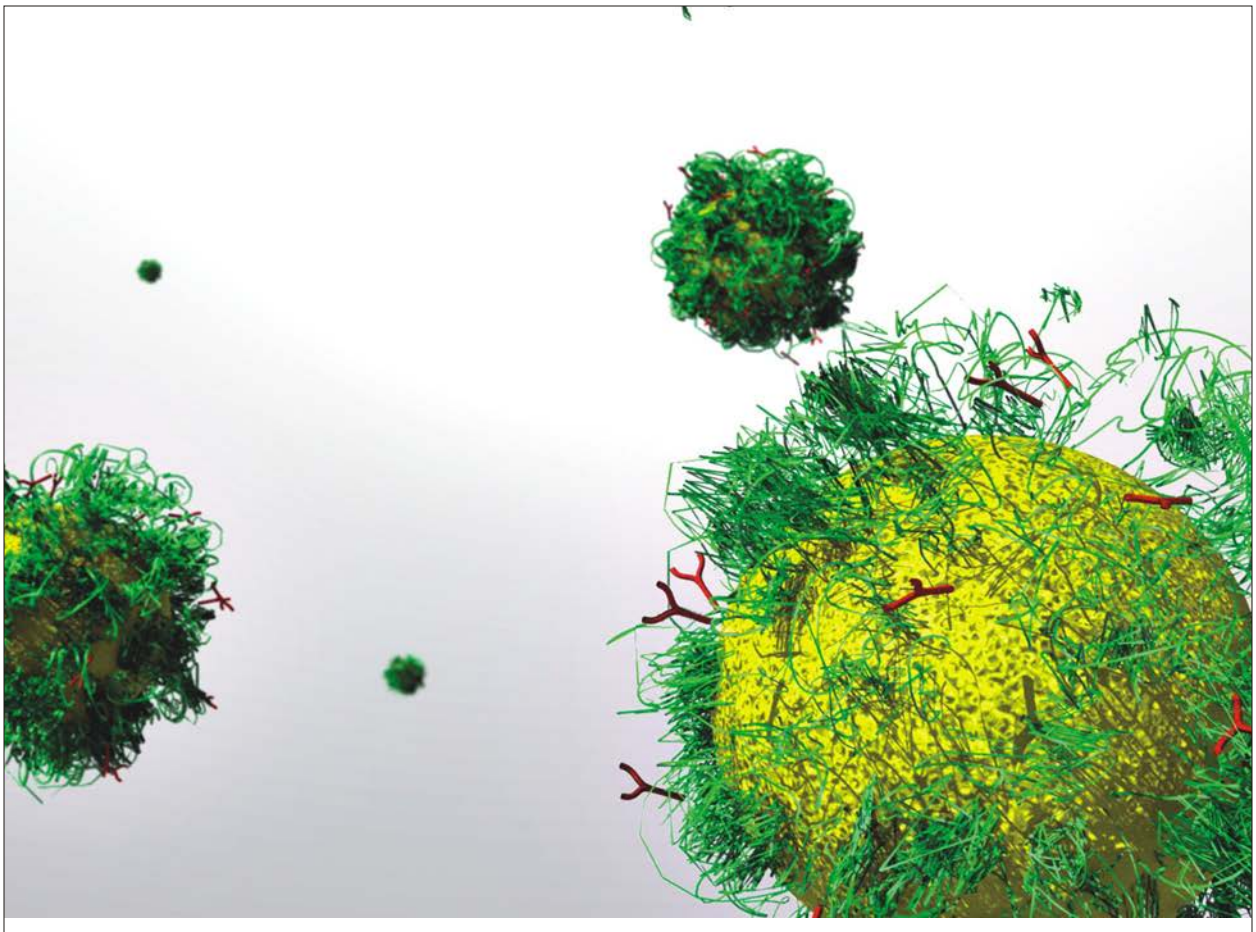

American Journal of Drug Discovery and Development

ISSN 2150-427X

Academic Journals Inc.

www.academicjournals.com

American Journal of Drug Discovery and Development 2 (3): 110-123, 2012
ISSN 2150-427x / DOI: 10.3923/ajdd.2012.110.123

Chemical Characterization of a Gold and Mercury based Siddha Sasthric Preparation-*Poorna chandrodayam*

