# Characterization of Hg-based ayurvedic drug *Kajjali*: classical and contemporary approaches

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**This communication presents characterization of *Kajjali*, a mercury (Hg) based Indian traditional ayurvedic drug, by both ancient and contemporary methodology. For preparation of *Kajjali*, 225 g each of *Shuddha Parada* (purified mercury) and *Shuddha Gandhak* (purified sulphur), initially purified through traditional methods of purification as described in ayurvedic literature, were manually triturated using stone mortar and pestle until it became a very fine black coloured powder. This preparation process took up to ~78 h. Thereafter, *Kajjali* was characterized by classical and contemporary methods. *Kajjali* passed the classical tests like *Rekhapurnatwa*, *Slakshanatwa*, *Nishchandratwa* and *Varitara*. XRD study confirmed that *Kajjali* contains mercury sulphide (HgS), identified as metacinnabar (cubic form of HgS), in addition to free sulphur. Composition of *Kajjali* has been determined by EDXA method, which validated the presence of 88.84% mercury and 11.16% sulphur. SEM studies substantiated particle size distribution of *Kajjali*, which varied from 60 nm to 2 μm range. Such integration of classical and contemporary studies is important, because such vital medicines should be characterized properly for safety and efficacy before their appropriate use for diseases.**

**Keywords:** *Kajjali*, metacinnabar, sulphur, XRD.

AYURVEDIC medicines are the treasures of natural herbs, animal products, metals and mineral drugs, which are frequently used to treat diseases since 1500 BC (ref. 1). *Rasa Shastra* is the branch of ayurvedic pharmaceutics, where metals or minerals are processed and converted into biomedicines by adopting specified procedure (*Shodhana*, *Marana*, etc.) with or without combination of herbs/botanicals. This special group of formulations is known as *Rasaushadhies* (herbo-mineral formulations), which are well known for their superior therapeutic potency over pure herbal formulations[2].

The word *Rasaushadhi* emphasizes the importance as a prime ingredient *Rasa* (synonym of *Parada*), mercury in English[3]. Another element, which plays an important role next to *Rasa* among *Rasaushadhies* is *Gandhaka* (sulphur)[4]. *Kajjali* (black collyrium of mercury sulphide (HgS)) is the primary compound of these two basic ingredients[5,6]. It is important to mention that the nomenclature of *Kajjali* is due to its black colour like *Kajal* (collyrium). Various other forms of HgS are also used in ayurvedic therapeutics (Table 1), however, among them, *Kajjali* is most commonly used in many ayurvedic formulations. Mercury is a well-known highly toxic element and its chemical forms are commonly grouped as elementary (mercury vapour; $Hg^0$, liquid mercury; Hg), inorganic (*cinnabar*; HgS, *calomel*; $Hg_2Cl_2$, etc.), and organic (methyl mercury; $CH_3Hg^+$, ethyl mercury; $C_2H_5Hg^+$, etc.) mercurials[7]. HgS, an inorganic mercurial, is commonly used in Indian Ayurvedic and Chinese traditional medicines[7,8]. However, therapeutic uses of these heavy metals are much alarming for public health[9].

In India, the medicinal use of *Parada* and *Gandhak* is known since 10th to 5th century BC, since the period of Acharya Charaka and Sushruta, but their medicinal use in the form of *Kajjali* is elaborately evidenced since 8th century AD, the period of Acharya Nagarjuna, the pioneer of *Rasa Shastra* (Indian alchemy). *Kajjali* is used frequently in the treatment of most diseases[10]. *Kajjali* has *Rasayana* (counteracting the effects of ageing) and *Yogavahi* (which potentiates the action of the drug and also carries the drug to its action site) properties when prescribed with the particular adjunct (*Sahapan*) and vehicle (*Anupan*)[10,11]. *Kajjali* is also used as one of the main ingredients in about 80 formulations mentioned in the Ayurvedic Formulary of India (AFI), which covers a wide range of therapeutic applicability[12–14].

