Standardization of Metal-Based Herbal Medicines

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Abstract: Problem statement: Standardization of herbal drugs is a burning topic in herbal drug industry today. Standardization is difficult because they are usually mixtures of many constituents and the active principle in most cases is unknown. However it is possible to generate a physico-chemical fingerprint for the standardization of these drugs with reference to authentic drugs, for monitoring variation between preparations from different companies and for evaluating batch to batch changes during long term storage. The present study was on the characterization of some of the metal based herbal medicines which were in traditional use for treating infectious diseases. **Approach:** Modern techniques such as X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Analysis (EDAX), Infrared spectroscopy (IR), Thermogravimetric Analysis (TGA), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Transmission Electron Microscopy (TEM) were used to generate physico-chemical fingerprint. **Results:** Our results showed that the metals in most of the herbal medicines were in the oxide or sulfide form. Some of the medicines contained metal particles in the nano range. **Conclusion:** In order to bring out high quality herbal products, herbal drugs must be standardized using modern techniques.

Key words: Bhasmas, chendurams, XRD, EDAX, ICP-OES

INTRODUCTION

Traditional herbal medicines have been widely used for thousands of years in many countries. Metals have been used in disease treatment since time immemorial. Gold in medicine was mentioned by Roman physician Pliny and Greek philosopher Dioscrides. Hippocrates, the father of modern medicine, explained the beneficial healing and anti-disease properties of silver. In olden days, people used silver bottles for storing water, wine and milk and to prevent spoiling. Siddha medicine is a form of South Indian medicine which is believed to have been developed by the Siddhars, the ancient supernatural spiritual saints of India. In siddha medicines, apart from gold and silver, mercury, sulphur, mica, arsenic, zinc and several other minerals, gems, shells, horns are treated with indigenous herbs and are given as bhasmas and chendurams. A bhasma means a fine ash obtained though incineration. Chendurams are prepared by the process of sublimation and they are much more potent than bhasmas. Siddha medicine has immense faith in the miracles of mercurial drugs and in the prolongation of life through rejuvenating treatments and intense

yogic practices[1]. Silver, gold, zinc, copper and other metals which are well known to have anti-microbial effect in modern medicines have been used as wonderful life saving drugs against infectious diseases for thousands of years without any adverse effects. Role of these herbo-mineral preparations for curing skin diseases such as psoriasis, eczema, alopecia, diabetic ulcer, warts, vitiligo and leprosy are well studied[2].Most of the medicines are mixture of compounds and because of its synergistic action, toxicity is being diminished, thereby increasing bioavailability through the cells of the body. Treating the minerals with herbal juices may lead to reduction (trituration) in particulate size even up to nano levels (less than 100 nm) enabling increased potency. These drugs are known to be effective even in low concentration.

Various commercially available medicines such as Linga Chenduram, Poorna Chandrodaya Chenduram, Kshaya Kulanthaga Chenduram, Velli Parpam, Naga Chenduram and Naga Parpam which contain noble and other metals were used for analysis. These medicines are particularly used for treating infectious diseases. So far, nothing is known about the chemical composition of these medicines and the scientific basis of its application

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in treating infectious diseases. Proper standardisation techniques for checking the quality are inadequate on these medicines to meet the criteria to support its use worldwide. The aim of our present study is to:

- Analyze the different brands of medicines (bhasmas or powder forms) using several modern analytical techniques to help in quality control and standardization of the drugs
- Collating the data from multiple approaches to suggest the likely speciation of metals present in these complex powders and their bio-absorption potential
- To assess the content and likely potency of very expensive preparations based on noble and other metals

MATERIALS AND METHODS

Siddha medicines like Poorna Chandrodaya Chenduram (PCC), Kshaya Kulanthaga Chenduram (KsKc), Velli Parpam (SP), Naga Chenduram (NC), Naga Parpam (NP) and three different popular brands of Linga Chenduram (LC1, LC2 and LC3) were procured from government and private market. These commercially available formulations are used for treating various diseases in traditional clinical practice in India and are usually prepared from purified metal, triturated with decoction of herbal juices. They are generally prescribed in the dose of 100-200 mg day^{-1} and recommended to be taken with a suitable adjuvant.

