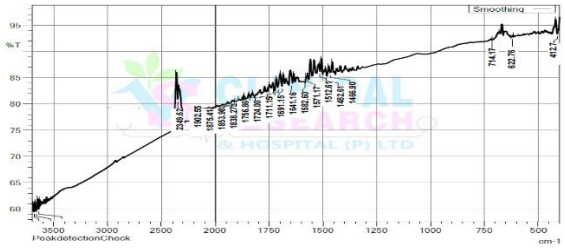


Figure 1: FTIR spectrum of Unpurified Thalagam (T1)

Similarly, FT-IR spectrum of purified thalagam (T2) reveals the presence of absorbance peak at 434 and 468 cm-1 due to Si-O bending and Zn-O stretching. Peak at 1364 cm-1 and 1421 cm-1due to S=O stretching and O-H bending, Peak at 2848 cm-1 and 1519 cm-1due to C-H aromatic bending and N-O stretching. As shown in Table 2 and spectrum Figure 2.

Table 2: Peak Table analysis of Purified Thalagam – T2

S.N	Peak	Intensity	Corr. Intensity	Base(H)	Base(L)	Area	Corr. Area	Comment
1	457.07	90.06	0.91	471.35	419.93	480.632	35.085	C-C stretching cyclohexane
2	491.35	90.09	0.39	509.92	471.35	375.147	8.012	C-I Stretching, Iodo compounds
3	529.91	90.09	0.22	544.20	509.92	334.366	2.773	C-Br stretching halo compound
4	588.48	90.17	0.00	598.47	587.05	112.127	0.034	C-Br stretching halo compound
5	709.88	91.08	0.21	715.60	685.60	257.164	4.666	C-H bond aromatic, mono-substituted benzene
6	1326.93	91.38	0.25	1338.35	1316.93	181.722	2.367	S=O stretching, sulfone
7	1351.21	91.32	0.43	1368.35	1338.35	252.383	5.234	S=O stretching, sulfonamide
8	1384.06	91.68	0.22	1395.49	1379.77	126.475	1.262	C-H bending, alkane gem dimethyl
9	1408.34	91.78	0.61	1419.77	1395.49	190.934	6.027	S=O stretching, sulfonyl chloride
10	1484.04	92.39	0.39	1491.18	1471.19	148.339	3.785	C=C bending, aromatic
11	1529.75	93.23	0.78	1539.75	1522.61	108.443	6.341	N-O stretching, nitro compound
12	1548.32	93.37	0.81	1558.32	1539.75	116.252	7.961	N-O stretching, nitro compound
13	1605.45	90.79	0.27	1616.88	1592.60	220.145	3.201	C=C stretching, conjugated Alkene
14	1625.45	90.67	0.22	1629.73	1616.88	118.299	1.484	C=C stretching, α,β unsaturated ketone

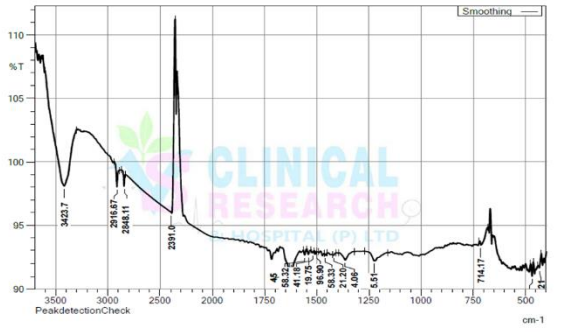


Figure 2: FTIR spectrum of Purified Thalagam (T2)

The FT-IR spectrum of purified thalagam (T2) projects the spectrum of absorbance peak at 1408 cm-1 and 1326 cm-1 due to presence of S=O stretching, Peak at 529 cm-1 and 491 cm-1 due to C-Br and C-I stretching. Peak at 1351 cm-1 and 1326 cm-1due to S=O stretching. Peak at 1548 cm-1 and 1529 cm-1due to N-O stretching. As shown in Table 3 and spectrum Figure 3.

