Journal of Postgraduate Institute of Indigenous Medicine (JPGIIM) Vol. 1, No.1, December 2025, ISSN: 3051-522X (Online)

Standardization of *Lokanatha Rasa* - A Classical Mercurial Preparation

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ABSTRACT

Rasa Shastra is a main pharmaceutical branch in Ayurveda. *Lokanatha Rasa* (LKN), is a mercurial preparation, indicated for various liver and spleen disorders. This study was aimed to conduct standardization of LKN used in Ayurveda treatments using physicochemical and modern technical parameters, employing advanced instruments like particle size analyzers, XRD, SEM, and ICP-MS. Organoleptic characteristics of the ingredients and final product in the powder form were tested based on classical parameters such as *Varna*, *Gandha*, *Sparsha*, *Varitharathwa*, *Rekapoornathwa* and *Gatha Rasathwa*. Results showed the total ash value of 85.25±0.1%, water-soluble ash value of 9.08±0.04% and acid-insoluble ash value of 3.50±0.2% which are within the acceptable range for a mineral powder form preparation. Loss on drying of 0.34±0.2% shows low moisture content and pH of 8.1±0.1 shows an alkaline pH suitable for the gastric mucosa. Microbial testing indicated absence of E. *coli*, *Coliforms*, *Pseudomonas* and *Salmonella*, with acceptable limits for aerobic plate count, yeast and molds. The average particle size of the LKN samples was <4.57 µm. FTIR analysis revealed characteristic absorbance peaks between 1792cm^{-1} and 417cm^{-1}, while SEM micrographs revealed various crystallite shapes (rod, round, square, and cubic). XRD confirmed the presence of inorganic mercury (HgS), with no elemental mercury. ICP-MS analysis revealed absence of Arsenic and Cadmium, Lead level below the WHO limits and Mercury level above the WHO limits. However, an ICP-MS analysis indicates the total mercury level of a product. But in this preparation, Mercury is present as HgS, which is proven as a non-toxic compound to the human body in previous research studies. These findings establish a fingerprint profile for LKN, aiding its standardization and quality control.

Key words –*Lokanatha Rasa*, Mercury, *Rasa Shastra*, ICP-MS, X-ray diffraction

Recommended Citation: Herapathdeniya, S. K. M. K., Paranagama, P. A., & Ranasingha, J. G. S. (2025). Standardization of *Lokanatha Rasa* – A classical mercurial preparation. *Journal of Postgraduate Institute of Indigenous Medicine*, 1(1), 11–34. Postgraduate Institute of Indigenous Medicine.

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Introduction

Rasa Shastra is a key branch of Ayurveda pharmaceutics, with mercury serving as a primary material. In addition to mercury, *Rasa Shastra* describes the use of various minerals, metals, gems, materials of marine origin, and some toxic substances and their preparations. These preparations, collectively known as *Rasaushadies*, are valued for their low dosage, absence of unpleasant taste, and rapid effectiveness, making them superior therapeutic options. (Satputae, 2003). Four types of *Rasaushadies* are traditionally described in *Rasa Shastra*: *Parpati* (prepared as small flakes), *Kupipakwa* (Prepared inside glass bottles), *Kalveeya* (also prepared inside glass bottles) and *Pottali* (scattered and comprehensive preparation) (Jha, 2000).

Lokanatha Rasa (LKN) is a mercurial preparation mentioned in several *Rasa Shastra* texts, with variations in its ingredients and preparation methods. In this study, LKN was prepared following the textual reference from *Rasendrasara Sangraha* (RSS), where its preparation method aligns with the *pottali* process. The *Rasendrasara Sangraha* (RSS) specifically indicates LKN for treating liver and spleen disorders (*yakrit pleeha roga*), oedema (*shotha*) and inflammatory bowel diseases (*grahani*) (Satputae, 2003). The prescribed dosage is 1 *raththika* (125 mg) per day.

To date, no studies have reported the standardization and characterization of LKN prepared according to RSS. Therefore, this study aims to develop a standardization profile for LKN and to conduct a thorough physicochemical analysis using both modern and conventional methods.

Materials and Methods

Preparation of LKN

All the ingredients used for the preparation of LKN (table 1) were purchased from the registered drug supplier of the Faculty of Indigenous Medicine, University of Colombo and their authenticity was confirmed based on the classical parameters. (Somadev, 2004).

Table 1: Ingredients used for the preparation of *Lokanatha Rasa* (LKN) (Satputae, 2003)

Ingredient	Amount
Shuddha Parada (purified Mercury)	50g
Shuddha Gandhaka (purified Sulphur)	50g
Abhra Bhashma (incinerated mica)	100g
Lauha Bhashma (incinerated Iron)	200g
Thamra Bhashma (incinerated copper)	200g
Varatika Bhashma (incinerated cowrie)	600g
Betel juice (Juice of *Piper betel* leaves)	Quantity sufficient

According to classical textual reference, ingredients of LKN were taken as 50g of each *Shuddha Parada* (purified Mercury) and *Shuddha Gandhaka* (purified Sulphur), 100g of *Abhra bhashma*, 200g of *Lauha Bhashma* (incinerated iron) and *Thamra Bhashma* (incinerated copper) and 600g of *Varatika Bhashma* (incinerated cowrie).

Beetle juice (*Pipper beetle* L.) which was needed for the grinding process in the preparation of LKN was purchased from the local market.

Kajjali was made by grinding *Shuddha Parada* and *Shuddha Gandhaka* together and was mixed with *Abhra*, *Thamra*, *Kaparda* and *Lauha Bhashmas* in relevant proportions and the mixture was ground 7 times using fresh beetle juice. The ground mixture was subjected to *Gajaputa*, where the mixture was placed inside a crucible and closed with another crucible of the same size. The joint of the two crucibles was sealed using a rag and mud and was subjected to heating at 800 – 1000ºC for one hour. Three samples of LKN were prepared following the same procedure.