Perkin-Elmer 5300 DV ICP-OES was used for the determination of elements. About 0.1-0.2 g of the sample was digested using HNO_3: Perchloric acid (2:1). After the digestion was complete, the flask was allowed to cool and the contents were transferred to a beaker and heated to remove all the acid. The resulting solution was diluted to 50 mL with deionized water. Perchloric acid was used because it does not form complexes with metals and metalloids[3].

The powder XRD patterns of the solid samples were recorded on X'pert pro analytical X-ray diffractometer using CuKα radiation filtered by a nickel foil over the range of diffraction angle 10-70°. The wave length of the radiation used was 1.5405A°.

The elemental composition of the samples was determined by JEOL ASM 3500 SEM with EDAX. A representative portion of each sample was sprinkled onto a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM and EDAX examination.

IR spectra in the region (4000-450 cm^{-1}) were recorded on Perkin-Elmer FTIR Spectrophotometer in KBr pellets. Thermograms were recorded in air atmosphere on a NETZSCH simultaneous thermoanalyzer.

The presence of nanoparticles was tested by Dynamic Light Scattering (DLS) method using Brookhaven 90 plus particle size analyzer, after suspending the materials in aqueous dispersing phase and filtering through 0.45 μ membrane filter. TEM was used to confirm the presence of nanoparticles.

RESULTS

ICP-OES analysis: The different samples show variation in metal content (Table 1). Hg is the main component in all brands of LC. There is a wide variation in content of different elements such as As, Ca, Cd, Cr, Cu, Fe and Mg between the three different brands LC1, LC2 and LC3 from three different companies. This could be due to the difference in the amount of plant juices added during preparation by different manufacturers. But these elements constitute only less than 1% of the samples. PCC contains Hg as the main constituent and 0.8 mg g^{-1} of the noble metal gold. KsKc showed the presence of many elements, particularly Ca, Fe, Mg and As in higher levels, 7.524 mg g^{-1} gold and Hg was absent. SP contains Ag in large amount and also Ca. NC and NP contain large quantities of Zn, Ca and Mg.

Table 1: ICP-OES analysis of metal based medicines

Element	LC1 (mg g^{-1})	LC2 (mg g^{-1})	LC3 (mg g^{-1})	PCC (mg g^{-1})	KsKc (mg g^{-1})	SP (mg g^{-1})	NC (mg g^{-1})	NP (mg g^{-1})
Ag	1.340	0.002	0.002	0.1790	0.1312	325.5000	0.00397	0.00926
Al	BDL	0.584	0.820	0.8449	7.4592	BDL	2.84800	3.10100
As	4.730	2.770	4.360	2.0920	32.4640	0.0195	0.06230	0.02500
Ca	0.480	1.551	1.590	12.5690	117.9680	98.0530	35.81400	39.74500
Cd	0.100	0.070	1.100	0.0546	0.8496	BDL	0.00390	0.00390
Cr	0.230	0.010	0.010	0.0377	0.0481	0.0310	0.01120	0.00390
Cu	0.120	0.006	-	0.0286	0.2672	0.2530	0.15510	0.04960
Fe	0.180	0.250	0.680	1.8250	117.5680	1.4800	4.38370	4.28300
Mg	0.030	0.230	0.270	0.5038	47.5680	2.2120	10.47600	15.50210
Mn	BDL	-	-	0.0208	0.5680	0.0338	0.19290	0.27340
Ni	0.006	-	-	0.0090	0.2256	0.0130	0.01320	0.02380
Pb	0.009	0.030	0.010	0.0830	0.0320	0.0351	1.67760	0.10130
Zn	0.105	0.110	0.140	0.2290	0.5040	0.1575	388.90000	388.38000
Hg	374.800	412.300	488.100	143.7400	BDL	1.3220	4.24100	BDL
Au	-	-	-	0.8000	7.5240	-	-	-