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ABSTRACT

Poorna chandrodayam (PC) is an elixir of mercury and gold widely used in Siddha Medicine prepared by kupi pudam process. It is a bright scarlet red coloured powder, soluble in Aqua regia. Content of gold, mercury and sulphur were found to be 9.78, 78.11 and 11.95 g, respectively and 280 ppm of free mercury. XRD and XPS studies revealed the presence of HgS, SEM studies revealed difference in size and agglomeration due to repeated cycles of calcinations, EDAX demonstrated HgS as a main component and a small percentage of Hg as oxide form and a small percentage of gold. IR spectra confirmed the pattern of Mercury sulphide and FTIR confirmed that PC is free of organic compounds. DTA revealed decomposition of water molecules and TGA revealed as mercury sulphide. ICP-OES analysis revealed the presence of mercury (141.76 mg g^{-1}), Calcium (11.68 mg g^{-1}) and gold (0.84 mg g^{-1}). TEM revealed nano range of particles of irregular size in the range of 60-70 nm. Presence of HgS in nanoparticles was further confirmed by EDAX attached with TEM. DLS revealed the particle size near nano range and only 10% were below 112.6 nm. AAS studies revealed the presence of Mercury (143.12%), gold (0.92%) and many other metallic compounds. The studies confirms that PC is converted into an amalgamation process and mostly present as Mercury sulphide along with gold and a small portion of mercury oxide.

Key words: *Poorna chandrodayam*, mercury, gold, amalgam, alchemy, siddha

INTRODUCTION

Mercurial and Gold preparations are very vital in Siddha Medicine (Anonymous, 1989) which are advocated for various debilitating diseases (Anonymous, 1989) since time immortal. This unique blend of alchemical process was practiced by our Saint Siddhars, even before the advent of this scientific era (Ramachandiran, 1998). These drugs are mostly a mixture of compounds and because of its synergistic action and purification process (Austin *et al*., 2002), toxicity is being diminished (Hardy *et al*., 1995), thereby increasing bioavailability through the cells of our body (Sudha *et al*., 2009). Treating the metals with herbal juices leads to reduction (titration) in particulate size even up to nano levels (less than 100 nm) enabling increased potency (Brown *et al*., 2007; Malarkodi *et al*., 2007), making them effective even in low doses (Kumar *et al*., 2006a). Proper standardization methods should be adopted for checking the quality to meet the criteria to support its use worldwide.

Poorna chandrodayam (PC) is a well-known, mercurial preparation with gold (Thiagarajan, 1992) widely used for many ailments like tuberculosis, jaundice, fever, rat bite, cancerous ulcer, sprue, arthralgia and male sterility (Muthaliar and Uttamarayan, 1987). In Ayurveda, it is termed as "*Makaradhwaja sindoora*" where, instead of banana stem juice during the process, aloe juice is added for titration (Mahdihassan, 1985). Apart from this, the methodology is same. Since the

methodology is quite complex and involves expertise, much work has not been carried out on this formulation. Hence, this study was carried out to evaluate the content of gold, mercury and sulphur in the preparation which would be very vital in chemical standardization of this formulation. Three separate batches of PC were made and compared. Further, this study is focused to analyze a widely used mercurial with gold preparations for the metals present in the complex powder and their bio-absorption potential, to ascertain the content and potency and validation through modern analytical techniques to help in quality control and standardization of the drugs.

MATERIALS AND METHODS

Preparation of *Poorna chandrodayam* (PC): PC was prepared from purified gold, purified mercury and purified sulphur in the ratio 1:8:16 titrated with red cotton flower for 2 days, dried and further triturated with Banana Stem juice for 2 more days, dried and processed by kupi pudam technique (Thiagarajan, 1992).

Chemical evaluation: It was subjected to physical, qualitative, quantitative and sophisticated Instrumental analysis according to standard methods (AOAC, 1980). Mercury was identified by the presence of white precipitate on treating with stannous chloride solution which on excess of reagent turns grey. When treated with dilute HCl and $BaCl_2$, white precipitate insoluble in concentrated HCl formed indicates sulphur.

Estimation of mercury, sulphur and gold: Presence of free mercury (Hg) was qualified by adding 20 mg drug with 5 mL of dilute HNO_3 and filtered. Two millilitere of the filtrate was neutralized with NaOH and added 1 mL in excess yellow or black precipitate was formed indicating the presence of mercury. This was further confirmed by neutralizing 2 mL of the filtrate with NaOH and adding 1 mL of KI solution which produced scarlet or greenish yellow precipitate. Presence of free sulphur was analyzed by shaking 20 mg of the drug with 5 mL of hot pyridine. Filter, add 0.2 mL solution of sodium bicarbonate and boil which produced blue or green colour.

Quantitative estimation for mercury (Hg), gold (Au), sulphur (S), free mercury (Hg) and free sulphur (S) were estimated according to AOAC (1980). Mercury was quantified by adding 0.5 g of the drug with 15 mL of H_2SO_4 and connecting it to a reflux condenser. Two millilitere of HNO_3 was added through the condenser and refluxed until white fumes evolve. Two millilitere of HNO_3 was added periodically every 4 h, down the condenser, thrice. Cooled and wash-down the condenser with 50 mL of water. Contents of the flask were boiled until a brown yellow precipitate and clear supernatant, liquid was obtained and filtered through Whatman No. 41 filter paper. After that it was washed with dilute H_2SO_4 and made up to 250 mL. This solution was used for the estimation of mercury and gold.