Table 2: *Shodhana* processes of the ingredients

Ingredient	*Shodana* liquid	*Shodana* method
Parada	*Chornodaka* (lime water), *Rasona Kalka* (garlic paste), and *Saindawa Lavana* (Rock salt)	Grinding and filtering
Gandhaka	Milk, Ghee	Melting and pouring
Abhra	*Triphala Kwatha* (Decoction of *Triphala*)	Heating and quenching
Thamra	*Samanya Shodhana - Taila* (Sesame oil), *Thakra* (Butter milk), *Gomuthra* (Cow's urine), *Aranala* (Vinegar), *Kulaththa kwatha* (Decoction of *Kollu*) *Vishesha Shodhana - Triphala* decoction	Heating and quenching
Loha	*Samanya Shodhana* – same as the ingredients for *Thamra* *Vishesha Shodhana* – Rock salt and lime juice paste, *Nirgundhi* juice	Heating and quenching }
Kaparda	Lime juice	Steaming
Ingredient	*Shodana* liquid	*Shodana* method
Parada	*Chornodaka* (lime water), *Rasona Kalka* (garlic paste), and *Saindawa Lavana* (Rock salt)	Grinding and filtering
Gandhaka	Milk, Ghee	Melting and pouring
Abhra	*Triphala Kwatha* (Decoction of *Triphala*)	Heating and quenching
Thamra	*Samanya Shodhana - Taila* (Sesame oil), *Thakra* (Butter milk), *Gomuthra* (Cow's urine), *Aranala* (Vinegar),	Heating and quenching

	Kulaththa kwatha (Decoction of *Kollu*) *Vishesha Shodhana - Triphala* decoction	
Loha	*Samanya Shodhana* – same as the ingredients for *Thamra* *Vishesha Shodhana* – Rock salt and lime juice paste, *Nirgundhi* juice	Heating and quenching }
Kaparda	Lime juice	Steaming

Initially a *Shodhana* (purification) process as summarized in table 2, was followed for the raw materials as mentioned in the classical *Rasa Shastra* texts (Dolle, 2010; Satputae, 2003; Mishra, 2000).

***Shodhana* of *Parada* (Mercury)**

Initially 100g mercury was taken in a clean porcelain mortar and 50g lime powder was added to it slowly. Then the mixture was grinded continuously without spilling out mercury. This grinding process was continued up to 72 hours. Then, 500ml lukewarm water ($4C^0$) was put into the mortar and stirred it well by using spatula. Due to this all the mercury disintegrated lime mixed with water properly. Then after washing with lukewarm water removed the lime in the mortar. This washing continued until only mercury remained in the mortar. Then 450ml of water used for each time in the washing purpose. Thereafter, by using a two folded cotton cloth, the mercury was filtered by squeezing the cloth slowly and collected the filtered mercury into a clean porcelain mortar. Finally weighed the amount of the mercury.

Fifty grams of garlic cloves were taken and peeled them off. Then removed the small part inside the garlic clove and weighed it. The weight of the garlic cloves was equal with the mercury which was completed the grinding process of lime. Then these garlic cloves were put into the mortar and grinded it until become a fine paste. Half weight of mercury, similar weight of rock salt was taken and it also mixed with garlic paste properly. Then after this garlic-rock salt paste mixed with the mercury and continuously grinded until become black color paste. Then after this black color paste also washed by lukewarm water. The washing process continuous until only mercury remains in the mortar. After by using two folded cotton cloth filtered the mercury by squeezing the cloth slowly

and collected the filtered mercury into a clean porcelain mortar. Finally weighed the amount of purified mercury.

***Shodhana* of *Gandhaka* (Sulphur)**

Hundred grams of *Gandhaka* were taken and powdered it properly. Then 100ml cow's milk put into the stainless-steel vessel and ghee smeared piece of cloth was covered the mouth of the vessel. Powdered *Gandhaka* were taken into the ghee smeared ladle and heat it slowly until become sulphur melt. Then properly melted sulphur pours on the ghee smeared cloth which was wrapped on the stainless-steel vessel. Then the melted sulphur slowly goes through the cloth and mix with milk. After 10 minutes *Gandhaka* were taken from the milk and repeat this procedure six times again. Each time new fresh ghee and milk were used in this process. At the end Sulphur washed with warm water and dried it properly.

Preparation of *Kajjali*

Equal amount of *Shuddha Parada* and *Shuddha Gandhaka* were ground together without adding liquid and grinded it until become shining less soot like powder preparation called as *Kajjali*.

Preparation of *Abhra Bhashma*

Shodhana* of *Abhra

A sample of 250g *Abhra* was taken, separated into layers and broke them into small pieces. Then these small pieces were put into a large crucible. Then this crucible was put inside the muffle furnace and heated up to 750^0C until *Abhra* became red hot. Then 500ml cow's milk taken into the vessel and red-hot *Abhra* were immediately put into the milk vessel. After ten minutes *Abhra* was taken out from the milk contained vessel. This procedure was repeated six times. Each time a fresh sample of milk was taken for the process.

Preparation of *Dhanya Abhra*

From *Shoditha Abhra,* 200g was mixed with 50g paddy properly. Then 18''x18''x18'' piece of cloth was taken and *Abhra* - paddy mixture was placed on it. Then this mixture was wrapped by the cloth and prepared it as a bolus. Wide mouth vessel was taken and 250ml vinegar was added into it. The *Abhra*-paddy bolus was placed inside this vessel and kept it for 3 days continuously. After that, this bolus was vigorously rubbed by using both hands until all *Abhra* particles came out from the tiny holes of the cloth. This vessel was kept 24 hours without

disturbing to settle down *Abhra* particles on the lower part of the vessel. The upper portion collected in the vessel was decanted carefully and lower part settled down as *Abhra* was collected and completely dried in sunlight.