BDL: Below detectable limit; LC1, LC2 and LC3: Brand 1, Brand 2 and Brand 3 of Linga Chenduram: PCC: Poorna chandrodaya chenduram, kskc- kshaya kulanthaga chenduram, SP: Velli Parpam, NC : Naga Chenduram, NP: Naga Parpam
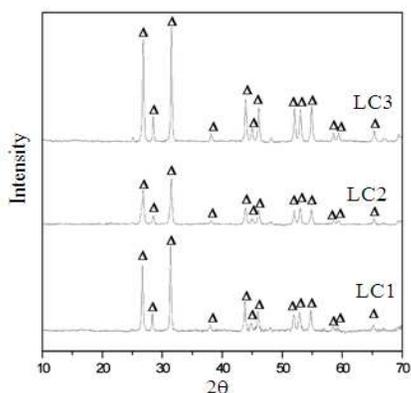
Fig. 1: XRD pattern of Linga Chendurams; LC1, LC2 and LC3: Brand 1, 2 and 3 of Linga Chenduram; Δ: HgS

Fig. 2: XRD pattern of standard ZnO and zinc based medicines; NC: Naga Chenduram; NP: Naga Parpam; *: ZnO 0: Unknown
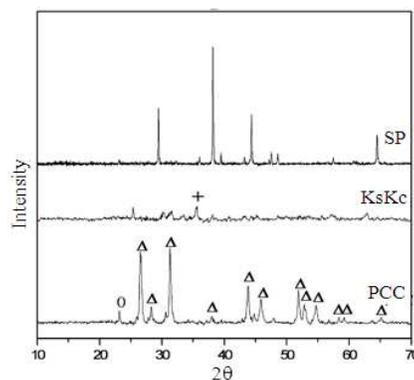

Fig. 3: XRD pattern of Gold and Silver based medicines; PCC: Poorna Chandrodaya Chenduram; KsKc: Kshaya Kulanthaga Chenduram; SP: Velli Parpam; Δ: HgS, 0: Unknown, +: Fe_3O_4
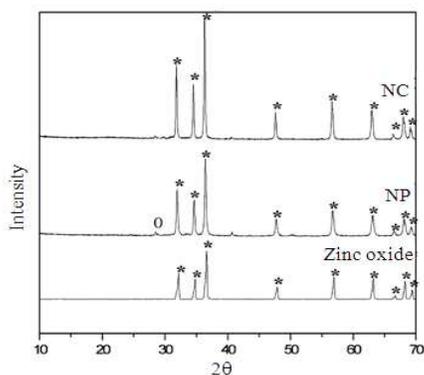
Table 2: EDAX analysis of metal based medicines

Elements present	LC1		PCC		KsKc		SP	
	wt (%)	At (%)	wt (%)	At (%)	wt (%)	At (%)	wt (%)	At (%)
Hg	79.89	38.59	30.27	5.59	-	-	-	-
S	18.58	56.15	53.55	61.88	3.40	2.76	-	-
O	-	-	11.86	27.48	34.32	55.94	30.26	46.49
Na	-	-	2.41	3.88	2.18	2.47	-	-
Si	1.53	5.26	-	-	11.13	10.33	0.86	0.75
C	-	-	-	-	-	-	15.03	30.75
Al	-	-	-	-	-	-	0.52	0.48
Ca	-	-	1.10	1.02	12.35	8.04	21.76	13.35
Au	-	-	0.81	0.15	0.94	0.12	-	-
Mg	-	-	-	-	10.16	10.90	1.26	1.27
Ag	-	-	-	-	-	-	30.31	6.91
Cl	-	-	-	-	3.19	2.34	-	-
K	-	-	-	-	0.69	0.46	-	-
Ba	-	-	-	-	0.25	0.05	-	-
Fe	-	-	-	-	11.04	5.16	-	-
Cu	-	-	-	-	0.45	0.18	-	-
Pb	-	-	-	-	9.90	1.25	-	-

LC1: Brand 1 of Linga Chenduram; PCC: Poorna chandrodaya chenduram; KsKc: Kshaya kulanthaga chenduram; SP: Velli parpam

XRD: The XRD patterns of the metal based herbal medicines are shown in Fig. 1-3. XRD scans of three different brands of LC (Fig. 1) are found to be similar (showed main peaks near 31.5 and 26.7°) and correspond to the standard peaks for HgS. NC and NP showed main peaks near 36.3 and 31.8°, which correspond to XRD pattern of standard ZnO (Fig. 2). XRD pattern of PCC coincide well with the standard peaks of HgS. KsKc showed the presence of many elements. The peak at 35.52° is due to Fe_3O_4. The form of gold, arsenic and silver in PCC, KsKc and SP could not be identified using XRD pattern (Fig. 3).