Table 3: Peak Table analysis of Purified Thalagam – T3

S.No	Peak	Intensity	Corr. Intensity	Base(H)	Base(L)	Area	Corr.Area	Comment
1	434.21	91.66	0.52	439.93	424.22	124.521	3.653	Si-O bending
2	468.49	91.00	1.05	475.64	462.78	108.487	6.235	Zn-O stretching
3	714.17	93.45	0.47	719.88	679.89	239.098	11.665	C=C bending, alkene
4	1225.51	92.21	0.71	1269.79	1161.24	797.869	28.990	C-O stretching ester
5	1364.06	92.28	0.61	1392.63	1321.21	522.977	15.484	S=O stretching, sulfonamide
6	1421.20	92.64	0.11	1425.48	1411.20	104.054	0.813	O-H bending, Carboxylic acid
7	1458.33	92.63	0.19	1466.90	1451.19	114.279	1.407	C-H bending, alkane
8	1496.90	92.91	0.02	1501.18	1495.47	40.420	0.113	C-H bending, alkane
9	1519.75	92.85	0.20	1528.32	1514.04	100.995	1.702	N-O stretching, nitro compound
10	1541.18	92.71	0.41	1549.75	1528.32	151.024	3.449	N-O stretching, nitro compound
11	1558.32	92.67	0.44	1565.46	1549.75	111.431	3.219	C=C stretching cyclic alkene
12	1625.45	91.60	0.05	1631.16	1622.59	71.657	0.211	C-H bending, aromatic
13	2391.04	95.97	14.48	2835.25	2359.61	1065.661	3508.485	C-H bending aromatic compound
14	2848.11	98.09	1.04	2875.24	2835.25	41.580	9.218	C-H stretching, alkane

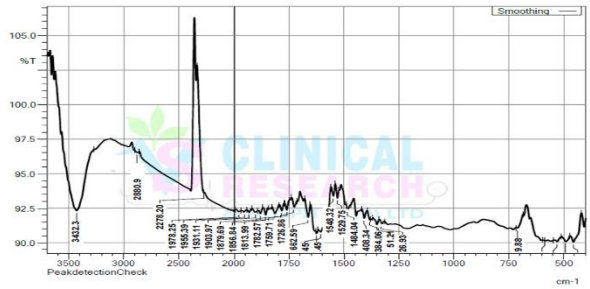
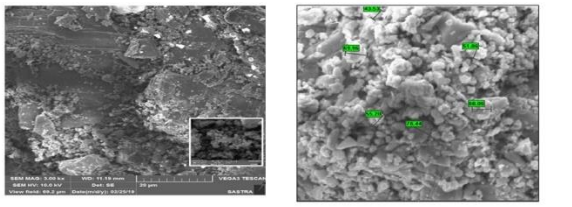


Figure 3: FTIR spectrum of Purified Thalagam (T3)

3.2. SEM analysis of Unpurified and purified Thalagam

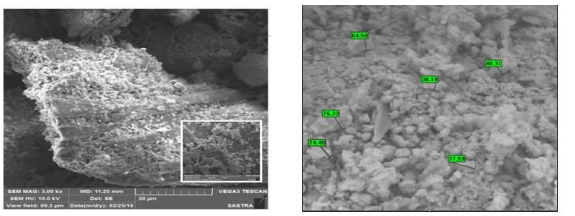
It was observed from the SEM analysis of unpurified Thalagam(T1) that the average particle size was found to be 59.84 ± 12.65 µm and the size of the particles ranges from 43.53 µm to 78.44 µm. As shown in Figure 4.



Un purified Thalagam (T1) - Cluster view Un purifed Thalagam (T1) – Categorised View

Figure 4: SEM analysis of Un-Purified Thalagam (T1)

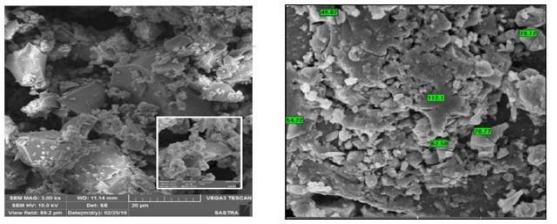
It was observed from the SEM analysis of purified form T2 that the average particle size of the sample was found to be 62.22 ± 18.44 µm and the size of the particles ranges from 40.31 µm to 77.59 µm. As shown in Figure 5.



Purified Thalagam (T2) - Cluster view Purifed Thalagam (T2) – Categorised View

Figure 5: SEM analysis of Purified Thalagam (T2)

It was observed from the SEM analysis of sample T3 that the average particle size of the sample was found to be 71.34 ± 22.47 µm and the size of the particles ranges from 49.82 µm to 112.1 µm. As shown in Figure 6.



Purified Thalagam (T3) - Cluster view Purified Thalagam (T3) – Categorised View

Figure 6: SEM analysis of Purified Thalagam (T3)

3.3. Results of XRF analysis of Unpurified and purified Thalagam

XRF analysis results of the Unpurified and purified Thalagam indicates the presence of Arsenic, Lead, Copper, Silicon, Calcium, Zinc, Iron, Barium, Magnesium, Molybdenum and Sulfur in native form and further it was also documented the oxide forms of Ferric oxide, Sulfur trioxide, Magnesium oxide, Calcium oxide, Lead monoxide, Silicon Dioxide, Barium oxide, Zinc oxide etc in both purified and unpurified form. As shown in Table 4 to 6.

