Journal of Ayurveda and Integrative Medicine 10 (2019) 262–268

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original Research Article (Experimental)

Chemical characterization of an *Ayurvedic* herbo-mineral preparation- *Mahalaxmivilas Rasa*

N. Srikanth [a], A. Singh [a, *], S. Ota [a], B. Sreedhar [b], Galib [c], K.S. Dhiman [a]

[a] *Central Council for Research in Ayurvedic Sciences, 61-65, Institutional Area, Opp. D-Block, Janakpuri, New Delhi, India*
[b] *Indian Institute of Chemical Technology, Uppal Road, Tarnaka, Hyderabad, India*
[c] *All India Institute of Ayurveda, Sarita Vihar, New Delhi, India*

ARTICLE INFO

Article history:
Received 19 January 2017
Received in revised form
3 November 2017
Accepted 16 January 2018
Available online 20 November 2018

ABSTRACT

Background: To protect the massive trust of patient in Ayurveda, a need aroused for the researches to ascertain the quality, safety & efficacy of herbo-mineral preparations on scientific lines. The rasa-aushadhis are having qualities such as instant effectiveness, requirement in very small dosage and ample therapeutic utility. *Mahalaxmi Vilas Rasa* [AFI, 20:27] has been used for treatment of a variety of ailments since time immemorial.

Objective: To prepare *Mahalaxmi Vilas Rasa* as per standard operating procedures (SoPs) mentioned in classical text and to characterize it chemically using modern analytical techniques.

Materials and Methods: The drug (*Mahalaxmi Vilas Rasa*) in three batches was prepared in GMP certified pharmacy. Physico-chemical analysis, HPTLC, Assay of elements by AAS & ICP-AES were carried out as per Ayurvedic Pharmacopoeia of India. Powder X-ray diffraction (XRD) was conducted using Rigaku Ultima-IV X-ray diffractometer.

Results: The elemental analysis shown the presence of Mercury, Sulphur, Calcium, Copper, Gold, Iron & Tin etc. and HPTLC revealed presence of organic constituents from plant material. The XRD had indicated that prepared drug contained free sulphur, cinnabar (mercury sulphide added as Kajjali), cassiterite (tin oxide, Vanga Bhasma), orpiment (Hartal, arsenic III sulphide) and mica (Leucite/ Zeolite, Abhrak Bhasma). The drug was also tested for residual pesticide and microbiological contamination which were found within permissible limits.

Conclusion: Classical pharmaceutical procedures of *Mahalaxmi Vilas Rasa* showed converting the macro elements into therapeutically effective medicines of micro form. Standards laid down in this study certainly utilized as an important tool for standardization and quality assurance of this herbo-mineral formulation.

1. Introduction

A specialized branch in Ayurveda, which is “Rasa Shastra” having literal meaning as “Science of Mercury” deals with materials known as ‘Rasa dravyas’’. Rasa denotes mainly *Parada* (Mercury). Formulations made by mercury and incinerated metals and minerals are known as Rasa-aushadhis (Herbo-mineral-metallic preparations).

The rasa-aushadhis are having qualities such as instant effectiveness, requirement in very small dosage and ample therapeutic utility. There are four methods of preparation of these formulations i.e. *Khalviya Rasayana, Parpati Rasayana, Kupipakawa Rasyana, Pottali Rasayana. Mahalaxmivilas Rasa* is a herbo-mineral-metallic preparation comes under the *Khalviya Rasayana.*

It is well known for its use in *Urdhwa Jatrugata rogas* (Upper Respiratory Disorders) and *Amavata* (Rheumatoid arthritis). Important therapeutic uses are in *Kasa* (Cough), *Pinasa* (Chronic rhinitis/sinusitis), *Rajayakshma* (Tuberculosis), *Amavata* (Rheumatism), *Vajikarana* (Aphrodisiac), *Gala Roga* (Diseases of throat), lymphadenopathy, *Antra Vriddhi* (Hernia), *Kushtha* (Diseases of skin), non-healing wounds, *Atisara* (Diarrhoea), *Prameha* (Urinary disorders), *Shlipada* (Filariasis), *Vrana* (Ulcer), *Nadivrana* (Fistula), *Bhagandara* (Fistula-in-ano), *Arsha* (Hemorrhoids), *Udara* (Diseases of abdomen/enlargement of abdomen), *Raktavikara* (Disorders of blood), *Stri roga* (Gynaecological disorders), *Tvagroga* (Skin disease),

* Corresponding author. 61-65, Institutional Area, Opp. D-Block, Janakpuri, 110058, New Delhi, India.
E-mail address: arjunccras@gmail.com (A. Singh).
Peer review under responsibility of Transdisciplinary University, Bangalore.

https://doi.org/10.1016/j.jaim.2018.01.002

Nasa Roga (Disease of nose), *Netraroga* (Eye disorder), *Mukha Roga* (Disease of mouth) and *Shula* (Colicky Pain) [1].

The adjuvant or *anupan* with which medicine should be taken depends on diseases to be treated (Table 1). It is used in Vati/tablet form. As it is an important and commonly used formulation, the documentation on chemical characterization and safety/toxicity studies is essential for its global acceptance. Hence, this study was conducted under Golden Triangle Partnership programme with the objectives to evolve physicochemical profile along with characterization. This paper is dealt only with preparation and chemical characterization.