From the above, 50 mL is pipetted and N/10 potassium permanganate was added drop wise until faint permanganate colour persist. Then it was decolorized (neutralized) with drop wise addition of H_2O_2 solution (10 volumes). Two millilitere of ferric ammonium sulphate indicator was added and triturated with N/10 Ammonium thio cyanate. Each mL is equivalent to 0.01003 g of mercury.

Take the residue obtained in the above filter paper in a tarred silica crucible. Dissolve if there is any residue adhering on the wall of the Kjeldahl flask with Aqua regia (HCl 3 parts and HNO_3 1 part) and add to the same crucible. Evaporate the acid on hot plate. Ignite it to constant weight from the weight of residue and calculate the content of Gold.

Sulphur was estimated by taking 0.2 g in 100 mL of N/10 iodine and 5 mL of HCl. After ½ an hour 5 mL of HNO_3 was added and allowed to stand for 1 h. Then it was evaporated in a hot plate to almost dryness. Two millilitere of HCl was added and again evaporated to dryness. Cooled and the residue was dissolved in 20 mL of water and filtered. It is washed, thrice, with 10 mL of water. Combined filtrates were heated and 10 mL of 25% $BaCl_2$ solution was added drop wise into the boiling solution for one minute. It was covered and allowed to stand overnight, undisturbed and filtered through Whatman No. 42 filter paper. Wash the filtrate until it was free from chloride. The residue and filter paper are taken in a tarred crucible and ignited to constant weight. Each gram of the residue is equivalent to 0.1374 g of sulphur.

Sophisticated instrument analysis: The processed drug was further subjected for detailed evaluation using Modern techniques like X-Ray Diffraction (XRD), X-ray photoelectron spectra (XPS), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Analysis (EDAX), Infrared spectroscopy (IR), Fourier transform infrared spectroscopy (FTIR), Differential Thermal Analysis (DTA), Thermogravimetric Analysis (TGA), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and Atomic Absorption Spectrometer (AAS) to obtain physico-chemical fingerprint (Chuu *et al*., 2001; Rai *et al*., 2009; Sudha *et al*., 2009).

XRD studies: Powder XRD studies of the solid samples were recorded from Philips 1710 X-ray diffractometer using CuKα radiation (λ = 1.5405A°) filtered by a nickel foil over the range of diffraction angle 10-70° operating at 30 kV and 20 mA. Pattern was recorded for the angle (2θ) ranging from 5-80 degree at a scanning rate of 3 degree/second.

XPS analysis: X-ray photoelectron spectra (XPS) measurement was performed with ESCLAB MKII instrument using none monochromatized MgKα X-ray as the excitation source. 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. Infrared (IR) spectrum in the low frequency region (50-400 cm^{-1}) was recorded on a Broker IFS 66 V/S vacuum Fourier transform Interferometer, where as the spectra from 400-4,000 cm^{-1} region were recorded using Perkin-Elmer Fourier Transform Infrared (FTIR) Spectrophotometer in KBr pellets.

DTA, TGA and ICPOES analysis: Thermograms DTA and TGA were recorded in a Nitrogen atmosphere on a Pyris Diamond thermal analyzer EXSTAR 6000, Perkin Elmer. The samples were placed in an alumina crucible and the temperature varied from 40-400°C. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) studies were carried out using Perkin-Elmer 5300 DV ICP-OES 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 (Wadekar *et al*., 2006).

TEM, EDAX and DLS analysis: The characterization of nanostructure and the defined phases in the sample was evolved by Transmission Electron Microscope (TEM). Thermograms were

recorded in air atmosphere on a NETZSCH simultaneous thermoanalyzer. EDAX attached to TEM (CEM, CM-12) was used for the detection of various elements in the sample. Presence of nanoparticles was further tested using Dynamic Light Scattering (DLS) method in Brookhaven 90 plus particle size analyzer, after suspending the materials in aqueous dispersing phase and filtering through 0.45 μ membrane filter.

Heavy metal analysis by AAS: Free Mercury, Sulphur and Gold were estimated using Atomic absorption spectrophotometer. For estimation, the formulations were digested by heating at 120°C in an electric oven till a constant weight was obtained. It was ground well, weighed and kept separate flask, treated with 5 mL. HNO_3 side by side and add 5 mL. HNO_3 into an empty flask which serves as blank. It was covered with watch glasses and heated to reflux in an electric hot plate at 80-100°C. After heating for 1 h the contents of flasks were treated with additional 5 mL. of HNO_3 followed by 2 mL. of 30% H_2O_2 and the heating with gentle reflux is continued till clear solution is obtained. It was then diluted with deionized water and filtered using Whatman 42 into volumetric flasks marked as sample solution. Working standard solutions of Hg was prepared from stock standard solution of 1000 ppm from Merck. The samples in the form of a homogeneous liquid, was introduced into the flame, flame intensity was calculated using Lambert-Beer law, i.e., Absorbance = log 10 I_0/I_t = K.C.L., Where, I0 = Intensity of incident radiation emitted by the light source, I_t = Intensity of transmitted radiation, C = concentration of sample (free atoms), K = constant (can be determined experimentally) and L = path length. Quantitative detection of trace elements is measured in parts per million (ppm) (Arvelakis and Frandsen, 2005).