Table 4: Element in oxide form

Formula	Concentration (%)
SO_3	47.29
As_2O_3	44.28
MgO	5.46
CaO	0.84
SrO_2	0.44
Sb_2O_3	0.37
PbO	0.34
BaO	0.34
Fe_2O_3	0.18
SeO_2	0.13
Al_2O_3	0.08
Ru	0.08
MOO_3	0.05
Cl	0.05
K_2O	0.03

Element in Native Form

Formula	Concentration (%)
As	61.77
S	32.00
Mg	2.69
Ca	0.92
Pb	0.60
Ba	0.48
Sb	0.40
Si	0.32
Fe	0.20
Ru	0.16
Cl	0.12
Na	0.08
MO	0.08
Al	0.08
K	0.04
Cu	62 PPM

Table 5: Element in oxide form

Formula	Concentration (%)
SO_3	48.94
As_2O_3	43.59
MgO	5.27
CaO	0.79
PbO	0.59
SiO2	0.29
BaO	0.29
MOO_3	0.05
Fe_2O_3	0.05
Al_2O_3	0.05
Cao	96 PPM
ZnO	69 PPM

Element in Native Form

Formula	Concentration (%)
As	60.80
S	33.63
Mg	2.70
Pb	1.09
Ca	0.93
Ba	0.42
Si	0.29
MO	0.08
Fe	0.08
Al	86 PPM
Ca	85 PPM
Zn	60 PPM

Table 6: Element in oxide form

Formula	Concentration (%)
SO3	49.82
As2O3	48.89
MgO	4.12
CaO	0.52
PbO	0.21
BaO	0.17
I	0.05

Element in Native Form

Formula	Concentration (%)
As	63.21
S	34.72
Mg	1.31
Pb	0.58
Ca	0.81
Ba	0.22

4.Discussion

The evaluation of physicochemical properties such as color, odor, microscopic examination, loss on drying, moisture contents and ash values of herbal materials is the important for quality control of raw materials. Heavy metal testing is of prime importance because poisoning has been reported following the ingestion of herbal remedies [15-17]. Nevertheless, the residues of fertilizers, pesticides and herbicides should also be monitored by using well accepted methods. Fourier Transform Infrared (FTIR) or Attenuated Transform Infrared (ATIR) spectroscopy is an important non-destructive quality assessment tool which facilitates the analysis of crude powders on solid matrix. The comparison of FTIR spectra of the sample with the reference is a powerful tool for the acceptance or rejection of samples. It was observed from the FT-IR spectra analysis of the purified and purified thalagam that presence of aliphatic ketones and esters are more in unpurified form than in purified samples, similarly purified form of thalagam reveals the presence of C-Br and C-I stretching which are potentially active functional group

XRF is an elemental analysis technique, which relies on recording the characteristic secondary X-rays

emitted from specific atoms when the materials are irradiated by a focused X-ray beam [18]. XRF has attracted increasing consideration and the relevant technique has been widely used for non-invasive imaging of thick and deep biological specimens with high spatial and temporal resolution [19].X-ray fluorescence (XRF) imaging is a powerful technique for the quantitative mapping of distributions and dynamics of elements and chemical species at the spatial sub micrometer resolution within biological samples [20].XRF analysis results of the Unpurified and purified Thalagam indicates the presence of Arsenic, Lead, Copper, Silicon, Calcium, Zinc, Iron, Barium, Magnesium, Molybdenum and Sulfur in native form and further it was also documented the oxide forms of Ferric oxide, Sulfur trioxide, Magnesium oxide, Calcium oxide, Lead monoxide, Silicon Dioxide, Barium oxide, Zinc oxide etc in both purified and unpurified form.

Scanning electron microscopy (SEM) has been almost universally applied for the surface examination and characterization of both natural and man-made objects. Although an invasive technique, developments in electron microscopy over the years has given the microscopist a much clearer choice in how invasive the technique will be. It was observed from the SEM analysis of the purified and purified thalagam that the average particle size of the purified form was high when compare to that of the un purified form.

5.Conclusion

From the data's of the present investigation it was concluded that the purified form of thalagam has reveals the presence of active functional groups like SO,OH,NO,CBr and CI, presence of these groups may be responsible for the desired activity pharmacological activity . XRF analysis it was observed that the elemental composition is greatly reduced in the purified form when compare to that of the unpurified form. It was concluded that the increased particle size may be due to formation of agglomeration between inter particular attraction that may happened due to the process of purification.

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