Preparation of *Abhra Bhashma*

Kasamarda Panchanga was collected from the herbal garden Faculty of Indigenous medicine, University of Colombo. The sample was washed and grinded with using little amount of water. The juice was taken and filtered it. Prepared *Dhanyabra* was put into the mortar and continuously grinded by adding *Kasamarda Panchanga* juice little by little, until it becomes a thick paste. For completion of this process 8 days were required. After that, small pellets were prepared by using this thick paste and dried them well in sunlight. The dried pellets were placed inside an earthen crucible and covered it with same size crucible. Then the joint of the two crucibles was sealed by using piece of cloths and clay. (*Sharava Samputa*). Then this apparatus was kept inside the muffle furnace, allowed to increase the temperature up to 900^0C and then kept in this same temperature for one hour. It was allowed to self- cooled and the pellets were taken out. This process was repeated for 28 times. After completion of each *Puta*, *Bhashma Pariksha* was conducted and recorded.

Preparation of *Lauha Bhashma*

Shodhana* of *Lauha

A sample of 500g *Lauha* pieces was obtained and it was subjected to *Samanya Shodhana* and *Vishesha* Shodhana. For *Samanya Shodhana* five different liquids, *Taila* (sesame oil), *Takra* (buttermilk), *Gomutra* (cow's urine), *Aranala* (vinegar) and *Kulaththa Kwatha* (decoction of *Dolichos biflorus* L.) were used. The sample of *Lauha* pieces were red hot and dipped in each of the above liquids seven time consecutively. For each time fresh liquid sample was taken. At the end of *Samanya Shodana*, the iron pieces were used for *Vishesha Shodhana* process and heated up to 850^0 C using a muffle furnace until the iron pieces were red hot. Red hot iron pieces were put into 200 ml of *Triphala* decoction and kept for two minutes. Then iron pieces were separated from the *Triphala* decoction. This step was repeated seven times using fresh *Triphala* decoction for each step. After that, iron pieces were dried properly.

Preparation of *Lauha Bhashma*

This preparation consists of three steps as *Bhanupaka*, *Sthaalipaka* and *Putapaka*. (*Rasendrasara Sangrahaya*);

i. *Bhanupaka* method – *Vishesha Shoditha Lauha* particles were put into a clay pot which contains *Triphala* decoction and kept it in direct sunlight for 7 days continuously and filtered.
ii. *Sthaalipaka* method – *Lauha* particles subjected to *Bhanupaka* method were taken and put into a clay pot with *Triphala* decoction. It was continuously heated in fire for 7 days consecutively and filtered.
iii. *Putapaka* method – *Lauha* sample subjected to *Sthaalipaka* method was taken and grinded using *Triphala* decoction. Then the paste obtained was made into pellets and dried. It was taken in an earthen vessel and covered with another vessel of the same size. The space between the two vessels was covered with a layer of cotton cloth smeared with mud. The prepared *Sharava Samputa* was heated for 650^0C in the muffle furnace.

Preparation of *Tamra Bhashma*

A sample of 500g of copper wire (*Thamra*) was taken and the same procedure of *Lauha Samanya Shodhana* was followed. For the *Vishesha Shodhana* of *Thamra, Snuhi Ksheera* was mixed with *Arka Ksheera* and *Saindhava Lavana* and prepared into a paste. This paste was applied on *Thamra* fragments and allowed to dry. These *Thamra* fragments were put into an earthen pot and kept it inside the muffle furnace. Then the sample was red hot in 850^0C temperature. Then red hot *Thamra* sample was quenched into the *Nirgundhi Kwatha* 3 times. Finally, this sample was washed with water to remove extra *Snuhi Ksheera*, *Arka Ksheera* and *Saindhava Lavana* to obtain *Vishesh Shodhitha Thamra*.

Then *Kajjali* was put into a mortar and *Vishesha Shoditha Thamra* was added to it. This was continuously grinded until it became a homogenous mixture. Then lime juice was mixed into it and continuously grinded till it became a paste. Then little by little paste were taken out from the mortar and prepared small pellets by using it. All these pellets were properly dried in sunlight. Dried pellets were arranged in an earthen vessel and same size another vessel was inverted on it. Joint of the two vessels were covered with mud and cloth strips. Then this apparatus (*Sharava Samputa*) was kept inside the muffle furnace and subjected it to 900^0C for 45 minutes (*Gaja Puta*). After that, this apparatus was allowed to self-cooling. Next day the crucible was opened and the *Thamra* powder was removed from the crucible. Equal amount of *Kajjali* was mixed again with it and properly grinded using lime juice. The same process was continued for 3 times. From the 4th time onwards, heat was applied to *Thamra* powder by grinding only lime juice without adding *Kajjali*. After completion of 8th *puta Thamra Bhashma* obtained with required characteristic features.

Preparation of *Varatika Bhashma*

Shodhana of *Varatika*

The 750g sample of *Varatika* was boiled with lime juice in *Dolapaka Yantra* (swing apparatus), filtered and powdered.

Preparation of *Varatika Bhashma*

Purified *Varatika* were powdered and grinded with aloevera juice. Then *Chakrika* (pellets) were prepared and heated at 350^{0}C in the muffle furnace by placing the pellets inside a crucible and closing it with another crucible of the same size.