The safety and efficacy are parallel needs of any medicament and their characterization is very much essential from both points of view. The aim of this study is to prepare *Kajjali* through ayurvedic traditional method and to characterize its physicochemical properties with ancient (confirmatory tests like *Rekhapoorna*, *Nishchandra*, *Varitara*, etc.) and contemporary analytical (XRD, SEM and EDXA) approaches.

*Parada* and *Gandhak* were procured from local regular suppliers. Both samples were tested for authenticity as per Ayurvedic acceptable and contemporary properties. The other required materials used in processing of *Kajjali* were also procured from the local market. The pharmaceutical study was conducted in the laboratory of the Department of Rasa Shastra, Faculty of Ayurveda,

**Table 1.** Different forms of HgS used in ayurvedic therapeutic

| Variety of HgS compounds | Broadly known as |
|---|---|
| *Kajjali* | Black sulphide of mercury |
| *Hingula* | Red sulphide of mercury |
| *Rasa Parpati* | Black sulphide of mercury |
| *Rasa Sindoor* | Red sulphide of mercury |

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Initially *Parada*[15] and *Gandhak*[16] were detoxified (*Shodhana*) according to methods quoted in the Ayurvedic Formulary of India, i.e. *Rasa Tarangini* 5/27-30 and *Rasamruta* 2/3 respectively, followed by the preparation of *Kajjali*[17]. The protocol has been adopted as described earlier by Sharma *et al.*[18].

The purification of *Parada* was completed in two steps: (i) *Parada* was triturated with equal proportion of lime powder for 36 h and then it was squeezed through a double-folded cloth. The collected material was washed with hot water and strained through double-folded cotton cloth; (ii) *Parada* (obtained from first step) was triturated with equal part of garlic and half part of rock salt until the paste turned completely black. Finally, the mixture was washed with hot water for complete removal of fibres of garlic. The purified mercury (*Shuddha Parada*) was then collected by straining through a double-folded cotton cloth and dried well. For purification of *Gandhaka*, crystals of sulphur were crushed to coarse powder (40 mesh size) and was later melted in a ghee smeared stainless steel pan on mild heat. After melting, it was poured quickly through ghee-smeared cloth into a vessel containing cow's milk (four times of *Gandhaka*). Then, milk was then decanted and *Gandhaka* collected from the bottom of the vessel. The same procedure was repeated seven times by taking fresh cow's milk. Finally, granules of *Shuddha Gandhaka* (the purified sulphur) were collected by washing with warm water and preserved after drying thoroughly. For preparation of *Kajjali*, 225 g of each of *Shuddha Parada* and *Shuddha Gandhaka* were taken and triturated manually using stone mortar and pestle until the mixture turned into a completely black, lustre free powder. It took ~78 h to pass the classical confirmatory test of *Kajjali*. At the end, it was weighed and preserved in a glass bottle for use.

XRD study of the *Kajjali* sample was carried out at the Centre of Advanced Study, Department of Geology, Institute of Science, Banaras Hindu University (Varanasi, India). The diffraction pattern was obtained on a PANalytical X'Pert Pro diffractometer fitted with a copper tube (CuK$\alpha$ radiation) and xenon detector. It was scanned over a $2\theta$ range of 5° to 70° using a 1/2° fixed divergence slit and 1/4° receiving slit with a step size of 0.0250, 1.20 s/step and a total run time of 56 min at 45 kV and 40 mA (ref. 19). The standard inorganic crystal structure database (ICSD) was used for comparison of the measured data in a Panalytical X'Pert High Score (Plus) v3 X database. SEM and EDXA studies were carried out at the Central Instruments Facility, Indian Institute of Technology (Banaras Hindu University), Varanasi, India. The sample was analysed on a Penta FET Precision OXFORD Instruments – X-act ZEISS model no. 51-1385-046 after Gold grid coating by Coater–Sputter QUORAM Q-150RES. Particles with different patches (spots) were analysed by EDXA to ascertain the elements present. Similarly, for SEM study, a very fine-grained powder of the sample was examined under electron microscope of 15,000 resolutions. The surface picture and the particle size thereof single particle, as well as clusters of particles were analysed.

