Indian Journal of Traditional Knowledge
Vol. 8 (3), July 2009, pp. 346-351

Preparation and characterization of a mercury based Indian traditional drug—*Ras-Sindoor*

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Received 29 August 2008; revised 7 April 2009

The mercury based Indian traditional drug Ras-Sindoor is administered for the various ailments such as syphilis, genital disorders, and for rejuvenation. Pharmaceutical processing of *Ras-Sindoor* involves treating metallic mercury with sulfur and the juice of the aerial root of Banyan tree *(Ficus benghalensis* Linn.*)* and then controlled intermittent heating so that the metallic state is transformed into the corresponding sulfide form. In the study, synthesis and systematic characterization of this drug using various techniques, viz. X-ray diffraction (XRD), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), far infrared spectroscopy (FIR), Fourier transform infrared spectroscopy (FTIR), differential thermal analysis (DTA), thermogravimetry analysis (TGA), energy dispersive X-ray analysis (EDAX) and atomic absorption spectroscopy (AAS) have been reported. Drug contains mercury in the mercury sulfide form (Hg^{2+}) being nanocrystalline (20-50 nm) in nature and associated with the organic contents of the aerial root of the *Ficus benghalensis* Linn. Some specific findings were also made which could be of help for the interpretation of therapeutic value, none-toxicity of *Ras-Sindoor* and for the standardization of such kind of traditional herbo-metallic drugs.

Keywords: *Ayurvedic* drug, *Bhasma*, Traditional medicine, *Ras-Sindoor*
IPC Int. Cl.[8]: A61K36/00, A61P15/00, A61P15/02, A61P31/00, A61P31/04

Ayurveda make use of herbal preparations for their curative effects. Use of metallic herbal preparations (*bhasma*), in which a process termed *bhasmikarana* used to prepare the drug, is unique to the *Ayurveda*. It is believed that the *bhasmikarana* process converts the metal into its specially desired chemical compound, which eliminates the toxicity of the metal and has the necessary medicinal benefits[1-2]. *Ayurvedic* texts provide a list of tests for the efficacy of the *bhasmikarana* process. The tests which are essentially qualitative ensure that the resulting drug is very fine (small grains), has no metallic shine and does not alloy with silver even at higher temperature to which it was subjected[3-6]. However, these qualitative tests do not provide any quantitative information about the composition and the structure of the final drug. For any drug containing heavy metals (for example mercury), such structural information is an absolute necessity[7]. *Ras-Sindoor* is a well known mercury based *bhasma* prescribed for certain diseases, viz. syphilis, genital disorders and also for rejuvenation purposes[4]. The combination of sulfur is believed to have neutralized the toxicity of mercury[8]**.** Some researchers utilized Hg^{203} as tracer to study the pharmaco-kinetics and bio-distribution of *Kajjali* (a sulfur and mercury preparation)[9]. Several others have also worked on the efficacy and safety aspects of mercurial preparations in such traditional drugs[10,11]. None of the work presents the elemental and structural characterization of the drug, which is essential requirement to discuss the non-toxicity and therapeutic value of the mercurial preparations. In the report, the composition and the structure of *Ras-Sindoor* using various techniques, viz. X-ray diffraction (XRD), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), far infrared spectroscopy (FIR), Fourier transform infrared spectroscopy (FTIR), differential thermal analysis (DTA), thermogravimetry analysis (TGA), energy dispersive X-ray analysis (EDAX) and atomic absorption spectroscopy (AAS) have been characterized.