2. Materials and methods

2.1. *Preparation of* Mahalaxmivilas Rasa

All the raw materials were procured by M/s. Maharishi Ayurveda Pharmacy, Noida and authenticated by Dravyaguna expert/Botanist, while the metal/minerals were certified by Rasa Shastra expert. The composition of *Mahalaxmi Vilas Rasa* is provided in the Table 2. The process involves following steps viz. Preparation of *Bhasmas* (*Abhraka/Swarna/Vanga/Tamra*), Preparation of *Kajjali,* Purification of *Haritala,* Purification of *Dhatura Bija,* Preparation of *Nagavalli Swarasa, Bhavana* with *Nagavalli Swarasa*.

2.1.1. Preparation of Bhasmas (Abhraka/Swarna/Vanga/Tamra)

2.1.1.1. Preparation of Abhraka bhasma [2]. The *Vajrabhraka* was heated to red hot in an iron pan and immersed in *Triphala kwatha.* This process was repeated for seven times. The processed *Abhraka* was bundled in a jute bag with 1/4th quantity of paddy and immersed in *kanjika* for 3 days. Thereafter, the bundle was rubbed thoroughly and squeezed in the liquid itself so that only fine *abhraka* particles can escape through the holes of the bag. Bag was removed from the *Kanji* and the contents were allowed to settle down. The supernatant liquid layers were separated carefully to collect fine particles of *Dhanyabhraka* [3] that were settled down in the container.

Dhanyabhraka (fine Biotite mica obtained through above process) was levigated with *Arka ksheera* (latex of *Calotropis procera* (Ait) R. Br.) for one day; *chakrikas* (thin, flat cakes) were made, and dried in sun rays. These *chakrikas* were placed in a *sharava samputa* (earthern plates) and the junctions were sealed properly. This sealed apparatus was subjected to *Gaja puta* (method of heating with specific temperature pattern). The material thus obtained at the end of the *puta*, was processed in the similar way for six more times. At the end of 7th *puta*; *abhraka* was levigated with *Nyagrodha-mula kwatha* (Decoction of root of *Ficus bengalensis* Linn.), dried and three *Gaja putas* were given. The *Abhraka* was next levigated with *Kadali rasa* (juice of rhizome of *Musa paradisiaca* Linn.) and 07 *Gaja putas* were given. After completion of *putas* it was grounded and preserved in air tight glass jar or porcelain containers [4].

2.1.1.2. Preparation of Tamra bhasma. Thin *patras* (plate) of *shodhita Tamra* [5,6], (processed copper) were boiled in *Nimbu swarasa* for 3 days. One fourth quantity of *shuddha parada* (processed mercury) was mixed with *Tamra patra* and grounded well for 3 h in presence of *Nimbu swarasa* (lemon juice). *Shuddha gandhaka* (processed sulphur) was added to this, levigated by adding *Nimbu swarasa* and made into bolus (*golaka*). This bolus was covered carefully with the paste of *Punarnava root* (*Boerrahvia diffusa* Linn.) and allowed to dry completely. This dried bolus was placed in *sharava* (earthen saucer), covered by another *sharava* and junction was sealed carefully with clay smeared cloth. This *sharava* was then placed in heating device and heated in *kramavriddhi agni (*gradually increased heat) for 12 h. When cooled, the *sharava* was removed from the pot. The product thus obtained was grounded with juice of *Surana kanda* (corm of *Amorphophallus campanulatus* Blume) for a day. A bolus of this was prepared by adding half part of *Gandhaka* and a little quantity of cow ghee. This was placed in *Sharava* and after sealing the joint of two *Saravas* heated in *Gajaputa*. This procedure was repeated for two times more [7].

2.1.1.3. Amrutikarana of Tamra bhasma [8]. The process was performed to remove the remnant impurities present in the *tamra bhasma* and to enhance the therapeutic efficacy by inducing nectar like properties [9]. For this, *Shuddha gandhaka* was added to *tamra bhasma* and levigated well with *Nimbu rasa* for 3 h. This was made into a bolus and placed into *S. kanda* scooped in the middle to accommodate the bolus. The upper portion was covered with *S. kanda* and wrapped with clay smeared cloth and dried. This was

Table 1
List of adjuvant or *anupan* to be taken for various diseases.

No	Diseases/Roga	*Anupan*
1	Heart palpitation, heart pain	Arjuna bark Kwath or Arjunarishta.
2	Sinusitis	Triphala decoction
3	Chronic Coryza	Mulethi powder and ghee, honey mixed unequal quantity
4	Tuberculosis/Kshaya roga	Chausath Prahri Pippal + Honey
5	Prameha, Impotency, Spermatorrhoea	Shilajita + Milk
6	Vitiation of Tridosha	Betel leaf juice
7	Rheumatism	Dashmoola Kwath
8	Diseases of abdomen	Punarnava Ras
9	Diarrhoea, Dysentery	Dry ginger powder + honey
10	Cold, coryza	Betel leaf juice
11	Fever due to cough	ginger juice + Honey.

Table 2
Composition and proportion of each ingredients of the formulation [1].