Statistical analysis: The Statistical data are expressed as Mean±Standard deviation (SD) and Mean±Standard error of the means (SEM) and statistical analysis was carried out using student's t-test (Kulkarni, 2006).

RESULTS

PC is a popular metallic preparation (Kumar *et al*., 2006b) considered to be a best alchemical drug of longevity (Patgiri and Prajapati, 2006). These 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 at a dose of 100-200 mg day^{-1} and to be taken with suitable adjuvant.

The total weight of *kajali* was 255.68±2.47 g which yield 92.40±0.77 g of the drug, respectively. The total duration of heating process was 32.03±0.47 hours and the time taken for self cooling stage (50°C) was 50.53±1.85 h. In total 34.66±0.24% process loss was observed. Similar findings were observed with *Makaradhwaja* (Patgiri and Prajapati, 2006), which further confirms the similarity of these formulations.

Physical properties: All were fine powders and appeared as bright scarlet red powder, odourless and crystalline powder, which are insoluble in water and organic solvents like HCl, HNO_3 and H_2SO_4 but soluble in Aqua regia. On heating in open test tubes they were sublimating as a black coloured precipitate of mercury sulphide and produced fumes of SO_2. Similar findings were observed in Cinnabar (Prakash *et al*., 1995) and *Linga chenduram* Number 1 (Austin and Jegadeesan, 1999).

Table 1: Content of individual and free elements in *Poorna chandrodayam*

Elements	Lab sample
Gold (Au)	9.78±0.26
Mercury (Hg)	78.11±0.47
Sulphur (S)	11.95±0.43
Free mercury (Hg)	280±2
Free sulphur (S)	Nil
Free gold (Au)	Nil

Results are expressed in percentage, Results are Mean±SEM of 3 values

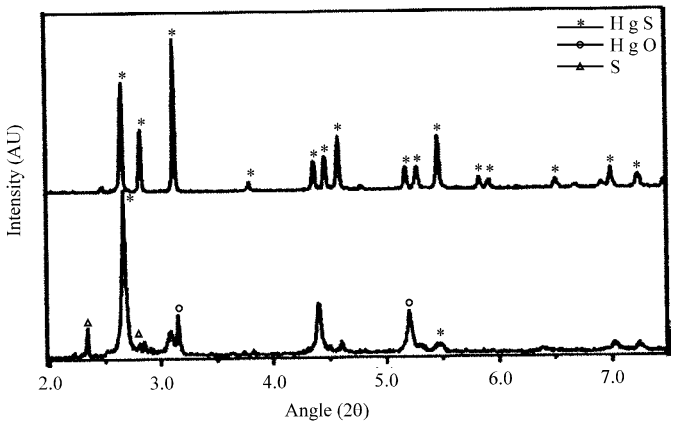

Fig. 1: XRD pattern of *Poorna chandrodayam*

Chemical analysis: Qualitative analysis confirms the presence of mercury, gold and sulphur in both the preparations. Quantitative evaluation revealed the percentage of gold, mercury and sulphur (Table 1). From this it is evident that the content of mercury is high and gold is low. The drug was found to be present in a complex process, termed as amalgamation process (Remy, 1989). Free sulphur was not found in both the formulation, whereas free mercury was observed (Table 1). It was found to be 280±2 ppm. The variation among the observations might be due to the difference in the heating methodology (Lenihan and Fletcher, 1977).

XRD patter of kajjali shows peaks due to free sulphur, mercury oxide and mercury sulphide, whereas XRD pattern of PC show only the peaks of Mercury sulphide (Fig. 1). The form of gold could not be identified using XRD pattern because of the amalgamation process involved. Further no extra diffraction peaks were observed in *Poorna chandrodayam*, confirming that the initial stages of processing of kajjali before the heating process mercury oxide and free sulphur were significantly present while after the heat treatment only mercury sulphide is present which confirms the crystallinity of the sample. The crystallite size was calculated from XRD pattern following Scherrer equation:

$$t = \frac{\lambda 0.9}{\beta} \times \text{Cos}\theta$$

where, t is the crystalline 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