Methodology

Preparation of *Ras-Sindoor*

Ras-Sindoor sample was prepared from raw materials obtained from the pharmacy of the Institute

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of Medical Sciences, Banaras Hindu University, Varanasi. Mercury so obtained was purified through sublimation. For purification of the sulfur, the traditional method using cow's milk and ghee (a milk preparation) was employed. In this method, sulfur mixed with *ghee* was heated up to its melting temperature and the resulting liquid is poured through a filter into a vessel containing boiled milk. Sulfur was on the bottom of this vessel. This process was repeated seven times and the final deposited product was taken out, washed with hot water and dried. Mercury and sulfur thus purified in the ratio (1:6) were mixed with the juice of the aerial root of Banyan tree (*Ficus benghalensis* Linn.). This mixture was placed in an iron mortar and crushed till the whole mixture was converted into a fine black, lusterless powder (*Kajjali*). This fine powder *Kajjali* was filled in a glass bottle (*Kach Kupi*) and heated in a controlled intermittent manner with gradually increasing temperature till the blue flame emerging from the pot disappear and the bottom of the bottle becomes red hot. A red hot iron rod was repeatedly inserted in the neck of the bottle so as to burn any accumulated sulfur at the neck of the bottle. After adequate cooling, the sublimate deposited at the neck of the bottle was collected. The whole heating process required is 7-8 hrs and the highest needed temperature was 650^{o}C.

Structural and chemical characterization of *Ras-Sindoor*

For powder X-ray diffraction (XRD) a Philips 1710 X-ray diffractometer with CuKα radiation (λ=1.5418 A°) operating at 30 KV and 20 mA was used. Pattern was recorded for the angle (2θ) ranging from 5-80 degree at a scanning rate of 3 degree/second. For the characterization of nanostructure if any and the defined phases in the sample, a transmission electron microscope (TEM) was used. X-ray photoelectron spectra (XPS) measurement was performed on ESCLAB MKII instrument, using none monochromatized MgKα X-ray as the excitation source.

The Infrared (IR) spectrum in the low frequency region (50-400 cm^{-1}) was recorded on a Bruker IFS 66 V/S vacuum Fourier transform interferometer, where as the spectra from 400-4,000 cm^{-1} region were recorded using FTIR spectrometer. Thermograms DTA and TGA were recorded in a Nitrogen atmosphere on a Pyris Diamond thermal analyzer EXSTAR 6000, Perkin Elmer. The sample was placed in an alumina crucible and the temperature was varied from 40-400^{0}C. EDAX attached to TEM (CEM, CM-12) was used for the detection of various elements in the sample. For quantitative detection of trace metals in parts per million (ppm) an atomic absorption spectrophotometer was utilized. Sample preparation for AAS was done as per the laid procedure[12].

Results

XRD, TEM and XPS analysis

XRD pattern of *Kajjali* shows peaks due to free sulfur, mercury oxide and mercury sulfide (JCPDS File number-20-1227, 01-0896, 02-461, respectively) while the XRD pattern of *Ras-Sindoor* shows peaks only due to mercury sulfide ((JCPDS File number-02-461) (Fig.1). No extra diffraction peaks were observed in the case of *Ras-Sindoor* confirming that while in the initial stages of the processing of the medicine (before the heat treatment) mercury oxide and free sulfur are present in significant amount while after heat treatment only mercury sulfide remains in the product. The diffraction peaks in the XRD pattern of *Ras-Sindoor* corresponding to mercury sulfide become sharper and intense compared to *Kajjali* sample as well as some new peaks appears due to mercury sulfide, which were not present in the *Kajjali* sample. This observation confirms that the heat treatment of *Kajjali* helps in the formation of mercury sulfide and increases the crystallinity in the sample. The crystallite size was calculated from XRD pattern following the Scherrer equation $t = \lambda \times 0.9/ (\beta \times \text{Cos}\theta)$. Here, t is the crystallite size for (h k l) plane, λ is the wavelength of the incident X-radiation [CuK_{α} (0.1542 nm)], β is the full width at half maximum (FWHM) in radians and θ is the diffraction angle for (h k l) plane. The above equation yields t = 25-50 nm. It is notable here that the FWHM in case of *Kajjali* is high in comparison to the finally prepared *Ras-Sindoor* samples that confirms that the size of the crystallite increases. It is obviously due to heat treatment of the *Kajjali* sample.