Physicochemical characterization of LKN

Physicochemical analysis of LKN was conducted using both classical and modern parameters. Organoleptic evaluations of *Kajjali*, *Bhashmas,* and LKN were performed respectively. Following this, the analysis included the determination of total ash, acid-insoluble ash, water-soluble ash, loss on drying at 110 ºC, pH, microbiological load, particle size, FTIR analysis, Scanning Electron Microscopy (SEM), X-RAY Diffraction (XRD**),** and heavy metal analysis of LKN by using high-resolution Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).

Organoleptic characteristics of LKN

Ash colour very fine powder was obtained as LKN. The evaluation of *Varna* (color), *Sparsha* (touch), *Gandha* (smell), *Varitharathwa* (floating on water), *Rekhapoornathwa* (when a pinch of powder was taken between the index finger and thumb, all particles enter the furrow of fingers), and *Gatha Rasathwa* (absence of a specific taste) were performed according to both classical and modern parameters.

Determination of ash values

Determination of total ash

A pre-weighed silica crucible containing 2g of powdered drug material was placed in a muffle furnace and heated to a maximum temperature of 450 ºC until it was free of Carbon. The crucible, now containing the total ash, was cooled in a desiccator and weighed multiple times to ensure a constant weight. The total ash value was then calculated as a percentage (%w/w) relative to the initial weight (Ayurveda Pharmacopoeia of India, 2007).

Determination of acid-insoluble ash value

Two grams of the sample were digested with 25 ml of diluted Hydrochloric acid (HCl) for 5 minutes, then filtered through Whatman No. 42 filter paper. The residue was washed with hot water until it was free from chloride. The resulting residue was allowed to air dry and then incinerated in a muffle furnace along with the filter paper. Afterward, the crucible containing the acid-insoluble ash was cooled in a desiccator and weighed (Ayurveda Pharmacopoeia of India, 2007).

Determination of the water-soluble ash value

Two grams of ash from LKN were boiled with 25 ml of distilled water. The insoluble matter was collected in a crucible and washed with hot water. The residue was then ignited at 450 ºC until a constant weight was achieved. After cooling in a desiccator, the residue was weighed. The weight of the insoluble matter was subtracted from the initial weight of the ash. The percentage of water-soluble ash, with reference to the sample, was then calculated. (The Ayurvedic Pharmacopoeia of India, 2007)

Determination of the loss on drying at 110 ºC (moisture content)

One gram of accurately weighed sample was placed in a previously weighed silica crucible and heated in a muffle furnace at 110 ºC until a constant weight was achieved. The crucible was then removed and allowed to cool. After cooling, the crucible was weighed again. The percentage of loss on drying was calculated from the weight loss and expressed as % w/w (Ayurveda Pharmacopoeia of India, 2007)

Determination of pH

Ten grams (w/w) of accurately weighed LKN were extracted with 100 ml (w/v) of distilled water by macerating for 2 hours. The mixture was then filtered, and the filtrate was used to determine the pH using a pH meter (Eutech pH700, Thermo Scientific) (The Ayurvedic Pharmacopoeia of India, 2007).

Microbiological load analysis of *Lokanatha Rasa*

For safety inspection of the preparation, it is necessary to analyze the microbiological load. This analysis was carried out at the accredited microbiological laboratory at the Industrial Technology Institute, Colombo, following the protocol in the Indian Ayurveda Pharmacopeia (Anonymous, 2007). Aerobic plate count, Yeasts and Molds, *Coliform, E.coli* count, *Salmonella, Staphylococcus, Pseudomonas aeruginos*a were analyzed.

Particle size analysis

Determination of the particle size was carried out using the Fritch Analysette 22 NanoTech particle size analyzer, with a measuring range of 0.01µm to 2100 µm (Particle sizing, static light scattering and detailed product of laser particle sizerb analysette-22 next nano)

FTIR analysis (Fourier Transform Infrared Spectrophotometer)

The possible functional groups and organic components present in the test drug were analyzed using a Fourier Transform Infrared Spectrophotometer (FTIR). Multiple beam internal infrared reflection spectroscopy was employed to identify the chemical nature of the molecules. For each preparation, characteristic, intense, sharp, well-defined peaks were observed. The presence of these characteristic peaks helps define a sample and provides a fingerprint for the relevant preparation. FTIR spectral analysis is typically performed in the region 400 – 2000 cm^{-1}. The FTIR analysis of LKN was conducted at the Instrumental Center of the University of Sri Jayewardenepura. Perkin-Elmer frontier optical system with a KBr beam splitter for MIR, with a spectral range of 4000-400 cm^{-1} at a resolution of 0.4 cm^{-1}. Well dried fine powder of LKN was used for the FTIR scanning. The samples were optimized to ensure minimal water content and to be moisture-free. Each sample was mixed with KBr in a 1:100 ratio (1 part of sample drug to 100 parts of KBr), and pellets were formed through the press pellet technique using hydraulic pressure. The IR spectroscopy system was calibrated, and the prepared pellet was scanned under the same conditions. (Stuart, 2004), (Olohigbe *et al.,* 2018)

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is an advanced technique of microscopy that captures high-resolution images of a sample. SEM produces detailed images of the surface of a sample by scanning it with a focused beam of electrons. This technique provides detailed view of the surfaces of the cells and entire organisms. (Goldstein *et al.,* 2003)

The fine powdered LKN was made conductive by gold coating. A layer of 5-10 nm gold was applied to the sample particles using a sputter coater. The sample was then placed on the specimen holder and examined under the microscope (EVO MA 15, Carl Zeiss, Germany (ZP) at magnifications of 50 kX, 25 kX,10 kX, and 1kX. Micrographs were captured using the built-in camera

X-RAY Diffraction (XRD) Study

X-ray diffraction (XRD) is a rapid analytical technique primarily used for phase identification of crystalline materials and can provide information on unit cell dimensions. The material to be analyzed was finely grounded and, homogenized, and its average bulk composition is determined.