During *Shodhana* (which emphasizes classical method of purification or detoxification of minerals or metals) of *Parada*, it was observed that when *Parada* was triturated with lime powder, it was converted into a grey-coloured powder. It was difficult to procure the whole amount of *Parada* through double-folded cloth, according to the process mentioned in classics. Therefore, it was washed with hot water and strained through double-folded cloth to get maximum yield of *Parada*. Further, *Shuddha Parada* was obtained easily compared to the earlier step, by washing with hot water and straining through double-folded cloth. A total of ~89% of *Shuddha Parada* was obtained after purification. However, maximum loss (~7.33%) was observed while triturating with lime powder. The percentage of loss depends on spilling out of mercury from the mortar during trituration (*Mardana*) and through adjunct materials during washing (Figure 1 *a*). During purification of *Gandhaka*, crystalline dark yellow *Gandhaka* turned into granules of dull yellow colour, after completion of *Shodhana* process. Around ~94.5% of yield of *Gandhaka* was obtained in *Shodhana* process (Figure 1 *b*). A total of 447 g (~99.33%) of *Kajjali* was obtained from 450 g of *Shuddha Parada* and *Gandhaka* (225 g of each) by triturating for a duration of 78 h (Figure 1 *c* and *d*). Loss of 3 g may be due to adherence with and spill out of the sample from mortar. The chief desired characters of *Kajjali* were evaluated through ayurvedic standards as presented in Table 2. The XRD data of the studied sample is presented in Table 3 and Figure 2. In XRD scan of *Kajjali*, maximum numbers of peaks correspond to metacinnabar (HgS). Moreover, some peaks are also identified as sulphur. The EDXA data of the samples reveals that the percentage of mercury and sulphur is 88.84% and 11.16% respectively. Absence of any other elements confirms the purity of *Kajjali.* The SEM study of *Kajjali* sample shows that about 90% of particles range from 60 nm to 200 nm (Figure 3).

The safety profile for any medicine always comes as top priority than efficacy and it becomes more important when metals and minerals are used in therapeutics. Ayurveda itself has described various confirmatory markers to test the suitability of administration of a drug. However, scientific validation through contemporary analytical tools is crucial to establish the nature and effectiveness of ayurvedic medicines. Therefore, classical observational techniques, viz. *Varna*, *Shlakshantwa*, *Nischandratva*, *Rekhapurnatwa* and *Varitara* for *Kajjali* are applied and evaluated with relevant explanation (Table 2). Simultaneously, contemporary characterization tools like SEM, EDXA and XRD are also applied to evaluate particle

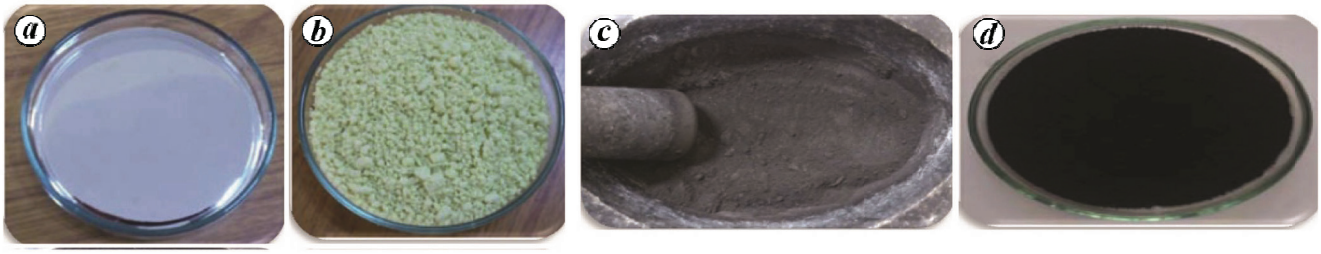


**Figure 1.** ***a***, *Shuddha Parada*; ***b***, *Shuddha Gandhaka*; ***c***, Preparation of *Kajjali*; ***d***, Prepared *Kajjali*.