S. No.	Materials used	Botanical/scientific names	Part/form used	Proportion
1	*Abhraka*	Biotite mica	*Bhasma* (Incinerated powder)	16 parts
2	*Gandhaka*	Sulphur	Processed powder	8 parts
3	*Vanga*	Tin	*Bhasma* (Incinerated powder)	4 parts
4	*Vriddhdaru*	*Argyreia nervosa* (Burmf) Bojer.	Dried seeds	4 parts
5	*Dhatura*	*Datura metel* Linn.	Processed seeds	4 parts
6	*Parada*	Mercury	Processed Mercury	2 parts
7	*Haritala*	Orpiment	Processed powder	2 parts
8	*Karpura*	*Cinnamomum camphora* Linn.	Sublimated Extract	2 parts
9	Jatikosa	*Myristica fragnans* Houtt.	Dried Aril	2 parts
10	*Jatiphala*	*Myristica fragnans* Houtt.	Dried Seeds	2 parts
11	*Swarna*	Gold	*Bhasma* (Incinerated powder)	1 part
12	*Tamra*	Copper	*Bhasma* (Incinerated powder)	1 part
13	*Nagavalli*	*Piper betel* Linn.	Leaf juice	Q.S.(for bhavana)

subjected to *gaja puta*. On cooling, the ashes were removed and *tamra* was collected, powdered and preserved.

2.1.1.4. Preparation of Vanga Bhasma [10]. *Vanga* was taken in a metallic spoon and heated was given till it was melted. The melted *Vanga* was immersed in a pot containing *churnodaka*. After cooling, the *vanga* was collected from the pot and the above process was repeated again for 6 times to obtain processed *Vanga*. The Processed *Vanga* was kept in an iron pan and heated. While it was melting, powders of *cincha* and *Asvattha twak* were sprinkled in small quantities and stirred with *loha darvi* (iron spatula). This process was continued till the melted *vanga* was reduced to powder form called *Jarita Vanga* [11]. *Jarita vanga* was mixed with haritala churna and grounded well in nimbu swaras. Small thin *cakrikas* were prepared, dried and placed in *sarava samputa* and *ardha gaja puta* was given. This process was repeated for 10 times. One fourth of *haratala* is added to *vanga* from the 2nd *puta* onwards. On cooling, the ashes were removed and *vanga* was collected, powdered and preserved. Fig. 1

2.1.1.5. Preparation of Swarna Bhasma [12]. The *Svarna* patras were heated to red hot state and immersed thrice in each of the liquids viz. *Tila-taila* (til oil), *takra (butter milk), kanjika, gomutra* (cow urine) *and Kulatha kashaya* (decoction of horse gram) consecutively [13]. The Processed *patras* so obtained were used for further processing. In *suddha parada*, *sodhita svarna* was added and levigated to make a bolus. Half the quantity of *gandhaka* was kept in a *sarava* and *swarna golaka* was kept on it. The *golaka* was covered with another *sarava*. *Sandhi lepa* of *sarava samputa* was done and dried in sun rays. *Puta* with 30 *vanyopalas* was given adding *gandhaka* each time in 1 part and the process was repeated 14 times. On cooling, the ashes were removed and *svarna* was collected, powdered and preserved [14].

2.1.2. Preparation of Kajjali (black sulphide of mercury) [15]

Hingulotha Parada [16] (Mercury obtained from Cinnabar) and *shuddha Gandhaka* [17] (processed sulphur) were taken in equal quantities in a *khalva yantra* (mortar pestle). The mixture was triturated thoroughly till a black colour, soft, lustreless fine powder like collyrium (*kajjali*) is obtained.

2.1.3. Purification of Haritala [18]

Small pieces of *haritala* were bundled in a piece of cloth and subjected to svedana in *Dola yantra* containing juice of *Kusmanda (Benincasa hispida* Thunb.) for 3 h.

2.1.4. Purification of Dhatura Beeja [19]

Dhattura seeds were soaked in go-mutra (cow urine) for 12 h and then they were washed with water and dried in the sun and subjected to *svedana* in a *dola yantra* containing *go-dugdha* (cow milk) for 3 h.

Fig. 1. Images of finished drug of *Mahalaxmivilas rasa.*

2.1.5. Preparation of Nagavalli Swarasa

Nagavalli patra (leaves of *Nagavalli*) were collected from the local market, washed under tap water and made into small pieces. The pieces of leaves were grinded and a fine paste was prepared. The paste was strained in to a stainless steel vessel through a clean cloth. Total 1700 ml of juice was collected. This *swarasa* was utilized for *bhavana.*

2.1.6. Bhavana with Nagavalli Swarasa [preparation of finished product (Tablet)]

This liquid (*nagavalli swarasa*) was added to the ground material in the end runner and trituration process [*Bhavana*] was carried out. The trituration was continued till the material dries completely. At the end of all levigation process, the material is removed from the

Table 3
Physico chemical analysis (Observations of three batch analysis).