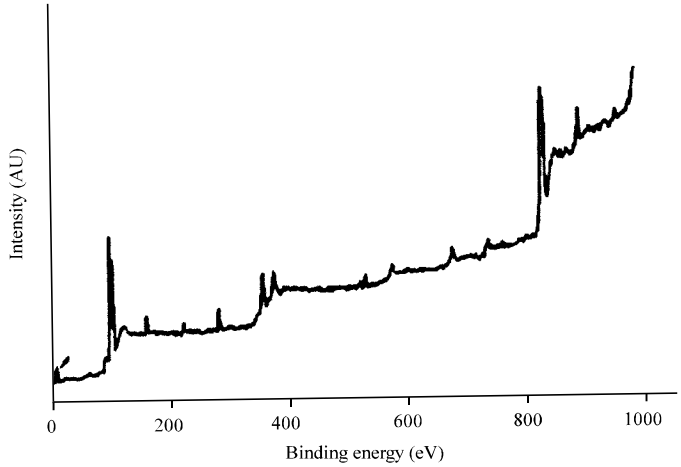

Fig. 2: XPS pattern of *Poorna chandrodayam*

diffraction angle for (h k l) plane. The above equation yields t = 30-70 nm. It is notable here that the FWHM in case of kajjali is high in comparison with PC which confirms that the size of the crystallite increases to heat treatment.

XPS analysis provides valuable information about the surface stage of the drug (Fig. 2). A typical survey spectrum of PC confirming the presence of Mercury and Sulphur was observed. Further, it also showed the presence of C peak and O peak. Although, the presences of other elements were observed 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 the organic molecules on the surface of the metallic compounds. High resolution spectra of Hg core level showed the presence of the peaks at 100.28 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. Thus XPS analysis also confirms the presence of HgS phase in the sample.

SEM images showed difference in size and agglomeration of the particles. Agglomeration of the particles is due to the repeated cycles of calcinations involved in preparation (Marrero *et al.*, 2007). The elemental composition of the drug samples were analyzed by EDAX (Table 2). Carbon and Nitrogen were found to be more in the Kajjali, where as Mercury was more in PC. Further it was interesting to note that the presence of Silicon, Silver, Chlorine, Potassium, Barium, Copper and Lead present in Kajjali were absent in PC. In PCC, HgS is the main component and a small percentage of Hg may also occur in oxide form. PCC contain small percentage of gold. 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 (Sudha *et al.*, 2009).

Abundance of C, N and O identified from the drug is obvious from the plant extracts used in the preparation. Sulphur is abundant in the Kajjali which is greatly reduced in PC. This is clear that this is due to the heat treatment which removes a large amount of free sulphur and only the sulphur bound to mercury remains in the preparation.

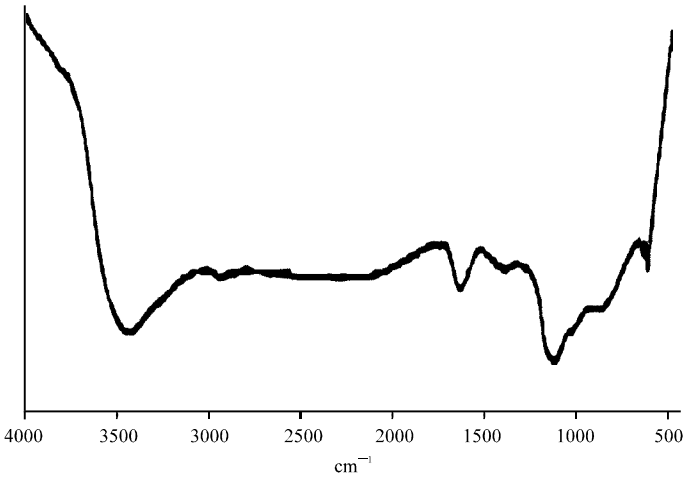

Fig. 3: Spectra of *Poorna chandrodayam* by Fourier transform infrared spectroscopy (FTIR)

Table 2: Energy dispersive X ray analysis of Kajjali and *Poorna chandrodayam*

	Kajjali		*Poorna chandrodayam*	
Element	Weight (%)	Atomic (%)	Weight (%)	Atomic (%)
Nitrogen (N)	27.44	29.45	21.37	21.84
Oxygen (O)	18.24	24.22	9.86	17.48
Sulphur (S)	6.13	6.88	9.53	11.88
Calcium (Ca)	0.45	0.13	1.10	1.05
Iron (Fe)	0.04	0.01	0.08	0.02
Mercury (Hg)	6.51	6.49	29.27	12.39
Gold (Au)	1.86	1.56	0.81	0.45
Sodium (Na)	4.28	6.72	1.12	3.88
Silicon (Si)	0.78	1.55	-	-
Carbon (C)	28.69	15.66	25.74	30.36
Aluminum (Al)	0.65	1.32	-	-
Magnesium (Mg)	0.56	0.34	1.12	0.65
Silver (Ag)	0.21	0.84	-	-
Chlorine (Cl)	0.72	0.34	-	-
Potassium (K)	2.58	3.58	-	-
Barium (Ba)	0.29	0.06	-	-
Copper (Cu)	0.35	0.74	-	-
Lead (Pb)	0.22	0.11	-	-
Total	100.00	100.00	100.00	100.00

An FTIR spectrum of PC was free of organic compounds (Fig. 3). Absence of organic matter is a 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 (Ma *et al.*, 2007). FIR Spectrum of PC (Fig. 4) is similar to the pattern of Mercury sulphide in mercurial preparations like *Linga chenduram* (Sudha *et al.*, 2009). The broad peaks at 3400 and 1600 cm^{-1} in the samples are due to characteristic OH stretching (γ-OH) H OH bending (δ-OH) vibrational bands due to adsorbed water in the sample (Predoi, 2007).