A10 mg sample of LKN was spread on to a piece of double-sided tape using a spatula, which was then placed on the aluminum sample holder. The X-ray diffractometer was set to its optimum settings. The sample holder was properly positioned, and the 2θ was initially adjusted to 70º. The sample was scanned slowly to ensure proper data recording till 8^{O} of 2θ. Once the scan was complete, the instrument was stopped. The recorded data was analyzed for the characterization for the sample. The 2θ values and the intensity of the peaks (counts) were represented on the X and Y axes, respectively. A higher count value indicates a higher degree of crystallinity of the phase. For phase identification, a minimum of three strong peaks were selected, and the results were compared with standard substances. (Kasai *et al.,* 2005). The study was conducted at the Instrumental Centre, University of Sri Jayewardenepura.

ICP-MS analysis

The ICP-MS analysis followed standard procedures as outlined by the Indian Institute of Technology, Mumbai, India. (Service and technical support of ICP-MS).

For the microwave digestion of the sample a 100mg of LKN was placed inside a cleaned quartz vessel. To this and 1ml of 30% hydrogen peroxide (Supra-pure grade, Merck, Germany) and 5 ml of 65% nitric acid (Supra-pure grade, Merck, Germany) were added. The samples were placed in the microwave (Multiwave, Anton Paar, Austria) and subjected to the following condition: (write the below without bullets).

- Starting at 200W for 2 minutes, followed by an increase in power to 500 W for 5 minutes.
- These conditions resulted in a digestion temperature of 250 ºC and a digestion pressure of 70 bar.

The sample was allowed to cool for 10 minutes and then transferred to 25 ml standard flask, which was filled with ultra-pure water (Millipore, USA) to a final volume of 25 ml.

In this study, a Perkin Elmer Sciex Elan DRC II quadrupole ICP-MS instrument (Perkin-Elmer, USA) was used. The instrument was operated with a glass

concentric spray chamber and a concentric nebulizer. A peristaltic pump was used for sample uptake.

ICP-MS parameters used for the study: (write the below in a paragraph)

Argon flow rate: Plasma gas – 15 L/min, Auxiliary gas-1.1 L/min, Nebulizer gas -1.0 L/min

RF forward power: 1300W

Cell gas/flow rate: NH_3 - 0.4 ml/min

Sample flush time: 60 s

Wash time: 60 s

Multi-elemental and individual standards used were obtained from Merck Germany.

Results and Discussion

Physicochemical and organoleptic characteristics of *Lokanatha Rasa* (LKN)

The organoleptic characteristics of *Kajjali* revealed a smooth, fine, jet-black powder without shining particles (Table 4). These features of *Kajjali* were due to the combination of mercury and sulphur subjected to continuous grinding without the addition of any liquid. The powder exhibited *Varitharathwa* and *Rekapoornathwa* properties, attributed to the fine combination of mercury and sulphur. *Kajjali* remained lusterless, as it did not contain free Mercury particles.

The *Abhra*, *Thamra*, *Loha*, and *Kaparda Bhashmas*, which are key ingredients in LKN, were also prepared according to classical *Rasa Shastra* texts. These *Bhashmas* showed acceptable colors and characteristic features. Specifically, *Thamra* remained brick-red, *Abhra* black, *Loha* purple-red, and *Kaparda* white, each showing classical features such as *Varithrathwa*, *Rekhapoornathwa* and *Gatharasathwa* (Table 4). The fine form of these *Bhashmas* was a result of their adherence to classical parameters. The final product, LKN, displayed a fine ash color, and demonstrated its fine consistency, with *Varithrathwa*, and *Rekhapoornathwa* features, reflecting the high quality of the preparation.

Table 3: Organoleptic characters of *Lokanatha Rasa* and its ingredients, including color, touch), smell, floating on water, fineness of powder, and taste

Ingredient	Parameters					
	Colour (*Varna*)	Touch (*Sparsha*)	Smell (*Gandha*)	Floating on water (*Varitha rathwa)*	Fineness of powder (*Rekhap oornath wa*)	Taste (*Gatha rasath wa*)
Kajjali	Jet-black	Smooth, fine powder	Smell of Sulphur	✓	✓	✓
Abhra Bhashma	Brick-red (*Ishtika*)	Smooth, fine powder	None	✓	✓	✓
Thamra Bhashma	Black (*Krushna*)	Smooth, fine powder	None	✓	✓	✓
Lauha Bhashma	Purple-red (*Pakwa Jambhawa Phala*)	Smooth, fine powder	None	✓	✓	✓
Kaparda Bhashma	White color (*chandrama*)	Smooth, fine powder	None	✓	✓	✓
Lokanatha Rasa	Ash color (*Dhoosara*)	Smooth, fine powder	None	✓	✓	✓

✓ Indicates positive for the parameter

The analytical assessment of LKN provides insights into its purity, accuracy, quality, safety, and chemical nature.

Ash values of LKN

Total ash value

The total ash value determines the metallic substances present in the sample. LKN is prepared using various inorganic materials, including mercury (Hg), Sulphur (S), Iron (Fe), Copper (Cu) and Calcium carbonate ($CaCO_3$). LKN is likely to contain a significant number of inorganic salts. The high content of inorganic salts in LKN is reflected by an ash value of 85.25±0.1%.

Acid insoluble ash value of LKN

The acid insoluble ash value indicates the amount of insoluble inorganic contents, such as silica, and sand, present in the preparation. The total acid insoluble ash value of LKN was 3.50±0.2%. The low content of sand and silica in LKN results in a low acid insoluble ash value. Additionally, LKN contains materials like *Abhra* (Mica) and *Kaparda* (Cowrie), which may contain small amounts of silica. The presence of silica contributes to the acid insoluble ash values.

