International Journal of Health Sciences and Research
Vol.13; Issue: 8; August 2023
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Analytical Study (XRD, XRF, EDXRF & PSA) on *Tarakeswara Rasa* - A Herbo-Mineral Preparation

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DOI: https://doi.org/10.52403/ijhsr.20230805

ABSTRACT

Tarakeswara rasa is a herbo-mineral preparation mentioned in Basavarajeeya Bahumutra prakarana containing Rasasindoora, Abhraka, Vanga and Loha bhasmas. Each of the above bhasmas and this unique combination is believed to be potent Rasa Rasayana useful in treating diabetes and its complications. The present study is conducted as a part of a clinical study on diabetic microalbuminuria, work done on analytical parameters of Tarakeswara rasa is detailed. The study drug was meticulously prepared and analyzed using both Ayurvedic bhasma pareeksha and modern analytical parameters like XRD, XRF, EDXRF, PSA, etc., and then administered to patients. The mean particle diameter of Tarakeswara rasa is 8.04 microns. XRD and EDXRF data reveal a multimineral Tin-rich Astrophyllite composition in the form of Tarakeswara rasa. The drug resembles the crystal astrophyllite which has been widely used in metabolic disorders under reiki system for healing, bringing positivity and aligning chakras.

Keywords: Diabetes Mellitus, Tarakeswara Rasa, Bhasma pareeksha, XRD, XRF, EDXRF, PSA

INTRODUCTION

Recent times have seen a significant upsurge in the usage of various herbo mineral preparations. With increasing demand there also arises concern about the of the quality of these drugs. For the medicine to serve its intended purpose they should be safe, effective and nontoxic. Classical methods of drug preparation ensure quality products but lack of standardization has led to downfall in the quality of medicines available in the market, compromising on its safety and efficacy. Standardization includes all measures which are taken during the manufacturing process and quality control procedures ensuring reproducibility. Rasa shastra classics have mentioned various methods for analyzing the quality of prepared bhasma. Though these methods are excellent and time tested and the bhasma passing all these tests are indeed of high quality, there is lack of knowledge regarding the structural changes and chemical composition of various bhasma's. Here the ancient methods become inadequate for the characterization of bhasma for its global acceptability.

The chemical composition of a metal/mineral drug is unique and not as divergent as an herbal drug. But there occurs disparity due to place of occurrence of the mineral and its availability in the market. With the advent of recent instrumental analyzing tools & techniques, one can easily understand the elemental composition of a mineral. It helps in identification, characterization and standardization of single drugs as well as formulations, also helps in identifying and ruling out adulterations and substitutions done to minerals in pharmaceutical procedures. This ensures effective chemical markers of bhasma prepared by a particular

method which can be differentiated from similar methods.

Some of the classical parameters as well as sophisticated instrumentation techniques that were used in the characterization of the study drug are enumerated below.

MATERIALS & METHODS

Tarakeswara rasa was prepared classically by mixing equal portions of Abhraka bhasmas,Loha bhasmas,Vanga bhasma and Rasasindoora. Parada (99.99% pure distilled mercury) shodana as mardana was done as per yogaratnakara[1] in kumari swarasa, triphala kasaya,trikatu kasaya,chitraka kasaya and nimbu swarasa for a period of 12 hours each. Gandhaka (laboratory grade pure Sulphur powder) shodana was done as saajya kurmaputa method as per Ayurveda prakasha[2]. Kajjali bhavana was done in kumari swarasa and subjected classically to kupipakwa vidhi[3] upto 720 degree Celsius of tikshnagni to obtain Rasasindoora. Vanga (99.5% pure Tin (metal) powder from Merck Life science) samanya shodana was done in churnodaka[4] and visesha shodana[5] in haridra nirgundi swarasa. Vanga jarana[6] was done with apamarga and marana[7] with kumari swarasa at 600 deg Celsius for one hour and a total of six putas were done to obtain vanga bhasama. Abhraka (krishnavajrabraka collected from Jharkand satisfying grahya lakshanas) shodana was done as nirvapa[8] in triphala kasaya. Abhraka marana was done as per sindooramanjari[9] where in dhanyabhraka nirmana is not required. There was also previous analytical study[10] conducted in the department on the Sindooramanjari based Abhraka bhasma which yielded good quality abhraka bhasma. Bhavana of shoditha abhraka in kumari swarasa and triphala kasaya to prepare chakrikas for marana. This was subjected to puta at 900 degree Celsius for one hour and a total of fifteen putas to yield abhraka bhasma. Loha (Iron metal powder electrolytic-100mesh LR of SD Fine chemical Ltd) was subjected to samanya shodana[11] in taila,takra,gomutra,aranala and kulatha kasaya and visesha shodana[12] was done in triphala kasaya. This was then subjected to bhanupaka, sthalipaka and putapaka[13] at in triphala kasaya at 600 deg Celsius for one hour and a total nine putas to yield loha bhasma.