TEM image of the drug sample shows spongy like structure with the particle size lying in the micro range (Fig.2). From the image it is clear that several crystallites are agglomerated in a signal particle giving rise to microcrystalline structure with loss of grain boundaries. XPS analysis provides valuable information for the surface state of the drug sample. A typical survey spectrum of the drug *Ras-Sindoor* confirming the presence of mercury and sulfur was

observed (Fig.3). In addition, it also shows the presence of C peak as well as O peak. Although the presence of the Mg, Ca, and Fe was shown in EDAX analysis, these ions were not observed in XPS analysis, indicating their absence on the surface. Presence of C and O, which are the building blocks or the organic molecules, on the surface of the drug by XPS supports the idea of the coating of organic molecules on the surface of the metallic compounds. High resolution spectra (Fig. 4) at Hg core level showed the presence of the peaks at 100.28 eV and 104.32 eV corresponding to Hg ($^4f_{5/2}$) and Hg ($^4f_{7/2}$) while S core level showed at 161.8 eV corresponding to S ($^2P_{3/2}$), respectively for HgS phase[13]. Thus the XPS analysis also confirms the presence of Hgs phase in the sample.

IR spectra analysis

FIR spectrum of *Ras-Sindoor* in the region (50-400 cm^{-1}) was studied (Fig.5). Crystalline mercury sulfide (HgS) is known to have absorption at 83, 92 and 100 cm^{-1} and their presence in the present FIR spectra indicate that *Ras-Sindoor* is essentially mercury sulfide[14]. This observation supports the XRD analysis. FTIR spectrum of *Ras-Sindoor* in the region from 400-4,000 cm^{-1} is shown (Fig. 5). There are fairly sharp peaks at 768, 1285, 1364, 1422, 2724 and 2870 cm^{-1} which indicate the presence of the organic compounds in the drug. These arise probably from the aerial root of the *Ficus benghalensis* Linn.[15-17]. FTIR spectrum of the powder of the aerial root also seems to confirm the conjection. The presence of appreciable concentrations of C, N, and O (Table 1) also suggests the presence of organic molecules in the drug. It would not be unexpected if the organic molecules also play an important role in the medicinal properties of these drugs. Therefore, a systematic study for their pharmacological activity would be desirable.

DTA and TGA analysis

The thermal analysis (DTA) plot for *Ras-Sindoor* shows three endothermic peaks in the range of 100-170 °C which could be indicative for the decomposition of water molecules (Fig. 6). On further heating another sharp peak is seen at 354°C which also corresponds to a weight loss of 9.54% (TGA). This may correspond to melting of mercury sulfide (melting temperature 344°C) present in the sample. Weight loss could also be due to the burning away of some attached organic molecules. Thus, thermal analysis supports the presence of mercury sulfide observed by XRD and FIR analysis and organic matter observed by FTIR analysis.

EDAX and AAS analysis

In addition to the metal Hg used in the drug, other metals are also expected in the drug which enters in it during its pharmaceutical processing. EDAX has been used to detect the elements present a considerable amount where as the AAS method is used to detect any elements in trace amount. Chemical compositions of *Kajjali* and *Ras-Sindoor* using EDAX (Table 1) and trace metal composition of Ras-Sindoor and for the aerial root of *Ficus benghalensis* Linn detected by AAS have been listed (Table 2). Abundance of C, N, and O in the drug was observed which is obviously from the plant extract used in the preparation of the drug. Sulfur is also abundant in *Kajjali* but proportion is greatly reduced in *Ras-Sindoor*. This is obvious as heat treatment removes a large amount of free sulfur and only the sulfur bound in mercury sulfide remains in the product. XRD analysis of *Kajjali* and

Table 1—Macroelement composition of *Ras-Sindoor* by Energy dispersive X-ray analysis

Element	*Kajjali*		*Ras-Sindoor*	
	Weight %[a]	Atomic %[a]	Weight %[a]	Atomic %[a]
C	35.69	44.66	25.74	30.36
N	27.44	29.45	41.37	41.84
O	23.56	22.14	30.14	26.68
Mg	0.56	0.34	1.12	0.65
S	6.13	2.88	0.88	0.39
Ca	0.07	0.03	0.04	0.04
Fe	0.04	0.01	0.08	0.02
Hg	6.51	0.49	0.63	0.04
Total	100.00	100.00	100.00	100.00