Water soluble ash value of LKN

The water-soluble ash value of LKN was 9.08±0.04%. These values can help determine the selection of the media for drug administration. Since the water-soluble ash content is relatively low in LKN, it suggests that LKN has limited solubility in fluids like saliva and gastric juice, which can affect the solubility and absorption of the drug.

Moisture content of LKN

LKN exhibited low value for loss on drying at 110 ^{O}C, with moisture content remaining under 0.34±0.2%. The low moisture content in all LKN samples reduces the risk of microbial contamination and prevents decomposition due to undesired chemical changes. Additionally, the low moisture content helps determines the stability of the samples overtime. Based on these results, all three LKN samples, with their low moisture content, are stable over extended periods. Therefore, LKN is less likely to be contaminated with common microbes such as bacteria and fungi.

pH of LKN

LKN exhibited basic pH value of 8.1±0.1. Due to these basic pH values, LKN is not cause any gastric irritation or a burning sensation. Considering the ingredients in LKN, the highest amount is *Kaparda bhashma* (cowrie), which has a chemical

composition of $CaCO_3$. Therefore, the basic pH of LKN is favorable for the gastric mucosa.

Microbiological load analysis of LKN

The microbial load of LKN showed that the aerobic plate count, yeast, and molds were below the limit of detection. E. *coli* and *coliform* were not detected, and *Pseudomonas* and *Salmonella* were absent. These results summarized in table 4, indicate that LKN is microbiologically safe to cause any harmful effects to the gastrointestinal (GIT) system and is in good hygienic condition.

Table 4: Microbial load analysis of *Lokanatha Rasa* (LKN), showing the test methods, results, and limits of detection for various microbial parameters, including aerobic plate count, yeast and molds, *coliforms*, E. *coli*, *Salmonella*, *Staphylococcus*, and *Pseudomonas aeruginosa*.

Test/Unit	Test method	Test Results	Limit of detection
Aerobic plate count CFU per g	SLS 516 -1/1:2013	$4.0X10^1$ est	_
Yeasts and Molds CFU per g	SLS 516 -2/2:2013	$8.5x10^1$ est	_
Coliform, MPN/g	SLS 516 -3/1:2013	Not detected	_
E. coli count, MPN/g	SLS 516 -12:2013	Not detected	_
Salmonella SP per 25g	SLS 516 -5:2017	Absent	_
Staphylococcus per 10g	CML/MM/01/02/004	Less than 10	_
*Pseudomonas aeruginos*a per 10g	BP 2016/V-A 486	Absent	_

Particle size analysis of LKN

The particle size analysis of LKN showed that 50% (D50) value represents the representative particle size of the sample. Therefore, the average particle size of

the mineral drug sample is less than 4.57 µm. This average was calculated by replicating the test three times.

Table 5: Variations in particle size at different percentages of *Lokanatha Rasa* (LKN), showing the average particle size (µm) with corresponding standard deviations for each concentration

Percentage	Particle size (µm)
10%	< 0.89 ± 0.10
20%	< 1.58 ± 0.14
30%	< 2.27 ± 0.17
40%	< 3.13 ± 0.26
50%	< 4.57 ±0.71
60%	< 7.50 ± 1.18
70%	< 11.99 ± 1.52
80%	< 15.61 ±1.61
90%	< 19.96 ±1.77

The bioavailability of metallic and mineral preparation mainly depends on the particle size of the relevant preparation. The average particle size of the LKN sample is less than 4.57 µm. According to the particle size analysis of LKN, most of the particles fall within the nano to micro particle range (Table 5). Particle size plays a critical role in determining the permeability of the drug through cells, tissues, and blood capillaries. (Goldberg *et al.,* 2007). Therefore, these nano and micro particles are responsible for the rapid absorption of the preparation.

FTIR analysis of LKN

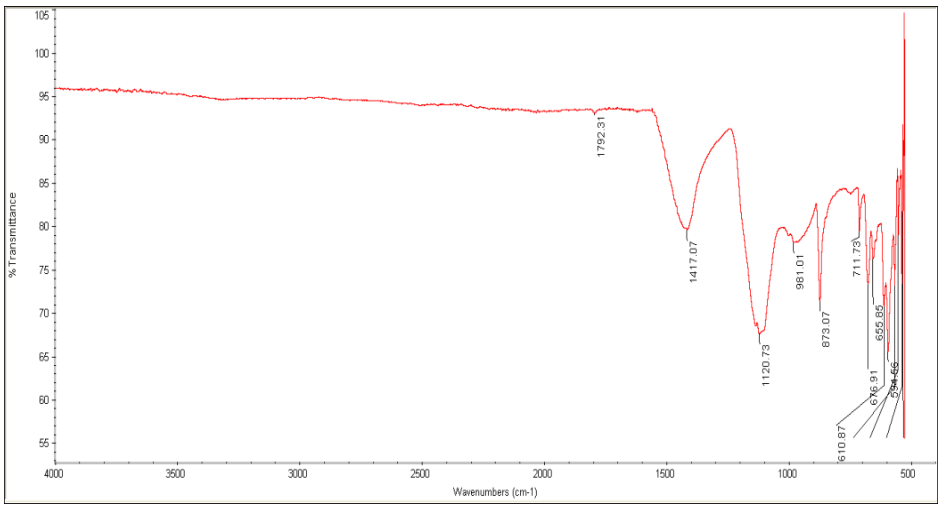

Figure 1: FTIR spectrum of *Lokanatha Rasa* (LKN), displaying key absorbance peaks at specific wavenumbers, which correspond to various functional groups and organic components within the preparation