In a khalva yantra 50gms of Rasasindoora was added. It was then powdered well using a pestle until the whole of the Rasasindoora turned to fine powder. To this 50gms of Vanga bhasma was added little by little mixed, powdered well with Rasasindoora to attain a homogenous mixture. Then add 50gms of Loha bhasma to this and repeat mixing. Finally, 50gms of Abhraka bhasma was added and the whole mixture was thoroughly mixed until homogenous, for a period of 3hours. In the text Basavarajeeya[14] it is mentioned to do bhavana in honey for 1 day. But this was not adopted considering the difficulty in storage and dispensing of the drug, if so, the drug becomes hygroscopic and semisolid in nature. The final product weighed 200gms, was stored in an airtight glass bottle. This formulation so prepared[15] was then subjected to analytical evaluation at Drug Testing and Standardization Unit (DTSU) at Government Ayurveda college Thiruvananthapuram, National Centre for Earth Science Studies (NCESS), Thiruvananthapuram & Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam

RESULT

The prepared Tarakeswara Rasa was subjected to organoleptic evaluation, pH estimation was done at Drug Testing & Standardization Unit of Government Ayurveda college, Thiruvananthapuram. Particle size analysis was performed at National Centre for Earth Science Studies Thiruvananthapuram and XRD, EDXRF & XRF data was performed at Indira Gandhi Centre for Atomic Research at Kalpakkam. This bhasma was also seen satisfying Ayurvedic bhasma pareeksha like varitaratwa (floats on still water) rekhapurnata (fine between fingerprints) niswadu(tasteless) sukshmatwa (fineness)

absence of any chandratwa (lack of shiny metallic elements) and not having dantagre kacha kachatwa(no grittiness on grinding between teeth).

1. Organoleptic characteristics of Tarakeswara rasa

The prepared Tarakeswara rasa was cocoa brown in color, tasteless, pungent odor and with fine smooth powdery texture.

2. Determination of pH values

100mg of Tarakeswara Rasa was diluted in a beaker with 100ml of distilled water. Electrodes of pH meter was introduced into the beaker and kept undisturbed for 30 minutes. The reading on pH meter fluctuated now and then and at the end of 30 minutes, it got stabilized and was noted down as pH = 6.94. pH of Tarakeswara rasa is near neutral, the drug doesn't irritate buccal or gastric mucosa on consumption.

3. Determination of XRD

XRD study was conducted in IGCAR, kalpakkam at Xray and crystal growth section of Material Science Group. PXRD spectrum at 2 theta angle is mainly matching with SnO_2 and Tin rich astrophyllite. Bhasma also shows the presence of HgO, HgS, Fe_2O_3 (maghamite) and SiO_2. In the final product all contents are present in Astrophyllite form. It is evident from the well-defined XRD peaks, that these particles were crystalline.