[a]Based on ZAF quantification (standardless)

Table 2— Trace element composition of *Ras-Sindoor* and Banyan aerial root

Element (in ppm)	*Ras-Sindoor*	Aerial root of Banyan tree
Na	27.5±2.062	71.50±2.22
K	19.8±1.039	59.66±1.74
Cu	0.64±0.039	5.66±0.311
Zn	04.3±0.352	33.06±0.353
Ni	0.07±0.005	00
Mn	0.21±0.015	3.28±0.82
Cd	00	00
Cr	0.09±0.018	0.60±0.0
Pb	01.7±0.135	3.66±0.84

Values are arithmetic mean ± standard deviation of three determinations in each caseppm- parts per million

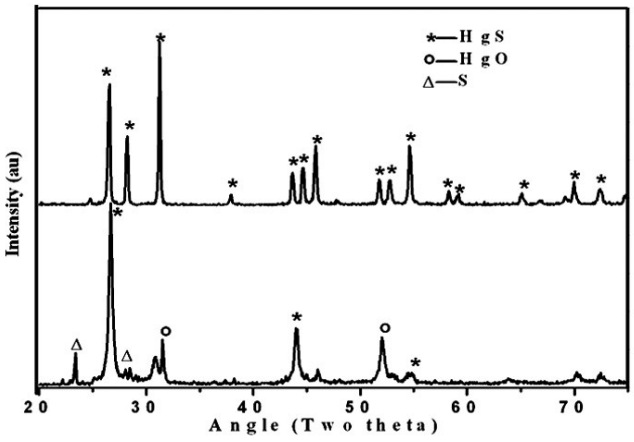

Fig.1XRD pattern of *Kajjali* & *Ras-Sindoor*

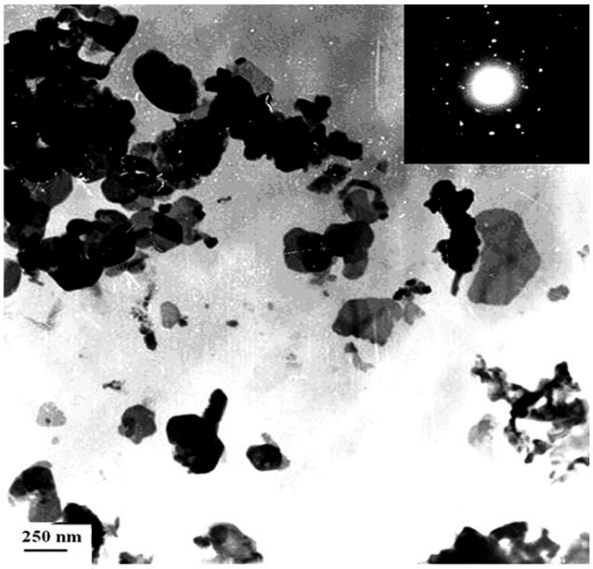

Fig.2 TEM image of *Ras-Sindoor*

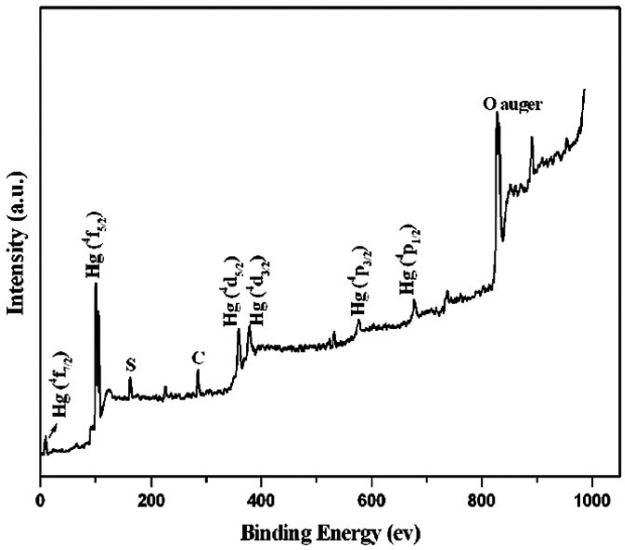

Fig.3 XPS spectrum of *Ras-Sindoor*

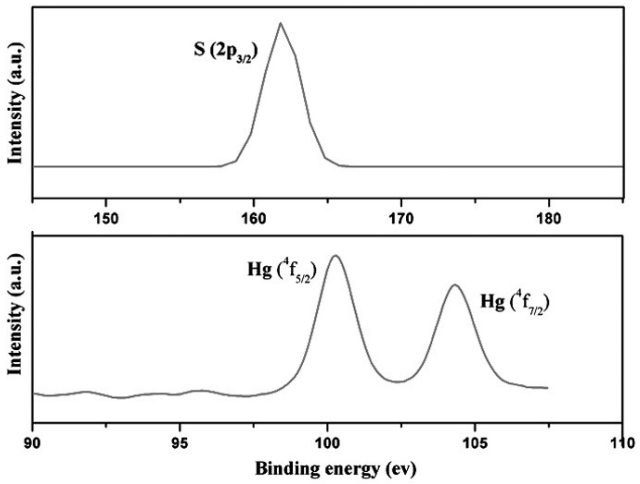

Fig.4 XPS spectrum of drug samples

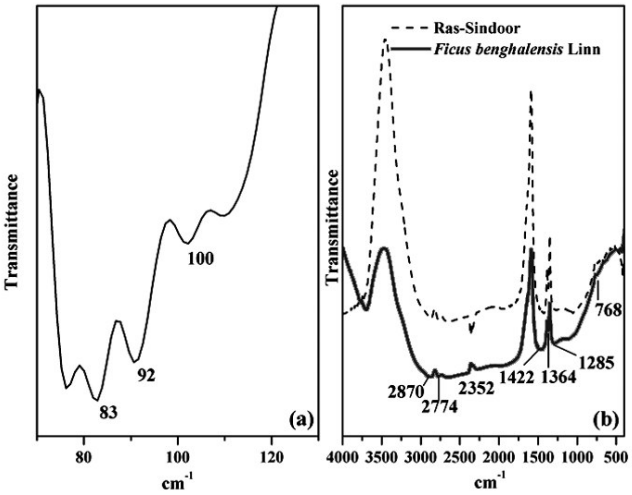

Fig.5 FIR and FTIR spectra

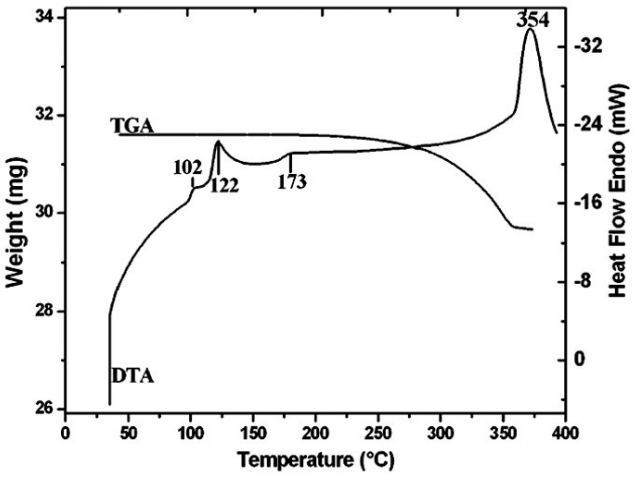

Fig.6 DTA & TGA plot of *Ras-Sindoor*

Ras-Sindoor also confirms this. Mg, Ca and Fe, conducive to healthy metabolism and preventives for stomach lesions, were also found to be present in the final *Ras-Sindoor* product in significant concentrations[18]. Na and K, needed for maintaining normal fluid balance are also present in the final product as is Zn useful for proper growth and immunity (Table 2). These elements (Mg, Ca, Fe, Na, K and Zn) act as additional supplement improving the curative properties of the drug. Several other heavy metals, e.g. Pb, Cd, Cr, Cu and Ni were also tested for their presence but their concentration (Table 2) was found to be well within the safe limits recommended by WHO[19]. The above beneficial elements are also present in significant concentrations in the aerial root of the *Ficus benghalensis* Linn. indicating that these elements enter in the *bhasma* along with other organic matter from the aerial root of *Ficus benghalensis* Linn. used in the preparation of the drug (Table 2). Thus, the additional element present in the drug is clearly due to plant part used and so may be called as bioavailable. It is notable that the proportion of mercury in *Kajjali* and in *Ras-Sindoor* does not seem to follow a consistent trend, though some of it is certainly lost during the preparation through direct spillage, vaporization or in the ignition process. This variability in the amount of mercury raises the safety concerns regarding the use of the drug and requires further work on the processing technique.

Discussion

Fundamental reaction for the generation of Mercury sulfide in a mixture of mercury and sulfur is $S + Hg \leftrightarrow HgS$ with $\Delta G° = -46$ KJ/ mol. This negative free energy change $\Delta G°$ shows the theoretical feasibility of making mercury sulfide by mixing elemental mercury and sulfur. Thus, even before the heat treatment some mercury sulfide is present in *kajjali* sample. To increase its proportion heat treatment seems essential. The pharmaceutical processing up to 650°C seems reasonable since sulfur boils at 392°C and mercury at 630°C. Juice of aerial root of *Ficus benghalensis* Linn serve as acidic medium and this acidic medium helps in formation of mercury sulfide[20]. Macro particle size of the preparation may be attributed to the grinding of raw materials for a long duration as well as the heat treatment which causes the change in the chemical nature of the raw materials. It is in general expectation that organic molecules will burn out at the processing temperature of the *bhasma* (above 400°C in most of these kinds of preparations). Here, the IR and thermal analysis shows the possibility of organic matter in the sample. This could be due to the formation of organo-metallic complexes in the drug sample that can sustain even at the high processing temperature of herbo-metallic drugs.

The studies discussed here are quite promising. Several significant possibilities and future prospects of the drug could be debated with these results. The macro particle size of the drug matches well with the colloidal size and this suggest the possibility that these colloidal particles are get attached to the human intestine and provide a large surface area thereby increasing the absorption of other nutrients and drugs, which are added to it during