S. No.	Parameter tested	Observations of three batch analysis
1.	Organoleptic characters	
	Colour	Brownish Black
	Taste	Slightly bitter
	Odour	Aromatic
	Appearance	Tablet
2.	Physico-chemical parameters	
	Identification	Yields the reaction characteristics of Sulphur, Mercury, Gold, Copper, Tin, Arsenic & Iron
	Loss on drying	2.5–4.0
	Organic and volatile matter	40.0–41.0
	Total Ash %w/w	52.0–55.0
	Acid Insoluble Ash %w/w	22.0–27.0
	Water soluble extractive	3.0–6.0
	Alcohol (90%) soluble extractive	3.0–5.0
	pH of aqueous extract	6.0–6.5
	Specific gravity	0.9999 ± 0.0004
	Particle size distribution	
	10%,	3.46–15.29 μm
	50%,	25.35–64.27 μm
	90%	119.37–210.50 μm
3.	Assay of Elements	
	Mercury (%w/w)	4.0–5.0
	Gold (%w/w)	1.5–2.0
	Copper (%w/w)	1.5–2.0
	Tin (%w/w)	5.0–6.0
	Arsenic (%w/w)	1.0–2.0
	Iron (%w/w)	7.0–8.0
	Sulphur (%w/w)	18.0–20.0
	Calcium (%w/w)	2.0–4.0
	Silica (%w/w)	1.0–4.0
	Magnesium (%w/w)	1.0–1.50
	Lead (%w/w)	0.50–1.50
	Boron (%w/w)	0.10–0.20
	Manganese (%w/w)	0.05–0.10
	Aluminum (%w/w)	0.10–2.0
	Chromium (%w/w)	0.02–0.04
	Cadmium (ppm)	0.10–0.20
4.	Residual pesticide (mcg/kg)	
	Alpha and beta HCH	Not detected
	Gamma HCH	Not detected
	Delta HCH	Not detected
	DDT and metabolites	Not detected
5.	D.T.	16-17 min
	Hardness	1.5 kg/cm^2
	Friability	0.60–0.70%
	Average wt.	125.0–125.30 mg/tab
6.	Microbiological examination	
	Total aerobic count	8000–20 000
	Coliform	Not detected
	E.coli	Not detected
	Salmonella sp.	Not detected
	Staphylococcus aureus	Not detected
	Yeasts	Not detected
	Moulds	Not detected
	Pseudomonas aeruginosa	Absent

Table 4
Observations/visualization/detection (Rf values).

Mahalaxmi Vilas Rasa			
	Batch I	Batch II	Batch III
At 254 nm	0.37, 0.48, 0.75	0.37, 0.48, 0.75	0.37, 0.48, 0.75
At 366 nm	0.35, 0.45, 0.78	0.35, 0.45, 0.78	0.35, 0.45, 0.78
At 520 nm	0.74, 0.81	0.74, 0.81	0.74, 0.81

end runner, shifted in to the clean trays and dried completely in Tray Drier at 50 °C. Dried material was shifted to tablet section and granules were prepared. 2% talcum powder was added to the granules and was compressed in to desirable size of tablets [125 mg] by passing through Rotary Tablet Machine.

2.2. Experiments & results of chemical analysis

2.2.1. Physico-chemical analysis

Physicochemical analysis is the important characteristics to evaluate the quality, standardization and safety of Ayurvedic drugs and provide information about correct identification and authentication of the raw drugs & formulations and may help in preventing its adulteration. Ash values are important quantitative standards and criterion to analyse the identity and purity of crude drugs. Moreover the total ash of a crude drug also reflects the care taken in drug preservation, and the purity of crude and the prepared drug. Acid insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Extractive values obtained using water and alcohol are useful for the evaluation of a crude drug as it gives an idea about the nature of chemical constituents present in it and is useful for estimation of chemical constituents, soluble in that particular solvent used for extraction. The pH conventionally represents the acidity and alkalinity.

Physicochemical parameters, *viz.* description, estimation of Loss on drying, Ash content, Acid insoluble ash, Water/Alcohol soluble extractive, *p*H, etc., qualitative/quantitative elemental testing, Residual pesticide, Microbiological examination and tablet parameters *viz.* Hardness, Friability, Average wt., Dissolution time etc. were carried out by following standard methods as per Ayurvedic Pharmacopoeia of India (API) [20–25] guidelines. The quantitative estimation of Heavy Metals viz. Hg, As, & Cd was carried out by Atomic Absorption Spectrometer (Perkin Elmer (USA) Analyst 400) and the other elements viz. Mg, Pb, Ca, B, Mn, Al, Cr were analysed on ICP-AES (THERMO EECTRON Corporation's model IRIS INTREPRID II XDL). However Sulphur, Silica, Sn, Au, Fe & Cu were quantified by using conventional methods [25]. The results of Physico-chemical analysis are mentioned in below Table 3.

2.2.2. HPTLC analysis

Sample preparation: 2 g powder each of three batches of MLV were soaked overnight separately in 20 ml of methanol. The solutions were continuously stirred for 6 h and kept for next 18 h and then filtered the samples, dried and made 10% solution.