1. 8 peaks corresponding to Tin rich astrophyllite (001,010, 02-2,022, 112, -212, -135, 1-51)
2. 11 peaks corresponding to SnO_2 (111,101,200,211,220,002,112,301,202, 321,222)

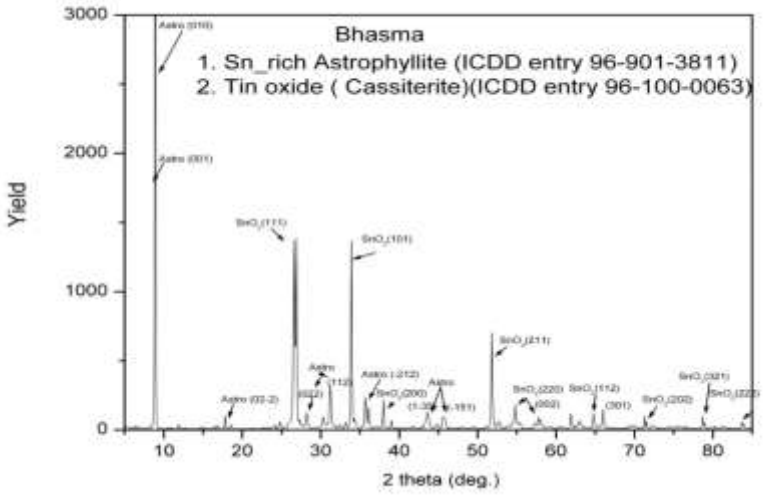

Figure 1: X-ray diffraction pattern of Tarakeswara Rasa

The general chemical formula of an Astrophyllite crystal is:-
$K_2 Na (Fe^{2+}Mn)_7 Ti_2Si_8O_{26}(OH)_4$. It consists of sodium, potassium, iron, manganese, titanium silicate with a hardness of 3.5. It is believed to have activated and aligning chakra in the body. It is traditionally believed to support detoxification at an energy level as the crystal protects against radiation and electromagnetic fields. Astrophyllite is said in folklore and crystal healing to be helpful for ADD, ADHD, anxiety, eliminating fat deposits, seizures and aids cellular regeneration. In addition of above elements, there is the presence of tin, mercury and Sulphur also converging into an astrophyllite form.

4. Determination of Particle Size

The particle size of Tarakeswara rasa was done using a Particle size analyser. As the bhasmas particles are smooth and very fine in nature, usually ranging from micro to nanometer in size, it usually floats on the surface of liquid medium. Here a uniform suspension of bhasma is initially acquired with a water and calgon mixture. It is then kept in a laser diffraction particle size analyser facility (CILAS-1180) to analyse sediment samples for particle size distribution. Mean diameter of the particles in Tarakeswara rasa is 8.04 microns. 5% of the sample shows particles less than 0.1microns. An average of 15% of particles is below 1 micron.

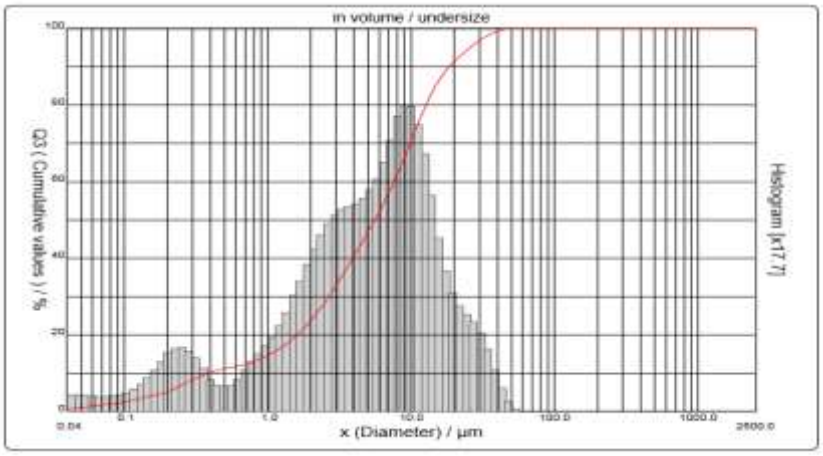

Figure 2: Particle size analysis of Tarakeswara rasa

5. Determination of XRF& EDXRF

Study for both XRF, qualitative as well as quantitative EDXRF analysis of the sample was done in IGCAR kalpakkam at Nuclear and Radioanalytical Chemistry Section. A detail sample study was undertaken followed by qualitative and quantitative estimation of the sample. The XRF data revealed Mercury concentration of above 48% followed by Iron of 33% and other minerals in this formulation.