SEM and EDAX analysis: SEM images of LC1, PCC, KsKc and SP showed difference in size and agglomeration of the particles (Fig. 4a-d). Agglomeration of the particles is due to repeated cycles of calcinations involved in preparation[4]. The elemental composition of the drug samples were analyzed by EDAX (Table 2).

From this analysis, it was confirmed that 79.89% Hg present in LC1 is equivalent to 92.63% HgS (as per the stoichiometry) while, other elements are S and Si. In PCC, HgS is the main component and a small percentage of Hg may also occur in oxide form. KsKc shows the presence of many elements, so the exact form could not be concluded. This is in correlation with ICP-OES and XRD data. PCC and KsKc contain small percentage of gold. SP showed higher levels of Ag, Ca and O_2. So, the most probable forms may be silver oxide and calcium oxide. SEM and EDAX provide good estimate of the concentration of main elements in the sample in a significantly faster way compared to ICP-OES method. Furthermore, it provides useful information on the distribution of the element forming the sample and their possible chemical form[5].
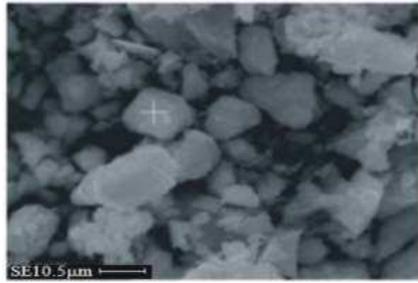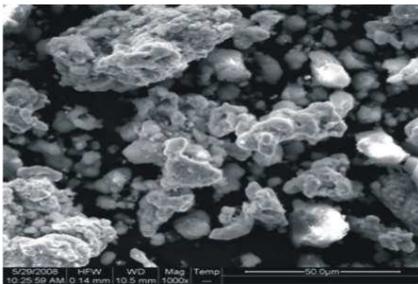
(a)
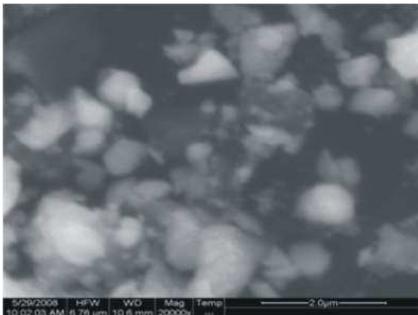
(b)
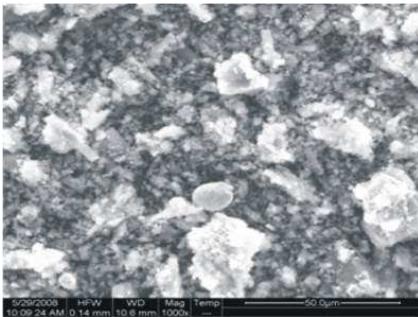
(c)

(d)

Fig. 4: (a): SEM of LC1; (4b): PCC; (4c): KsKc; (4d): SP
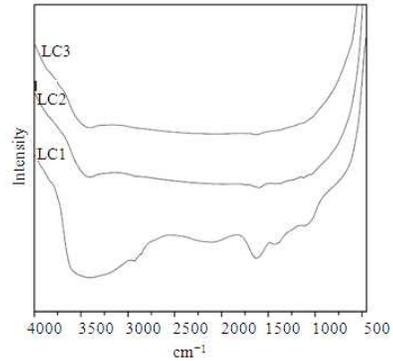