the process of preparation or prescribed to the patient along with them. Thus, these drugs act as the absorbent. Further, the presence of the organic matter on the surface of the drug suggests that these organic matter acts as the coating material on the surface of the metallic compound present in the drug and metal compound acts as the carrier of the organic mater (just like the concept of novel drug delivery in the modern medicine) derived from the herbs/plant used during the pharmaceutical processing. In short, *Ras-Sindoor* acts as a carrier for the organic contents from the aerial root of the *Ficus benghalensis* Linn. which is styptic and immunomodulator and is known to be useful in treatment of syphilis, dysentery, inflammation of lever, etc[21]. It could be concluded that mercury sulfide (HgS) in nano crystalline (20-50 nm) form associated with organic molecules probably plays an important role in making it biocompatible and non-toxic at low doses (dose of *Ras-Sindoor* is<125 mg/day). Other essential elements present in *Ras-Sindoor* act as additional supplement and help in increasing the efficacy of the drug. Even after all, the actual biological role of the metal present in such drugs is not very clear. In order to accept such kind of herbo-metallic drugs especially containing heavy metals, an extensive research is needed for the complete pharmacokinetic study on the animal system.

Conclusion

Ras-Sindoor is shown to contain mercury sulfide (crystalline in nature with crystallite size ranging from 25 to 50 nm) associated with several organic macromolecules derived from the plant extract used during the processing of the drug. Several macro/trace elements are also found to be present in different

amounts, which were bio-available and responsible for adding to the medicinal value of *Ras-Sindoor*.

Acknowledgement

Authors are thankful to Prof ON Srivastava, Department of Physics, Dr NP Singh, Department of Botany and Prof RK Mondal, Department of Metallurgy (IT), Banaras Hindu University for providing laboratory facilities. Financial assistance to SKS as JRF is gratefully acknowledged.