FTIR analysis of LKN revealed absorbance peaks at 1792 cm^{-1}, 417 cm^{-1}, 1120 cm^{-1}, 873 cm^{-1}, 711 cm^{-1}, 676 cm^{-1}, 655 cm^{-1}, 610 cm^{-1}, and 594 cm^{-1}as illustrated in figure 1. The strong peak at 1792 cm^{-1} in the IR spectrum corresponds to the C=O stretching vibration of anhydrides, and the peak at 1417 cm^{-1} corresponds to the OH bending of alcohol or carboxylic acids. The peak at 1120 cm^{-1} corresponds to the C-O stretching secondary/tertiary alcohols and aliphatic ethers. Two weak peaks at 873 cm^{-1} corresponds to the C-H bending (substituted), and the peak at 711 cm^{-1}1 corresponds to the C-H bending (benzene derivatives). The weak peaks at 676 cm^{-1}, 655 cm^{-1}, 610 cm^{-1}, and 594 cm^{-1} in the IR spectrum are associated with halogens, organometallic compounds, and inorganic compounds. FTIR analysis suggests the presence of organic matter in the formulation. These functional groups could be due to the formation of organometallic compounds in the drug sample, which can withstand high processing temperatures.

SEM analysis of LKN

The micrographs at 25 nm, 50.00K X (Figure 2, A) and at 1µm, 25.00K X (Figure 2, B) magnifications, shows the larger particles clearly, where the agglomeration of smaller particles is prominently visible. Additionally at 25 nm, 50K X magnification, various crystallites in different shapes, including rod-shaped, round, square, and cubic particles, are embedded within the lumps indicating

microcrystalline structure. The micrograph at 1µm, 10.00K X (Figure 2, C) reveals the arrangement of clusters of granules in the LKN sample. The larger particles are agglomerations of smaller particles. The micrograph at 10µm, 1.00K X (Figure 2, D) magnification shows particles of different sizes and shapes, distributed homogenously throughout the sample. Nearly 60% of the particles are small, resembling nanoparticles.

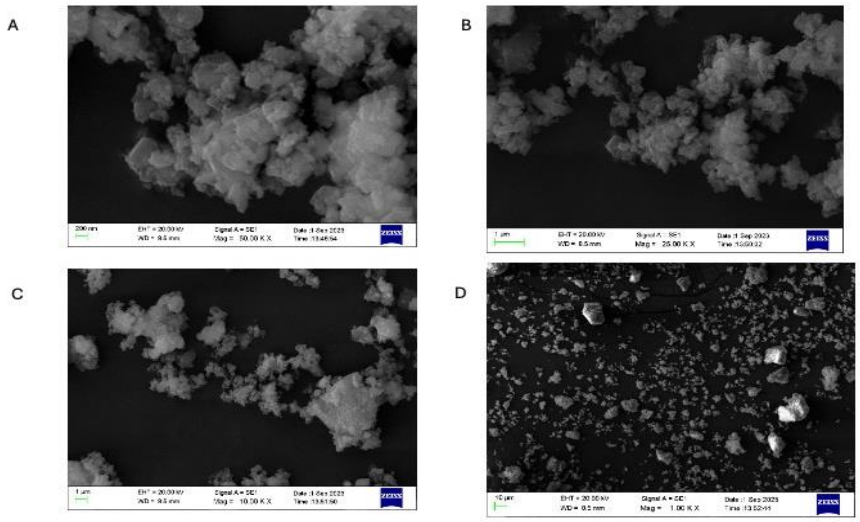

Figure 2: Scanning Electron Microscopy (SEM) images of *Lokanatha Rasa* (LKN) at various magnifications. (A) Image at 50,000X magnification showing the fine particulate structure of LKN. (B) Image at 25,000X magnification revealing the distribution and agglomeration of particles. (C) Image at 10,000X magnification highlighting the arrangement of granules. (D) Image at 1,000X magnification showing larger agglomerates and particle clusters. The scale bars represent 200 nm (A), 1 µm (B), 1 µm (C), and 10 µm (D).

XRD analysis of LKN

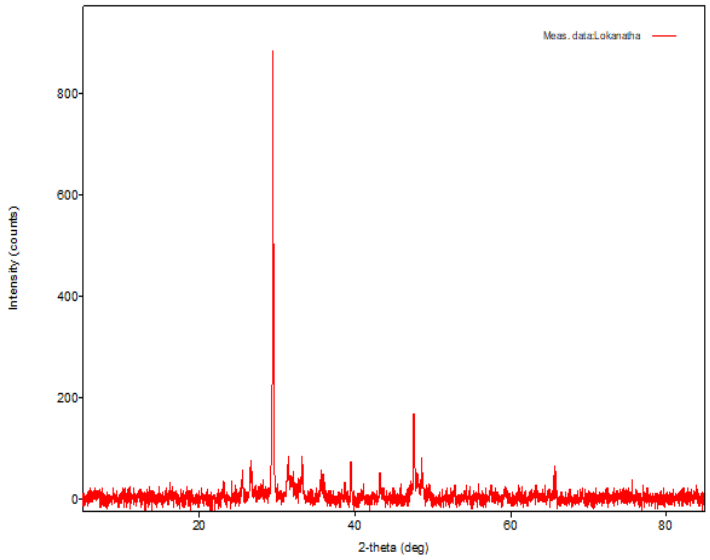

Figure 3: X-ray diffraction (XRD) pattern of *Lokanatha Rasa* (LKN) showing characteristic peaks corresponding to the crystalline phases present in the sample. The diffraction pattern indicates the presence of various crystalline structures, with prominent peaks representing specific compounds in the formulation