High Performance Thin Layer Chromatography was performed on TLC plates pre-coated with 0.25 μm thin layers of silica gel 60 F_{254} (E. Merck). 10 μL methanolic solution of formulation (three batches) were applied on the plates as bands 8.0 mm wide by use of a Linomat-IV applicator (CAMAG, Switzerland) fitted with a 100 μL syringe (Hamilton, Switzerland). The application positions X and Y were both 10 mm, to avoid edge effects. Linear ascending development to a distance of 80 mm with Toluene: Ethyl acetate: formic acid 10: 3:1 (v/v) as mobile phase was performed in a twin-trough glass chamber previously saturated with vapors of mobile phase for 20 min. The plates were dried in air and visualized under 254 nm and 366 nm for ultra violet detection and taken the fingerprints as evident. The same TLC plate was also derivatized with anisaldehyde-sulphuric acid reagent and visualized in white light. The HPTLC profile alongwith the R_f values are shown in the mentioned Fig. 2 and Table 4

2.2.3. X-ray diffraction study

Powder X-ray diffraction (XRD) analysis of *Mahalaxmivilas Rasa* was carried out using Rigaku Ultima-IV X-ray diffractometer with CuKα radiation (λ = 1.54 A°) operating at 40 kV and 30 mA. Pattern was recorded for angle (2θ) ranging from 10 to 100° at a scanning rate of 1°/second and scan step of 0.1°. Sample identification was done by matching d-spacing with the standard database. The detailed XRD data and XRD pattern are given in below mentioned Table 4 and Figs.3 and 4

3. Discussion

The organo-leptic observation shows that the prepared *Mahalaxmivilas Rasa* is in the form of Brownish Black coloured tablet having aromatic odour and slightly bitter taste. The qualitative analysis shows the positive test for the presence of Mercury, Sulphur, Calcium, Copper, Gold, Iron and Tin etc. The Chemical analysis revealed that it contains 4.34% of Mercury, 19.12% of Sulphur, 7.29% of Iron, 5.33% of Tin, 1.85% of Gold, 1.74% of Copper, 1.43% of Arsenic, 2.75% of Calcium, 1.3% of Magnesium, 2.5% of silica together with

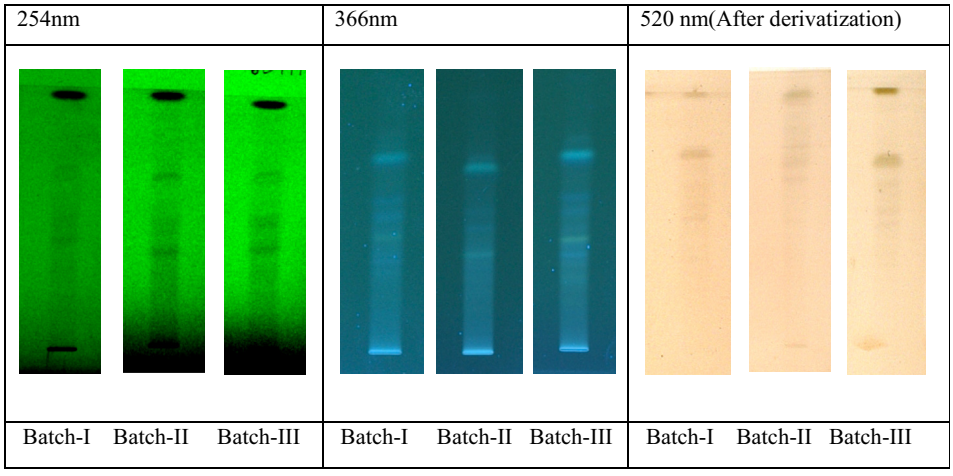

Fig. 2. -HPTLC profiles of *Mahalaxmivilas Rasa*.

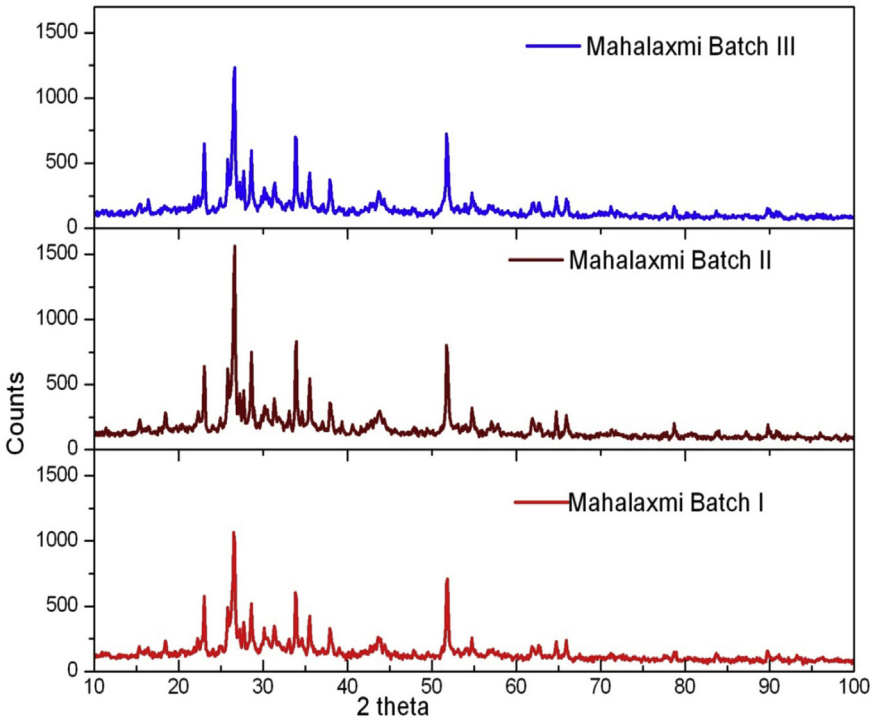

Fig. 3. XRD pattern of *Mahalaxmivilas Rasa* (batch –I, II, III).