References

1 Kumar A, Nair AGC, Reddy AVR & Garg AN, Availability of essential elements in *Bhasma*: Analysis of Ayurvedic metallic preparations by INAA, *J Radioanal Nucl Chem*, 270 (2006) 173.

2 Wadekar MP, Rode CV, Bendale YN, Patil KR & Prabhune AA, Preparation and characterization of a copper based Indian traditional drug; *Tamra bhasma*, *J Pharm Biomed Anal*, 39 (2005) 951.

3 Kulkarni-Dudhgaonkar SB, *Rasaratna Samuchyaya*, (Shivaji University Publication, Kolhapur), 1970, 158.

4 Patel NG, *Folk Medicine: The Art and the Science,* In: RP Steiner (Eds), (American Chemical Society, Washington), 1986, 41.

5 Shastry KN, *Rasatarangini*, English translation of original in Sanskrit, In: Sadananda (Eds), (Motilal Banarasi Das Press, Varanasi), 1979.

6 Svoboda RE, *Prakriti: Your Ayurvedic constitution*, (Bellingham Sadhana Publications), 1998, 169.

7 Saper R, Robert B, Stefanos NK, Paquin J, Burns MJ, Eisenberg DM, Davis RB & Phillips RS, Heavy metal contents of Ayurvedic herbal medicinal products, *J American Med Assoc*, 292 (2004) 2869.

8 Frawlay D, *Ayurvedic Healing, A Comprehensive Guide*, (Lotus Press, Twin Lakes, USA), 2000.

9 Subramanian S, Maral & Mukherjee A, (NUCAR, BARC, Mumbai), 2003.

10 Vohora, SB, Proceedings First international conference on elements in health and disease. In: MN Pal, RB Arora, MSY Khan (Eds), New Delhi, 1984, 269.

11 Singh AK, Studies on the toxicity of Mercury preparation (Ghandhaka-Jaritra Parada preparation): An experimental study, PhD Thesis, (BHU, Varanasi), 1986.

12 Mitra A, Chakraborty S, Auddy B, Tripathi P, Sen S, Saha AV & Mukherjee B, Evaluation of chemical constituents and free radical scavenging activity of Swarnbhasma (gold ash), an Ayurvedic drug, *J Ethnopharmacol,* 80 (2002) 147.

13 Ding T & Zhu JJ, microwave heating synthesis of HgS and PbS nanocrystals in ethanol solvent, *Mater Sci Engineer B*, 100 (2003) 307.

14 Ross SD, Inorganic infrared and Raman spectra, (McGraw Hill Ltd, UK), 1972, 101.

15 Cherian S & Augusti KT, Antidiabetic effects of a glycoside of leucopelargonidin isolated from *Ficus benghalensis* Linn., *Indian J Exp Biol,* 41 (1993) 26.

16 Manian R, Anusuya N, Siddhuraju P & Manian S, The antioxidant activity and free radical scavenging potential of two different solvent extract of *Camellia sinensis* (L) O. Kuntz, *Ficus benghalensis* L. and *Ficus racemosa* L., *Food Chem,* 107 (2008) 1000.

17 Mukherjee PK, Saha K & Murugesan T, Sceening of antidiarrhoeal profile of some plant extracts of a specific region of West Bengal, *Indian J Ethnopharmacol,* 60 (1998) 85.

18 Garg AN, Kumar A, Nair AGC & Reddy AVR, Analysis of some Indian medicinal herbs by INAA, *J Radioanal Nucl Chem*, 271 (2007) 611.

19 Anonymous, *WHO, Expert committee on specification for pharmaceutical preparation*s, 32 Reports, (WHO, Geneva), 1992, 44 and 75.

20 Svensson M, Duker A & Allard B, Formation of cinnabar-estimation of favorable conditions in a proposed Swedish repository, *J Hazard Mater*, B 136 (2006) 830.

21 Gabhe SY, Tatke PA & Khan TA, Evaluation of the immunomodulatory activity of the methanol extract on *Ficus benghalensis* root in rats, *Indian J Pharmacol*, 38 (2006) 271.