Fig. 5: FT-IR of Linga Chendurams; LC1, LC2 and LC3: Brand 1, 2 and 3 of Linga Chenduram
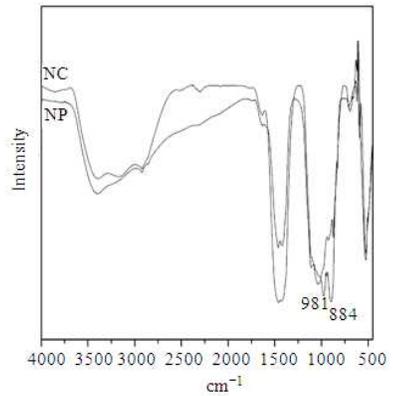

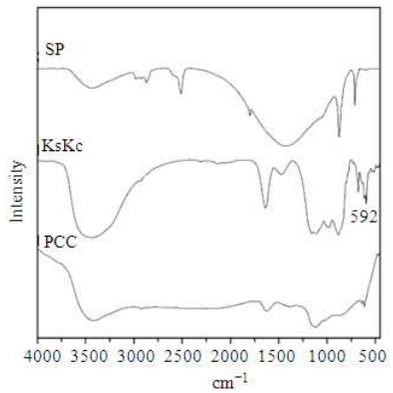Fig. 6: FT-IR of Zinc based medicines; NC: Naga Chenduram; NP: Naga Parpam

Fig. 7: FT-IR of Gold and Silver based medicines; PCC: Poorna Chandrodaya Chenduram, KsKc- Kshaya Kulanthaga Chenduram; SP: Velli Parpam

FTIR: FT-IR spectra of the drug samples show that they do not contain organic compounds (Fig. 5-7). The absence of organic matter is further proof of proper incineration during the preparation of these medicines and the absence of any external organic contamination. This is in agreement with the earlier infrared study of Bhasmas[6]. The three different brands of LC showed a
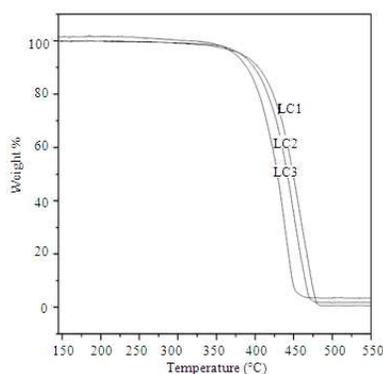
Fig. 8: Thermogravimetric Analysis of Linga Chendurams; LC1, LC2 and LC3: Brand 1, 2 and 3 of Linga Chenduram
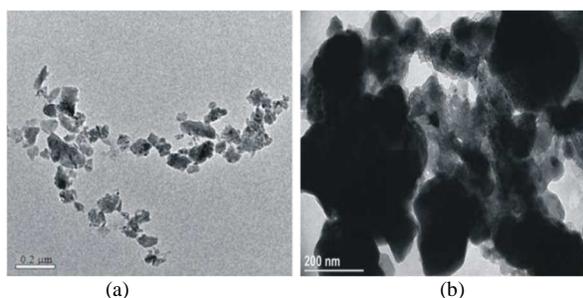

Fig. 9: (a): TEM of LC1; (b): PCC

similar pattern and it can be used as a chemical fingerprint (Fig 5). IR Spectrum of PCC is similar to Linga Chenduram. The peak at 592 cm^{-1} in KsKc is due to Fe_3O_4[7]. The exact form of silver in SP could not be concluded using IR Spectrum (Fig. 7). The broad peaks at 3400 and 1600 cm^{-1} in all the samples are due to the characteristic OH stretching (γ-OH) H OH bending (δ-OH) vibrational bands due to adsorbed water in the sample[8].

TGA: TGA curves for three brands of LC are shown in Fig. 8. When all the three brands of LC are heated in the presence of air, it showed complete decomposition at 500°C itself. Hardy *et al.*[9] report that if HgS is heated in air it is first converted to mercuric oxide and then decomposes to mercury vapour and oxygen at about 500°C. Since the TGA curves show the complete decomposition at 500°C, it confirms the presence of mercuric sulphide. The presence of only HgS in LC is also confirmed by XRD data.

DLS: Ancient literatures regarding the preparation of Bhasmas give importance to particle size.