trace elements *viz.*, Aluminium, Manganese, Lead, Boron, Chromium, Cadmium etc. which have been found in <1.0% range. Moisture content 3.70% was found when determined loss on drying at 105 °C. Total ash content (53.35%) representing the elemental composition is left after burning of organic/volatile matter (40.26%). The observations show that water soluble (4.67%) and alcohol soluble (4.12%) matter are also present in this formulation. The drug also tested as per API guideline for Residual pesticide and Microbiological examination which found in permissible limits.

The results from HPTLC profile as shown in Fig. 2 and Table 3 revealed the presence of few bands at different R_f representing organic constituents. Generally, the Herbo-Mineral preparations

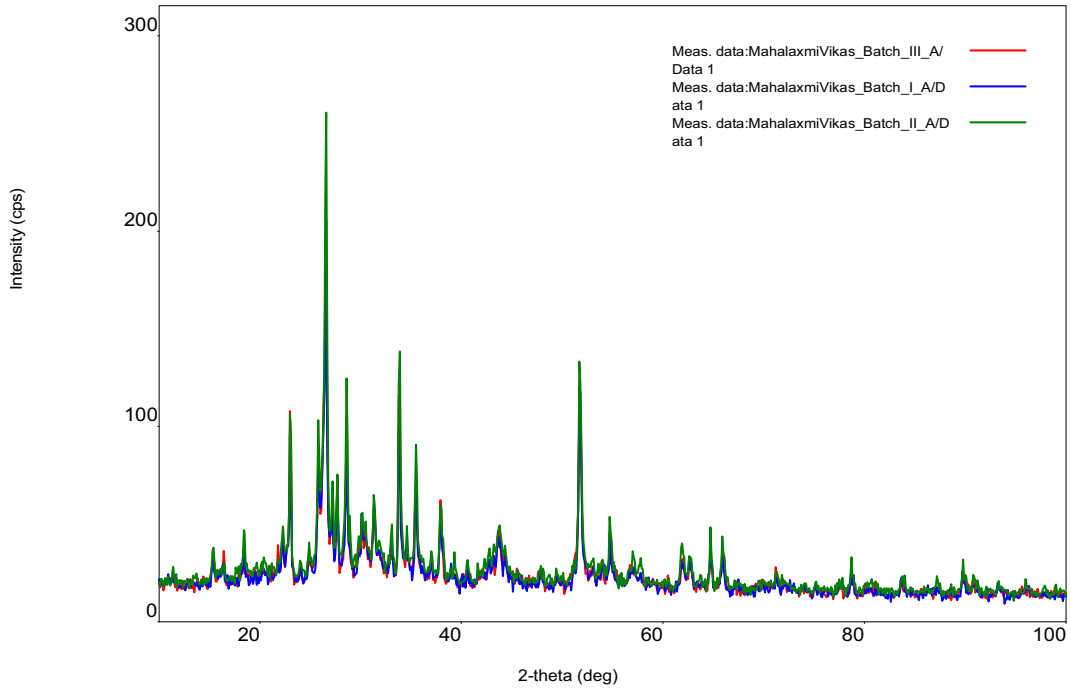

Fig. 4. Overlay of XRD pattern of *Mahalaxmivilas Rasa* (batch –I, II, III).

Table 5
d-spacing and 2-theta (deg) values of XRD analysis.