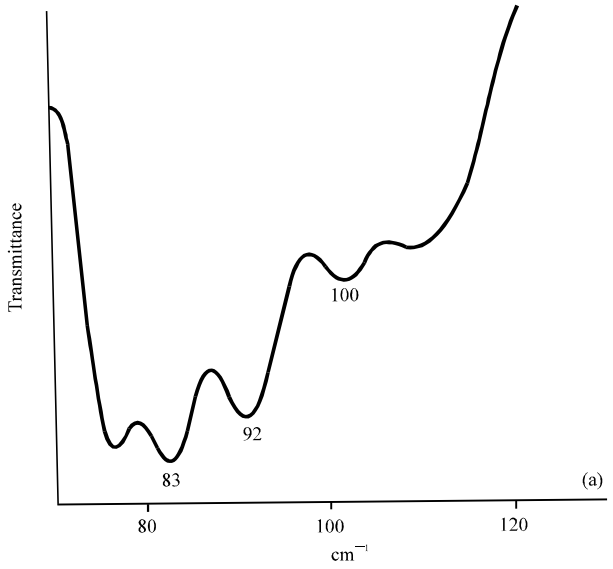

Fig. 4: Spectra of *Poorna chandrodayam* by Far infrared spectroscopy (FIR), X-axis: Absorbance ranging upto 120 cm^{-1}, Y-axis: Transmittance of vibrational brands

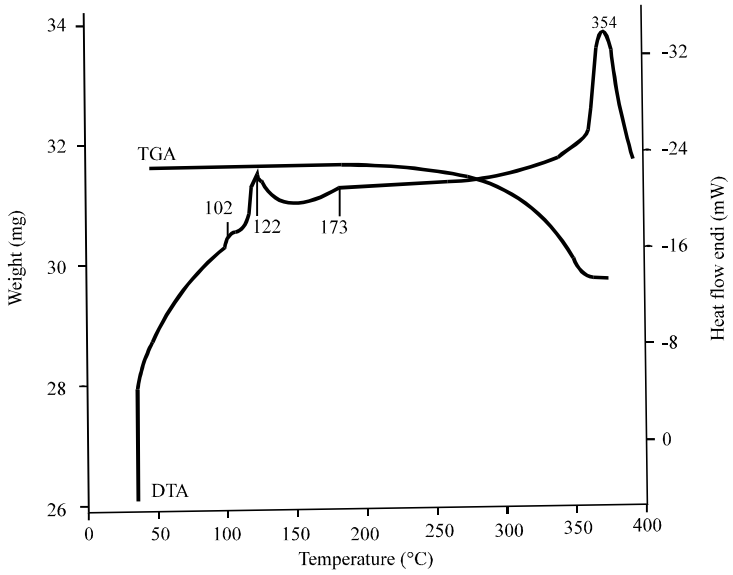

Fig. 5: Differential thermal analysis (DTA) and thermogravimetry analysis (TGA) of *Poorna chandrodayam*, X-axis: Weight/Heat flow, Y-axis: Temperature

Thermal analysis (Fig. 5) shows three endothermic peaks in the range of 100-170°C which is indicative of decomposition of water molecules. Further heating another sharp curve at 354°C corresponding to a weight loss of 9.54% (TGA) which correspond to melting point of mercury sulphide (melting point 344°C). The weight loss could be attributed to burning away of some organic molecules. Thus the thermal analysis supports the presence of mercury sulphide observed by XRD and FIR analysis and organic matter observed by FTIR analysis.

Table 3: Analysis of *Poorna chandrodayam* using inductively coupled plasma-optical emission spectroscopy (ICP-OES)

Element	*Poorna chandrodayam* (mg g^{-1})
Aluminium (Al)	0.8590
Arsenic (As)	1.9200
Calcium (Ca)	11.6850
Cadmium (Cd)	0.0524
Chromium (Cr)	0.0380
Copper (Cu)	0.0240
Iron (Fe)	1.8520
Magnesium (Mg)	0.5021
Manganese (Mn)	0.0200
Nickel (Ni)	0.0050
Lead (Pb)	0.0780
Zinc (Zn)	0.2180
Mercury (Hg)	141.7600
Gold (Au)	0.8420

Table 4: Analysis of *Poorna chandrodayam* by dynamic light scattering (DLS)

Particles size (mm)	Percentage in *Poorna chandrodayam*
10	112.6
20	164.5
30	198.7
40	203.6
50	246.3
60	298.3
70	302.3
80	402.5
90	501.6
95	602.2

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis revealed many metals in PC (Table 3). Mercury was found to be high and accounted for 141.76 mg g^{-1}, followed by calcium 11.68 mg g^{-1} and gold was found to be 0.84 mg g^{-1}.