Table 3: Particle size analysis of metal based medicines by dynamic light scattering

Percentage of particles	Particle size less than in (nm)							
	LC1	LC2	LC3	PCC	KsKc	SP	NC	NP
10	88.8	90.8	69.2	139.9	397.1	188.7	357.8	233.9
20	107.1	105.2	82.1	174.7	521.3	232.1	452.8	305.3
30	122.6	117.1	92.9	205.1	643.7	269.6	536.7	370.0
40	137.6	128.2	103.2	235.2	750.5	306.2	620.4	435.9
50	153.3	139.5	113.9	267.2	877.7	344.9	710.3	508.0
60	170.7	151.9	125.6	303.6	1026.5	388.5	813.2	592.1
70	191.6	166.3	139.5	348.1	1213.0	441.3	940.0	697.5
80	219.4	185.0	157.9	408.7	1477.7	512.5	1114.2	845.5
90	264.6	214.4	187.3	510.4	1625.0	630.4	1409.7	1103.3
95	308.8	242.1	215.6	613.0	2428.9	747.7	1711.8	1374.2

LC1, LC2 and LC3: Brand 1, Brand 2 and Brand 3 of Linga Chenduram; PCC: Poorna Chandrodaya Chenduram, KsKc: Kshaya Kulanthaga Chenduram, SP: Velli Parpam, NC: Naga Chenduram, NP: Naga Parpam

It is mentioned in the texts that the process of Marana (incineration) should be repeated to get the smaller particle size. All the medicines were screened for nanoparticles (Table 3). LCs alone show narrow range of distribution of particles. Brand 3 of LC had 10% of the particles below 69.2 nm. PCC had particle size close to nano. It is surprising that thousands of years old Indian system of medicine had knowledge of nanoscience, which modern science has just started exploring in the 21st century.

TEM analysis: The medicines LC1 and PCC which showed particles in the nano or near nano range were further analyzed using TEM (Fig. 9a-b). TEM Pictures show that particles are not of uniform size. There is more number of particles in the range of 40-50 nm in LC and the particles in PCC are slightly larger. In both LC1 and PCC, the presence of HgS in the nanoparticles was also confirmed by EDAX attached with TEM. So far there appears to be no reference for HgS in nanoform being used in traditional medicines.

DISCUSSION

LC is used to treat fevers, skin diseases and also venereal diseases. It is given along with honey in the dose of 50-100 mg day^{-1}. Particle size analysis by DLS and TEM showed the presence of nanoparticles (below 100 nm). This may enhance bioabsorption and efficacy. EDAX, XRD and TGA confirmed the presence of HgS as the main constituent. HgS is less toxic and less absorbed than other forms of mercury such as elemental mercury and organic mercury. Jiunn-Jye Chuu *et al.*[10] report that the low dosage of HgS (0.1 g kg^{-1} day^{-1}) for 7 consecutive days did not produce any toxic effects in experimental mice. The toxic effect of HgS (even 1.0 g kg^{-1}) disappeared completely in 5 weeks

subsequent to the cessation of its administration. But, methyl mercury (2.0 mg kg^{-1} day^{-1}) persisted at least 11 weeks subsequent to the cessation of its administration[10]. Long term use of HgS may lead to the accumulation of mercury in liver and kidneys. The correct preparation methods, appropriate dosage, disease condition, age and drug combinations greatly influence HgS toxicity. Three brands of LC showed a similar pattern in XRD, FTIR and TGA and indicate that these techniques can be used as rapid physico-chemical fingerprints for quality control of these medicines.

PCC is generally used for treating tuberculosis, jaundice, fevers and bronchitis. It is also used in rat bite, cancerous ulcers and myalgia. It is administered to patients along with Karpooraadi chooranam or honey or betel leaf juice. The prescribed dose is 0.1-0.2 g. Particle size analysis by DLS showed presence of 10% of the particles below 139.9 nm. TEM showed particles slightly larger than Linga Chenduram. EDAX showed the presence of Hg, S, O_2 and small percentage Au. XRD confirmed the presence of HgS as the main constituent. PCC did not show any characteristic peak in IR Spectroscopy and was similar to LC. HgS has been used for thousands of years in traditional medicines. Gold and its compounds have been used historically in drugs for treating wide variety of ailments. Nanosized gold particles proved to be effective in ameliorating the symptoms of mycobacterial-, collagen- and pristane- induced arthritis in rat models. Gold in the traditional Indian Ayurvedic medicine, Swarna bhasma (gold ash), has been characterized as globular particles of gold with an average size of 56-57 nm[6]. The form of the gold here in PCC could not be concluded.