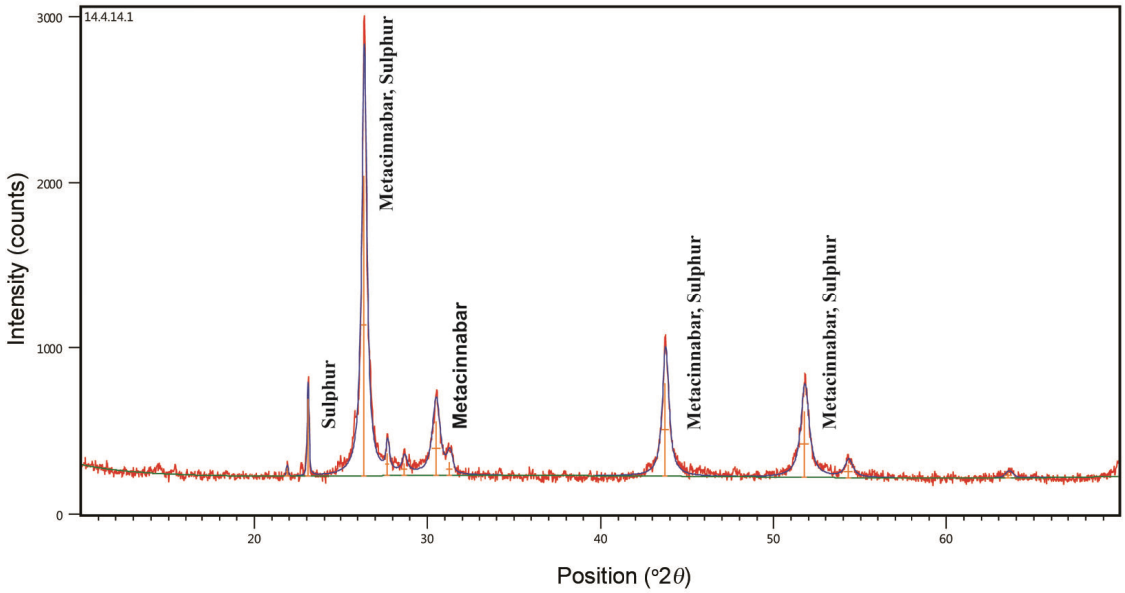


**Figure 2.** XRD scan of *Kajjali* where five most intense peaks are labelled.

size, morphology, elemental compositions, crystallinity, and the form of the compound respectively.

*Kajjali* was prepared from purified mercury and sulphur (purified initially following the classical methods). *Shodhana* is a process of purification and detoxification by which physical and chemical blemishes are eliminated and the purified substances are subjected to further use. *Parada Sodhana* was carried out according to the reference mentioned in the AFI; part-I (quoting the reference of *Rasa Tarangini* 5/27-30). This classical reference of *Parada Shodhana* portrays the complete removal of physical and chemical blemishes (*Parada-Dosha*), and then *Shuddha Parada* may be further used as a medicine. *Gandhaka Shodhana* was carried out according to the reference mentioned in the AFI; part-I (quoting the reference of *Rasamritam* 2/3). The principle adopted for purification of *Gandhaka* is *Dhalana* (melting and quick pouring in liquid media). After completion of this process, purified sulphur was thoroughly washed with hot water to remove the fat contents, which had adhered from milk and ghee media used during the process.