TEM image of the drug sample show spongy like structure with the particle size lying in the nano range. From the image it is clear that several crystallites are agglomerated on a signal particle giving rise to microcrystalline structure with loss of grain boundaries. TEM Pictures show that particles are not of uniform size. There is more number of particles in the range of 60-70 nm. The presence of HgS in the nanoparticles was further confirmed by EDAX attached with TEM.

Preparation of Bhasmas and Chendoorams in Siddha medicine gave much importance to particle size (Nayanar, 1926). It is mentioned in texts, that the process of Marana (incineration) should be repeated to get smaller particle size (Subbarayappa, 1997). PC was screened for nanoparticles by Dynamic Light Scattering technique (Table 4). PCC had particle size close to nano. Only 10% of the particles were below 112.6 nm. It is further interesting to note that our saints thousands of years ago had such a knowledge of nanoscience which the current scientific world has started exploring.

Atomic Absorption Spectrometer (AAS) studies revealed the presence of other metallic compounds given in Table 5. Presence of Mercury (143.12%) and gold (0.92%) is further confirmed. The presence of copper, zinc, nickel, iron, manganese, cadmium, chromium, aluminium, arsenic and

Table 5: Evaluation of *Poorna chandrodayam* by atomic absorption spectrometer (AAS)

Element	Concentration in *Poorna chandrodayam* (ppm)
Sodium (Na)	26.80
Potassium (K)	19.26
Calcium (Ca)	12.48
Mercury (Hg)	143.12
Gold (Au)	0.92
Copper (Cu)	0.04
Zinc (Zn)	0.20
Nickel (Ni)	0.01
Iron (Fe)	1.86
Magnesium (Mg)	0.48
Manganese (Mn)	0.02
Cadmium (Cd)	0.04
Chromium (Cr)	0.04
Aluminium (Al)	0.72
Arsenic (As)	0.90
Lead (Pb)	0.07

lead might be obtained from the raw materials, which are ores obtained and contain other metallic components. Presence of Sodium, potassium, calcium and magnesium might be arrived from the herbal extracts.

DISCUSSION

PC is a mercurial and gold preparation widely prescribed in Siddha medicine 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 (Nayanar, 1926). The prescribed dose is 0.1-0.2 g. The mercury present in the formulation is mainly as Mercuric sulphide and some are mercuric oxide conjugated with gold.

Among the mercurial compounds HgS is found to be the least toxic and less absorbed than any other form of mercury (Hardy *et al.*, 1995). Previous studies on mercurials reveal lower dose of HgS (100 mg kg^{-1} day^{-1}) for 7 consecutive days did not produce any toxic effects in experimental mice (Chuu *et al.*, 2001). Similar observations are reported in *Linga chenduram* No 1 (Austin *et al*, 2002). Even the toxic effect due to the dose of 1 g kg^{-1} day^{-1} disappeared completely after 5 weeks subsequently after cessation of its administration (Chuu *et al.*, 2001). But methyl mercury at a dose of 2 mg kg^{-1} day^{-1} persisted at least 11 weeks subsequent to cessation of its administration (Mahdihassan, 1985). Long term use of HgS might lead to accumulation of mercury in the liver and kidneys (Sanfeliu *et al.*, 2003; Patgiri and Prajapati, 2006; Amin *et al.*, 2009). The correct methods, appropriate dosage, disease conditions, age and drug combinations greatly influence HgS toxicity (Mahdihassan, 1985; Khaniki, 2007).

XRD, XPS FTIR and TGA indicated that these methods can be used for rapid physiochemical fingerprints. XRD analysis of kajjlli and PC confirm that it is mostly as mercuric sulphide and little quantity as Mercuric oxide. Presence of Mg, Ca and Fe in the formulation is conductive to healthy metabolism and preventives for stomach lesions were also found to be present in the final product in significant concentrations (Rai *et al.*, 2009). Na and K needed for maintaining normal fluid balance are also present in the final product as is Zn is useful for proper growth and immunity (Zhang *et al.*, 2008). Elements like Mg, Ca, Fe, Na, K and Zn present in PC also act as an

additional supplement in improving the curative properties (Predoi, 2007). On the contrary the presence of Pb, Cd, Cr, Cu and Ni were well within the safe limits (Singh, 2008; Princewill-Ogbonna and Ogbonna, 2011) prescribed by AYUSH and WHO (Sabdono, 2009).