KsKc is used to treat all diseases of the respiratory system including tuberculosis. It is generally administered to patients along with honey and Thirikadugu chooranam and is usually prescribed for 10 days. It has larger size compared to other Chendurams. EDAX and ICP showed the presence of mixture of many elements viz. As, Ca, Fe, Pb and a small percentage of Au. Arsenic sulphide and Arsenic trioxide have long been used in traditional medicines for various diseases, yet arsenic can be highly toxic and carcinogenic. Inorganic arsenic is now accepted in Western medicine as a chemotherapeutic agent against certain hematopoietic cancers. Orpiment (As_2S_3) and Realgar (As_4S_4) are used externally for various skin diseases. Realgar is frequently included as an ingredient in oral traditional remedies for its antipyretic, anti-inflammatory, antiulcer, anti-convulsive and anti-schistosomiasis actions. Total arsenic content alone

should not be considered for safety evaluation and the form in which it is present is important. The form of As and Au here in KsKc could not be concluded. XRD and IR Spectra showed the presence of Fe_3O_4 as one of the components. Chronic administration of Swarnabhasma (which contains gold, lead and arsenic) showed no sign and symptom of liver and kidney toxicity in experimental animal model. Chronic Swarnabhasma-treated animals showed significantly increased superoxide dismutase and catalase activity, two enzymes that reduce free radical concentrations in the body. These antioxidant properties may have some important role in the rejuvenation of health [11].

SP is used to treat cough, tuberculosis, piles, leucorrhoea, gonorrhoea and spermatorrhoea. It is given in the dose of 100 mg twice a day with butter. The patients are advised to avoid tamarind and tobacco during treatment. It has 10% of the particles below 188.7 nm. EDAX showed the presence of Ag and O_2 but the exact form in which Ag is present could not be identified by XRD and IR data. Silver is known to possess strong antimicrobial properties both in its metallic and nanoparticle forms. The Fe_3O_4 attached Ag nanoparticles can be used for the treatment of water. Silver sulfadiazine provides better healing of burn wounds. The silver nanoparticles are reported to show better wound healing capacity, cosmetic appearance and scarless healing when tested using animal model. The microorganisms are unlikely to develop resistance against silver as compared to antibiotics as silver attacks a broad range of targets in the microbes [12].

NC is used to treat ascites, piles, skin diseases, leprosy and white discharge. It is given in the dose of 100-200 mg. with three pungents powder in honey or ghee. NP is used to treat piles, fistula, diarrhea, cough, asthma and dysentery. It is administered along with butter or ghee or Thethan kottai lehyam in the dose of 100-200 mg twice a day. NC has 10% of the particles below 357.8 nm and NP has 10% of the particles below 233.9 nm. NP had overall less particulate size than NC. XRD confirmed the presence of ZnO in both. ZnO nanoparticles are proved to be effective against both Gram-positive and Gram-negative bacteria. They have antibacterial activity against even high-temperature resistant and high-pressure resistant spores. There appears to be an interaction between the bacterial cells and the ZnO particles, which is presumably due to the electrostatic forces [13].

CONCLUSION

Among the metal based herbal medicines studied here, Linga Chenduram is found to have more number

of particles in the nano range. TEM also confirmed the nano particles. They are generally taken with the adjuvants such as butter, ghee or honey. This can lead to better bio-absorption of some of these medicines. EDAX can be used to find the elemental composition at the surface level to identify the possible chemical form, but ICP-OES is a fully adequate technique for the multielemental determination of metals in Bhasmas. EDAX revealed that the medicines had metal mainly in oxide or sulphide form. XRD, IR and TGA analysis can be used as a physico-chemical fingerprint. These fingerprints could explain both the similarities and differences between various samples successfully and is a valuable primary tool for checking the quality control of metal-based medicines. Modern techniques are necessary to standardize and bring out high quality herbal products owing to their complex nature.

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