2 theta	d (A)	size(A)	Chemical formula	Phase data name	cps
23.1	3.9	438.0	S	Sulfur (2,2,2)	62.0
25.8	3.5	437.0	S	Sulfur (0,2,6)	47.0
26.6	3.3	302.0	Hg S, SnO_2, $K_{86\cdot5}Al_{86\cdot5}Si_{105\cdot5}O_{384}$	Cinnabar (1,0,1), Cassiterite, syn (1,1,0), Zeolite X, (X) (6,4,2)	156.0
27.2	3.3	552.0	$K_{86\cdot5}Al_{86.5}$ $Si_{105\cdot5}O_{384}$	Zeolite X, (X) (7,3,1)	31.0
27.7	3.2	563.0	S, As_2 S_3	Sulfur (1,1,7), Orpiment (2,1,1)	36.0
28.6	3.1	492.0	Unknown	Unknown	74.0
30.1	3.0	175.0	K $Al(SiO_3)_2$, $K_{86\cdot5}Al_{86\cdot5}Si_{105\cdot5}O_{384}$	Leucite (4,0,2), Zeolite X, (X) (8,2,2)	16.7
31.3	2.9	524.0	Hg S, S, K $Al(SiO_3)_2$	Cinnabar (1,0,2), Sulfur (0,4,4), Leucite (3,2,3)	28.0
37.8	2.4	281.0	Hg S, SnO_2, S, $KAl(SiO_3)_2$	Cinnabar (1,0,3), Cassiterite, syn (2,0,0), Sulfur (4,2,2), Leucite (4,0,4)	25.0
43.8	2.1	61.0	Hg S, SnO_2, S, As_2 S_3, $KAl(SiO_3)_2$, $K_{86\cdot5}Al_{86\cdot5}$ $Si_{105\cdot5}O_{384}$	Cinnabar (1,1,0), Cassiterite, syn (2,1,0), Sulfur (3,1,9), Orpiment (0,4,1), Leucite (2,2,6), Zeolite X, (X) (10,6,2)	12.3
51.7	1.8	461.0	SnO_2, As_2S_3, $K_{86\cdot5}Al_{86\cdot5}Si_{105\cdot5}O_{384}$	Cassiterite, syn (2,1,1), Orpiment (1,3,-2), Zeolite X, (X) (14,2,0)	98.0
54.7	1.7	578.0	HgS, SnO_2	Cinnabar (2,0,2), Cassiterite, syn (2,2,0)	28.0
57.0	1.6	141.0	Sn O_2, S, As_2 S_3, $K_{86\cdot5}Al_{86\cdot5}Si_{105\cdot5}O_{384}$	Cassiterite, syn (0,0,2), Sulfur (2,2,14), Orpiment (4,5,0), Zeolite X, (X) (15,3,3)	7.9
61.8	1.5	206.0	SnO_2, K $Al(SiO_3)_2$	Cassiterite, syn (3,1,0), Leucite (7,5,2)	14.9
64.7	1.4	734.0	S	Sulfur (5,3,11)	32.0
65.9	1.4	518.0	SnO_2	Cassiterite, syn (3,0,1)	22.4

are insoluble in common organic solvents such as Chloroform, Acetone, Methanol and water. In this case, the alcohol soluble extractive is very less (<5.0%). This soluble portion did not show any defined behaviour on TLC may be due to its non-homogeneous nature.

XRD pattern of *Mahalaxmivilas Rasa* (3 batches) as shown in spectra Figs. 3 and 4 and results mentioned in Table 5, have indicated that all the samples contained free sulphur, cinnabar (mercury sulphide added as *Kajjali*); cassiterite (tin oxide, *Vanga Bhasma*); orpiment (*Hartal*, arsenic III sulphide); and mica (Leucite/Zeolite; *Abhraka Bhasma*). *Abhraka bhasma* is the major inorganic constituent added by weight followed by free sulphur and *Vanga bhasma*, in the *Mahalaxmivilas Rasa* samples. No signature of *tamra bhasma* or *Swarna Bhasma* could be identified in these spectra, indicating that the methods used for their preparation, did not yield any crystalline products or their signature is buried under the strong lines of other constituents. But their presence in the sample could be identified from the chemical analysis showing the presence of gold, boron, copper etc. The crystalline form of *abhraka bhasma* remains inconclusive. It can be seen that the three XRD patterns on the samples are qualitatively same in the relative intensities of the peaks. *Kajjali* is meta-cinnabar and in its preparation elemental mercury observed to be absent. The presence of free sulphur ensures that the *kajjali* does not decompose into mercury and sulphur and also some partial conversion into mercury oxide due to exposure to air; the presence of free sulphur is confirmed by XRD. The assay of total ash content in these samples (52%–55%) almost matching the expected fraction of inorganic constituents by weight and the XRD results indicate that the inorganic contents have remained intact over time.

4. Conclusion

The inferences and the standards laid down in this study certainly utilized as an important tool for standardization and quality assurance of this herbo-mineral formulation. It may be useful for Researchers/Scientists/Academicians and also may be considered for laying down the pharmacopoeial standards of *Mahalaxmivilas Rasa*.

This study reveals that *Mahalaxmivilas Rasa* prepared by ancient classical pharmaceutical processes is safe and very effective in converting the macro elements into therapeutically effective medicines of micro form. Further, the sub-chronic oral toxicity studies in male and female rats was studied and found non toxic. As this paper is dealt only with preparation and chemical characterization of *Mahalaxmivilas Rasa,* the detailed data on safety/toxicity studies will be published separately.

Conflicts of interest

There are no conflicts of interest.

Sources of funding

None declared.

Acknowledgment

The Authors express heartfelt thanks and would like to acknowledge Dr SK Sharma, Former Advisor (Ayurveda), M/o AYUSH, Govt. of India, Dr MM Padhi, Former Dy. Director General, CCRAS, Dr Pramila Pant & Dr. Ravindra singh, Assistant Director (Chemistry) for valuable guidance, Dr VK Singh, M/s. Maharishi Ayurveda Pharmacy, Noida for technical inputs. The authors are thankful to Dr J Arunachalam, Former Director, National Centre for Compositional Characterization of Materials (BARC), Hyderabad for helping in data analysis and interpretation of the results. Thanks are also conveyed to Miss Bhavana Dwivedi, Dr.Aarti sheetal, Dr Arun Bhadula, Dr Praveen Sharma, Dr Suman Singh, Dr Divya Mishra and Dr Yadunandan Dey, Senior Research Fellows of CCRAS for technical assistance.