Particle size analysis by DLS showed presence of 10% of the particles below 139.9 nm. TEM showed particles slightly larger. EDAX showed the presence of Hg, S, O_2 and small percentage Au. XRD confirmed the presence of HgS as the main constituent. PC did not show any characteristic peak in IR Spectroscopy and the peaks were identical with mercury sulphide earlier reported with *Linga chenduram* (Singh *et al.*, 2009). The additional trace elements and compounds found in the final product are due to the addition of herbal ingredients and can make the formulation bioavailable (Mitra *et al.*, 2002).

HgS has been used thousands of years in traditional medicines (Austin *et al.*, 1999, 2001; Saxena *et al.*, 2006). Gold and its compounds have been used historically in drugs for treating wide variety of ailments (Mahdihassan, 1985). Nano-sized gold particles proved to be effective in ameliorating the symptoms of mycobacterial-, collagen- and pristane-induced arthritis in rat models (Brown *et al.*, 2007). Gold in the traditional Indian Ayurvedic medicine, Swarna bhasma, has been characterized as globular particles of gold with an average size of 56-57 nm (Mitra *et al.*, 2002; Aghabarati *et al.*, 2008). But in our study the form of the gold could not be concluded which needs detailed analysis on gold.

The pharmaceutical processing upto 650° C is justifiable, since sulphur boils at 392°C, mercury at 630°C and the metallic gold will be converted into an amalgamation process because the melting point of gold is 1064.43°C (Marrero *et al.*, 2007). The herbal juices added serves as acidic medium and this acidic medium helps in the formation of mercury sulphide. Macro particle size of the preparation may be attributed to the grinding for a long duration and also the heat treatment which causes the change in the chemical mature of the raw material used in the process. It is indeed general that organic molecules will burn out at the processing temperature above 400°C in most of these kinds of preparation. Whereas here IR and TGA show the possibility of organic matter in the sample which could be due to the formation of organo-metallic complexes in the drug which could sustain even high processing temperature.

Previously, some works were also been carried out on other mercurial preparations, like *Linga chenduram* (Number 1) and *Rasa chendooram* (Austin, 2003), used in Siddha medicine. From the studies it was clearly demonstrated that the content of mercury was 33.75±0.11% and 7.10±0.01% of sulphur in *Linga chenduram* (Austin and Jegadeesan, 1999) and 84.80±0.33% of mercury and 14.13±0.01% of sulphur in *Rasa chendooram,* when compared with the ore of mercury, cinnabar which contains 84-85.2% of mercury and 14.8-16% of sulphur. These findings are also *in lieu* with our findings, where there is a reduction in the content of the element from their ore/metallic element, due to the method of processing (Liu *et al.*, 2008). In this study the chemical nature of PC was found to be mostly as mercury sulphide and a small portion as oxide along with gold. Interestingly this study also demonstrated 9.78±0.26% of Gold, 78.11±0.47% of Mercury and 11.95±0.43% of Sulphur in PC. Similar observations were made with PC procured from The Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd., (IMPCOPS), Adyar, Chennai which was found to contain 8.76% of gold, 78.42% of mercury and 12.43% of sulphur (Austin, 2012). Free mercury was found to be 287% in IMPCOPS sample, when compared with our PC which had 280±2 and in both samples free sulphur and free gold was absent (Austin, 2012). The formulation was found to be a complex amalgamation process and though gold was not much traceable, other compound was mostly in the form of mercury sulphide and a small quantity of mercury oxide. The

oxidation process of the mercury would be due to the little amount of reaction with oxygen at a lower temperature of around 350°C. The studies further confirm the technique of nanotechnology which is being widely described nowadays, when comparing with the alterations on its particle size and nature of the element (Mitra *et al*., 2002; Shanmugam *et al*., 2007).

Studies revealed many interesting and promising observations to note with. The macro particle size of the drug matches well with the colloidal size and suggest the possibility that colloidal particles get attached to 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 making them adsorbent (Elham *et al*., 2011). Further the presence of Organic matter acts as a coating material on the surface of the metallic compound present in the drug and metal compound act as a carrier of organic matter similar to the recent concepts of drug delivery system. Further the drug in nano crystalline form associated with organic molecules probable play a significant role in making it biocompatible and non toxic in low doses less than 125 mg day^{-1}. Other essential elements present in PC acts as additional supplement and help in increasing the efficacy which needs further validation. Hence an extensive research will through a better understanding about the complete pharmacokinetic and pharmacodynamic activity of this drug.

CONCLUSION

PC is gold and mercury amalgamation drug containing chiefly mercury sulphide and a small proposition of oxide with gold. It is crystalline in nature and the crystalline size ranging from 40 to 70 nm along with several organic macromolecules derived from plant extracts used in the formulation. Several macro/trace elements found to be present in different amount make it bioavailable and enhance its activity and reduced the toxicity of the compounds present in them. The near nono size of PC enables better bio-absorption. The role of other trace elements would help in better systemic activity and reduce the toxic effects. The study would be useful as a physico-chemical fingerprint to understand about PC and would be a valuable tool towards checking the quality and bring out high quality metal based formulations owing to their complexity in nature.

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