Analysis Long Report
Spectrum: TR!-200220
S.N. AG0602
Ambient temperature 16 C°

Date 2/20/2020
Time 04:51:33 PM
MCA

Sl no	Element	Counts	Intensity	Concentration
	S	75473	29380.93	0.9956
	Rb	88097	14050.79	0.0723
	I	139596	71956.00	14.9178
	K	158282	8946.67	0.4743
	Sr	333379	51863.13	0.2587
	Kr	115004	5214.46	0.0459
	Ag	72435	0.00	0.0000
	Fe	3446469	2983320.75	33.2956
	Ni	100350	24548.20	0.2798
	Zn	124733	0.00	0.0000
	Cr	28242	3546.18	0.0567
	Cu	46788	9851.72	0.0962
	Al	11394	326.17	0.0888
	Si	18345	5483.84	0.6791
	Hg	2067913	1979099.00	48.7392

Figure 3: XRF data of Tarakeswara rasa

Qualitative estimation EDXRF shows the following elements in the sample of Tarakeswara Rasa. Further quantitative estimation was not done because of the previously obtained XRF concentration data to avoid unnecessary repetition and energy conservation.

Sl.No	The elements found to be present in the sample
1	Hg, S, Fe, Sn, K, Al, Si, Mn, Mg, Cu, Zn, Ti, Rb, Sr, Ni, V, Cr and Ba

Figure 4: EDXRF of Tarakeswara rasa

Viewing the above data in light of previously published studies, one can understand that the major elements like Mercury, Iron, Sulphur is present in Rasasindoora[16], in addition also has Manganese, Iron, Silica and Potassium.

Tin is the major constituent of Vanga bhasma, along with it Potassium, Silicon, Iron, Magnesium, Aluminium, Sulphur, Titanium, Copper, Chromium, Strontium and Nickel are seen in Vanga bhasmas [17]. The presence of potassium can be attributed to Vanga Jarana[18]. In here Apamarga used, has potash as the main content and potassium rich tin oxides are formed at end of Jarana.

In addition to Iron as major content, Magnesium, Aluminium, Silicon, Potassium and Titanium, are seen associated with EDXRF of Abhraka bhasma[19].

Aluminium, Manganese, Rubidium, Strontium, Copper, Zinc with major amount of Iron are seen in EDXRF of loha bhasma[20].

Other than the above, Vanadium and Barium is seen in EDXRF qualitative data of Tarakeswara Rasa but absent in XRF quantitative concentration. Without knowing its quantity, one cannot predict whether it is a major constituent of any one of the bhasmas or adulterant or from the utensils used or from the Dravadravyas used during shodana marana procedures. Although studies[21] are seen on presence of nickel and chromium leeching into food during preparation in stainless steel vessels, it can only be assumed and cannot be fully substantiated at this level that they leeched out into bhasmas during drug processing. Longer duration of exposure to high heat and repeated cycles reduce Ni and Cr leeching but doesn't eliminate them entirely.

Figure 5: Ayurvedic bhasma pareeksha

DISCUSSION

The use of modern analytical techniques is a must to characterize the drug, its interaction with specific body tissues/organs and to provide a molecular basis for insights into curative aspects. The interaction of biological systems with bhasma depends on various factors such as nature of bhasmas its size, shape, composition, cell type, other drug interactions and environment of drug action. Other factors governing the interaction are yet to be completely understood.