All the classical confirmatory tests are applied for *Kajjali*. It is black sulphide of mercury ($\beta$-HgS), which is considered as *Samaguna Sagandha Niragni Murchchana* of *Parada*. The possible fundamental reaction (S + Hg $\rightarrow$ HgS) between mercury and sulphur in the formation of *Kajjali* (HgS) shows the theoretical feasibility of making HgS by mixing elemental mercury and sulphur. As mercury is a highly toxic substance, addition of sulphur counteracts the toxicity of mercury (Hg + S).

The results of this study may be used as an explanation for some issues that have been raised recently on toxicity of different metallic and mineral preparations due to the presence of heavy metals in more than permissible limits. In this study, *Kajjali* passed all classical confirmatory tests. *Nishchadratwa*, meaning no shining particles, is the foremost confirmatory test for *Kajjali*, which indicates absence of free metallic particles in *Kajjali*. The classical *Tamra Patra Pariksha* (test of no precipitated line of free mercury observed while rubbing *Kajjali* on copper flake) of *Kajjali* also supports the above inference. XRD study confirms that most intense peaks correspond to HgS, called as metacinnabar ($\beta$-HgS) and a few peaks match with free sulphur (Table 3). This study counters the issues of heavy metal toxicity raised due to the non-existence of free mercury ($Hg^0$) in ayurvedic medicines,

**Table 2.** Classical confirmatory tests for *Kajjali* used in ayurvedic system of medicine

| Ayurvedic parameters | Method of test | Observations | Evaluation |
|---|---|---|---|
| *Varna* | Sample + by observing | Black colour | Identification of desired character of final form of *Kajjali.* |
| *Shlakshantwa* | Sample + appearance and touch by rubbing between index finger and thumb | Powdery with smooth texture | Fine powdery form with uniform distribution of particles showing nano to micro size of particles. |
| *Nischandratva* | Sample + drop of water, rub on palm with finger and see in sunlight | No brightness observed | Absence of metallic lustre of free mercury |
| *Rekhapurnatwa* | Sample + rubbed gently between index finger and thumb | Particles of *Kajjali* entered in furrows of finger | Lesser particle size. |
| *Varitara* | Sample + dropped over stable surface of water filled in glass beaker | Floating of each particle over stable surface of water | Lightness of particles due to expanded surface area. |
| *Tamra Patra Pariksha* | Sample + lemon juice, rub on copper sheet | Absence of silver like coating | Absence of free metallic mercury. |

**Table 3.** XRD data of *Kajjali*

| Position (°2$\theta$) | $d$-spacing (Å) | Relative intensity (%) | Mineral name |
|---|---|---|---|
| 26.360 | 3.378 | 100.00 | Metacinnabar, sulphur |
| 43.723 | 2.068 | 30.93 | Metacinnabar, sulphur |
| 23.053 | 3.854 | 25.51 | Sulphur |
| 51.759 | 1.764 | 21.94 | Metacinnabar, sulphur |
| 30.489 | 2.929 | 17.93 | Metacinnabar |

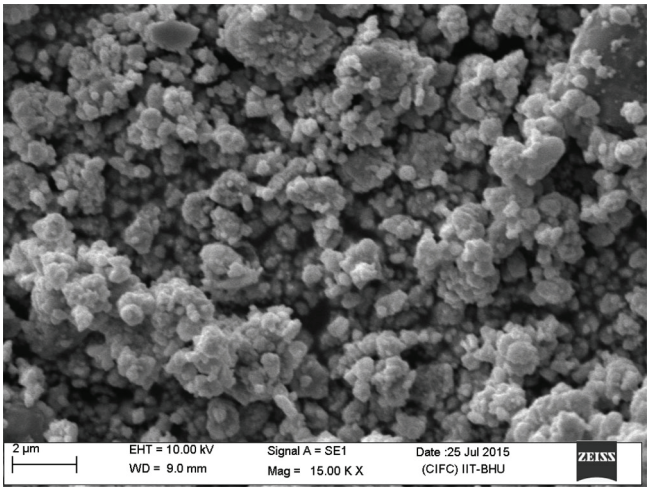


**Figure 3.** SEM image of *Kajjali*.

where this chemical form of Hg (e.g. *Kajjali*, *Rasa Sindoor* – see Table 1) is used as ingredient[20].