Table 6: X-ray diffraction (XRD) analysis of *Lokanatha Rasa* (LKN), showing the 2-theta values, corresponding d-spacing, peak height, full-width at half maximum (FWHM), crystallite size, and identified phases with their respective chemical formulas

No	2-theta (º)	d-spacing (Å)	Height (counts)	FWHM (º)	Size (Å)	Phase name	Chemical formula
1	23.223 (13)	3.827 (2)	128 (11)	0.09 (2)	951 (234)	Unknown	Unknown
2	26.488 (11)	3.3623 (14)	561 (24)	0.342(13)	249 (9)	Metacinnabar,	Hg S
3	30.68 (3)	2.912 (3)	83 (9)	0.54 (4)	159 (12)	Metacinnabar,	Hg S
4	31.37 (2)	2.849 (2)	59 (8)	0.44 (7)	194 (33)	Unknown	Unknown

5	43.893 (8)	2.0610 (3)	179 (13)	0.364(17)	245 (11)	Metacinnabar,	Hg S
6	51.93 (3)	1.7593 (9)	113 (11)	0.57 (3)	161 (9)	Metacinnabar,	Hg S
7	54.44 (2)	1.6840 (7)	20 (5)	0.54 (7)	171 (22)	Metacinnabar,	Hg S
8	63.79 (15)	1.458 (3)	8 (3)	1.3 (2)	77 (13)	Metacinnabar,	Hg S
9	70.03 (3)	1.3424 (5)	26 (5)	0.69 (11)	146 (23)	Metacinnabar,	Hg S
10	72.24 (4)	1.3067 (6)	14 (4)	0.71 (11)	144 (23)	Metacinnabar,	Hg S

XRD study revealed prominent peaks at the following 2θ angles: 26.488º (d = 3.3623), 30.68º (d = 2.912), 43.893º (d=2.0610), 51.93º (d=1.7593), 54.44º (d=1.6840), 63.79º (d=1.458), 70.03º (d=1.3424), and 72.24 º (d=1.3067), corresponding to the metacinnabar phase with the HgS chemical formula (Figure 2, table 6). Some metallic oxides were observed at lower intensities indicating unstable oxide states. The XRD results for LKN *Kajjali* showed clear peaks. Notably, elemental mercury was not detected in the XRD analysis. Therefore, as a mercurial product, LKN does not contain mercury in its elemental or free form.

ICP-MS analysis of LKN for following materials

Mercury is a toxic heavy metal for the human body, and its concentration in LKN sample was found to be 3.9 ppm as determined by ICP-MS (Table 7). The preparation was made and analyzed after following special procedures like *Shodhana*, *Jarana*, and *Amrithikarana*. According to classical textual references, these procedures are designed to remove toxic or unwanted components from the materials used.

Table 7: ICP-MS analysis of Lokanatha Rasa (LKN) for various heavy metals, showing the test results and corresponding limits of detection

Name of the metal	Test results	Limit of detection
Cadmium (Cd) mg/kg	Not detected	10 ppm

Lead (Pb)	8.2ppm	10 ppm
Mercury (Hg)	3.9 ppm	01 ppm,
Arsenic (As)	Not detected	10 ppm

Despite the ICP-MS analysis indicating that the Mercury content in LKN is slightly above the permissible level set by WHO guidelines, it is important to note that LKN does not produce any adverse or toxic effects on the human body. *Kajjali*, or black mercuric sulfide, is the mercury containing ingredient in LKN. Purified mercury, when ground with purified *Gandhaka* (sulphur) without any liquid, transforms into a lusterless (*Nischandrathva*) black powder with a soot like texture (*Kajjali*). The physicochemical characterization of *Kajjali* is well defined in classical *Rasa Shastra* texts, and modern analytical techniques, such as XRD and SEM, were used to confirm its properties.

The traditional method of *Mardana* (grinding) binds each free mercury atom with *Gandhaka* (Sulphur), which is confirmed through the XRD study by the presence of the HgS compound. During the *Shodana* process, Sulphur is subjected to heat, causing it to melt and subsequent pour into rhombic forms, as observed in XRD study. The classical *Nischandrathva* test was validated by XRD, showing the absence of free mercury in the LKN sample.

SEM analysis and particle size analysis confirmed that LKN consists of nano-sized particles. Therefore, *Kajjali* is also maintained in nano-sized particles within the preparation. The ICP-MS analysis only indicates the total mercury content of the preparation but does not specify whether it is in elemental or compound form. Since mercury is in the form of mercury sulfide (HgS), it is accepted by the body, which accounts for the high mercury content detected in the preparation.

Conclusions

This study provides a comprehensive pharmaceutical and analytical evaluation of *Lokanatha Rasa* (LKN), a mercurial preparation made according to classical Ayurvedic formulations. The findings demonstrate that LKN, prepared using traditional methods such as *Pottali Kalpana*, exhibits significant physicochemical properties, including a fine particle size, low moisture content, and the absence of free mercury. The analytical techniques used, including FTIR, XRD, SEM, and ICP-MS, confirm the presence of mercury in the form of mercury sulfide (HgS), which is deemed safe for the human body according to Ayurvedic

principles. Additionally, the study validates the absence of toxic impurities and highlights the stability and quality of the product. The results from this study contribute to the standardization of LKN and provide essential data that can be used for quality control and manufacturing processes. Future studies may further explore these parameters to refine and enhance the production of high-quality LKN for therapeutic use.

Conflicts of Interest

The authors declare that there is no conflict of interest and affirm that the content of this publication is original and not assisted by AI.

Acknowledgement

The authors acknowledge the Ministry of Environment, Sri Lanka and the United Nations Industrial Development Organization (UNIDO), Government of Switzerland, for providing grant assistance for this study.

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