References

[1] Sharma S. Hindi commentary by Pt. Kashinath Shastri on *Rasatarangini;* Taranga 10, verse 21-22. 11th ed. Varanasi: Motilal Banarasidas Publications; 1979. p. 225–6.
[2] Anonymous. The ayurvedic formulary of India (AFI) (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part I. 2nd ed. Delhi: The Controller of Publications; 2003. p. 267.
[3] Upadhyay M. (Reprinted- 2008). Hindi commentary by Shri Gulraj Sharma Mishra, Ayurvedaprakasha, Adhyaya 2, verse 113-115. Varanasi: The Chowkhambha Vidya Bhawan; 2008. p. 289.
[4] Sharma S. Hindi commentary by Pt. Kashinath Shastri on *Rasatarangini;* Taranga 10, verse 39-42. 11th ed. Varanasi: Motilal Banarasidas Publications; 1979. p. 229.
[5] Sharangadhara. By Pt. Parasurama Sastri Vidyasagar on *Sharangadhara* Samhita, Madhyama khanda; Dhatusodhan-Marna Kalpana: Adhayaya 11, verse 2-6. 5th ed. Varanasi: Chaukhamba Orientalia; 2002. p. 241.
[6] Upadhyay M. (Reprinted- 2008). Hindi commentary by Shri Gulraj Sharma Mishra, ayurvedaprakasha, adhyaya 2, verse 117-119. Varanasi: The Chowkhambha Vidya Bhawan; 2008. p. 368.
[7] Sharangadhara. By Pt. Parasurama Sastri Vidyasagar on Sharangadhara Samhita, Madhyama khanda; Dhatusodhan-Marna Kalpana: adhayaya 11, verse 28-35. 5th ed. Varanasi: Chaukhamba Orientalia; 2002. p. 244–5.

[8] Sharma S. Hindi commentary by Pt. Kashinath Shastri on *Rasatarangini;* Taranga 17, verse 48-50. 11th ed. Varanasi: Motilal Banarasidas Publications; 1979. p. 420–2.
[9] Sharma S. Hindi commentary by Pt. Kashinath Shastri on *Rasatarangini;* Taranga 02, verse 58. 11th ed. Varanasi: Motilal Banarasidas Publications; 1979. p. 24.
[10] Sharma S. Hindi commentary by Pt. Kashinath Shastri on *Rasatarangini;* Taranga 18, verse 1-104. 11th ed. Varanasi: Motilal Banarasidas Publications; 1979. p. 435–55.
[11] Anonymous. The ayurvedic formulary of India (AFI) (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part I. 2nd ed. Delhi: The Controller of Publications; 2003. p. 243.
[12] Anonymous. The ayurvedic formulary of India (AFI) (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part I. 2nd ed. Delhi: The Controller of Publications; 2003. p. 246.
[13] Sharma S. Hindi commentary by Pt. Kashinath Shastri on *Rasatarangini;* Taranga 15, verse 09-11. 11th ed. Varanasi: Motilal Banarasidas Publications; 1979. p. 363–4.
[14] Bhatt G. Hindi commentary by Pt. Narendranath Mitra on Rasendrasarasangraha; Paradvishya, verse 233–234. 4th ed. Varanasi: Motilal Banarasidas Publication; 1976. p. 63.
[15] Vagbhatta. Hindi commentary by ambikadutt Shastri on Rasaratna samuchchaya, adhyaya 08, verse 05. 9th ed. Varanasi: Amarbharti Prakashan; 1995. p. 135.
[16] Anonymous. The ayurvedic formulary of India (AFI) (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part I. 2nd ed. Delhi: The Controller of Publications; 2003. p. 372.
[17] Krishananand S. Rasa tantra sara siddha prayoga sangraha- Part I, Dravya sodhana prakarana. 18th ed. Rajasthan: Krishan Gopal Ayurved Bhawan; 2010. p. 27–39.
[18] Vagbhatta. Hindi commentary by ambikadutt Shastri on *Rasaratna samuchchaya*, adhyaya 03, verse73–75. 9th ed. Varanasi: Amarbharti Prakashan; 1995. p. 73–4.
[19] Y Trikamji Acharya. Rasamritam, parisista 8. Varanasi: Motilal Banarasidas publications; 1951. p. 146.
[20] Anonymous. In: The ayurvedic Pharmacopoeia of India, (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part I. 1st ed., vol. I. Delhi: The Controller of Publications; 1990.
[21] Anonymous. In: The ayurvedic Pharmacopoeia of India, (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part I. 1st ed., vol. III. Delhi: The Controller of Publications; 2001.
[22] Anonymous. In: The ayurvedic Pharmacopoeia of India, Minerals & Metals, (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part I. 1st ed., vol. VII. Delhi: The Controller of Publications; 2008.
[23] Anonymous. In: The ayurvedic Pharmacopoeia of India, (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part II. 1st ed., vol. I. Delhi: The Controller of Publications; 2008.
[24] Anonymous. In: The ayurvedic Pharmacopoeia of India, (Ministry of Health and Family Welfare, Govt. of India, New Delhi) Part II. 1st ed., vol. II. Delhi: The Controller of Publications; 2009.
[25] Anonymous. Pharmacopoeial standards for ayurvedic formulations, (CCRAS, Ministry of Health and Family Welfare, Govt. of India, New Delhi), revised edition. Delhi: The Controller of Publications; 1987.