Although sulphur has the largest number of observed allotropes (exists in two or more different forms in the same physical state), two most common allotropic forms are rhombic sulphur ($\alpha$-sulphur) and monoclinic sulphur ($\beta$-sulphur)[21]. The stable form of sulphur at room pressure and temperature, is the orthorhombic sulphur (also called as rhombic sulphur or $\alpha$-sulphur). Warren and Burwell[22] were the first to determine the crystal structure of orthorhombic sulphur consisting of symmetrically packed $S_8$ ring molecules. At about 96°C, the $\alpha$-sulphur (rhombic sulphur) transforms (reversibly) into $\beta$-sulphur (monoclinic sulphur) which is stable up to ~118°C (at melting point of sulphur). In the present study, XRD analysis confirms presence of metacinnabar and orthorhombic form of sulphur in *Kajjali*. During purification, sulphur is melted and poured into liquid media (viz. cow's milk and ghee) for sudden cooling and during the processing of *Kajjali*, a mild temperature is generated during rubbing, which may be the possible causes for the formation of orthorhombic sulphur in *Kajjali.*

*Varitara*, *Rekhapurnatwa* and *Shlakshanatwa* tests signify the fineness of particles with smooth texture of samples. SEM study suggests fine nature of *Kajjali*; particle size of most grains varies between 60 nm and 200 nm. Only a few particles range from 1 µm to 2.5 µm size. For the effective role in therapeutic bio-availability of metallic and mineral preparation, particle size is one of the important factors, as it decides the permeability of drug through cells, tissues and blood capillaries[23]. It has been reported that nanoparticles exhibited a size-dependent uptake from the intestine and its passage via the mesentery lymph supply and lymph nodes to the liver, with significant absorption of particles <100 nm (ref. 24). Therefore, uptake of *Kajjali* particle ranges of 60 nm through the intestine can be expected. Earlier, *Shah et al.*[11] reported that, in rats, co-administration with *Kajjali* increased the $C_{max}$, AUC and $t1/2$ of Rifampicin by ~1.75, 1.5 and 1.35 times respectively. In ayurvedic *Rasaushadhies*, HgS is used in herbomineral combination, where low percent of bioaccessibility also reduced the risk of this chemical form of Hg in medicine ingestion substantially[25]. Due to this important inherited property, *Kajjali* is used as *Yogavahi* in many *Rasaushadhies*.

EDAX study has been used to know the elemental composition of *Kajjali*; composition of mercury and sulphur is 88.84% and 11.16% respectively. Absence of other trace elements confirmed that *Kajjali* is the purest compound of mercury and sulphur. Additionally, XRD study revealed that this high percentage of mercury is completely bound with sulphur and formed HgS; which ensured that the classical steps followed to prepare *Kajjali* converted the purified mercury and sulphur into biocompatible form of mercury compound.

The present study illustrates the physico-chemical characterization of *Kajjali* attained through the well-defined processes of *Rasa Shastra*. Further, *Kajjali* produced is subjected to classical confirmatory test, followed by modern analytical techniques like XRD, SEM and EDAX. Traditional method of *Mardana* initiates the binding of each free mercury atom with sulphur, which is confirmed through XRD study by the presence of HgS compound. The heat generated during melting and subsequent quenching during purification of sulphur and preparation of *Kajjali* leads to the presence of rhombic form of sulphur as observed in the XRD study. *Nishchandra* test of classic method is validated by XRD which shows complete absence of free mercury. Thus, both ancient and contemporary tests complement each other and confirm that *Kajjali* is black HgS, known as Metacinnabar with nano-size range particles (confirmed through SEM study), which is authenticated in the classical tests of *Rekhapoornata*. Therefore, we suggest that classical methods integrated with contemporary techniques can be very useful for characterization of ayurvedic drugs.


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