Bhasma particles should be of optimum size for easy absorption and penetration into minute capillaries and assimilation inside the body. The rate of absorption of drug is directly proportional to the particle size of drug. Finer the particle size, quicker is the absorption and hence fast and efficient drug action is ensured. Particle size reduction is ensured by various techniques like subjecting to heat and water, dry and wet grinding, calcination at high temperatures during shodana, bhavana, jarana and marana. Particle size also depends on the nature of drug and number of calcination cycles. Mean particle size in Tarakeswara rasa is 8.04 microns. 5% of the sample shows particles less than 0.1microns. An average of 15% of particles is below 1 micron. Thus, particle size averages between few Nano to micro level. While comparing with previous standardization study[22] of Tarakeswara rasa, particle size ranges from 0.5 and 2 µ. It was taken from SEM studies. In SEM analysis, one would usually zoom in at the smallest particle size and take a SEM picture. Therefore, one wouldn't know the average size of the mixture of bhasmas. By PSA, it is evident that some of the particles are below 0.1 micron and 1 micron ranging up to 8 µ, thus they are mostly heterogenic and polydispersive. The disparity is due to different methodology adopted while drug preparation.

Regarding XRD, a unique finding of tin rich astrophyllite can be seen. The chemical composition of Tarakeswara rasa matches with naturally occurring astrophyllite crystal with a tin predominance. The crystal has been widely used in reiki system for healing, bringing positivity and aligning chakras.

Qualitative EDXRF data reveals the presence of all the usual elements cited previously from Abhraka, Vanga, Loha and Rasasindura, like Mercury, Iron, sulphur and Tin. Similar is the composition of Tarakeswara Rasa. In addition, it has other elements like Vanadium, Barium etc. Presence of Nickel and Chromium may be attributed to utensils used for the procedures.

Due to the acidic pH in the stomach, metal salts become insoluble. But by their minute particle size there occurs liposolubility, which allows them to penetrate the intestinal barrier either by endocytosis, phagocytosis, or persorption and transit to the bloodstream. Particles which are having the diameter of approx. 1.5 µm can be absorbed through the gap junctions and epithelial tissue in various locations of the middle gut. As Tarakeswara rasa is having a near neutral pH of 6.94, its absorption starts right from mouth in salivary fluids, while absorption will be hampered in acidic gastric pH, resorption continues when coming to bile fluids and intestinal juices.

While going through the Ayurvedic bhasma lakshanas, the classical standards enable us to qualitatively select proper bhasmas which are therapeutically enabled to produce desired effect. By various physical and chemical tests, aim is to produce a fit bhasmas which is biologically active. It appears that the classically prepared bhasmas are mostly therapeutically active metal oxide or metal sulfide particle complexes like kajjali, Rasasindura,Vanga etc.. Most oxides and sulphides are generally insoluble in water but soluble in strong acid. But when metal oxides and sulphides are made associated with organo chemicals from fresh/dried herbs, they are subjected to chelation by which their properties tend to change. Additionally, when these metals are repeatedly heated

with heterogenous organic compounds like swarasa, kasaya they stabilize into fine herbo-metallic particles as seen in bhasmas. These then have the potential to penetrate a living system with high bioavailability with its small size and peculiar alignment of particles in them. The idea comes from nature, where plants and animals do this while chelating copper or iron into enzymes and carrier metallo-proteins and metallo-proteases. It also includes the iron in hemoglobin and all heme proteins, copper in ceruloplasmin and plant copper protein plastocyanin, as well as the zinc-containing enzyme alcohol dehydrogenase in man. Method of preparation of Bhasma is similar to the modern-day surfactant-mediated nano-particle metal oxide production, which is highly costly, compared to classical methodology. As one's body is incapable of converting all needed metals and minerals for its use, by bhasmikarana the insoluble heavy metals can be turned to active biological products which can later be used for addressing issues in human body.

Rather than dismissing classically produced bhasmas formulations as toxic based on our lack of current understanding in them, there are opportunities to observe how they work chemically, analytically, and clinically to decipher their mechanisms of action at various levels. By doing so, one can add to the current knowledge, expand boundaries to help mankind in achieving complete health.

CONCLUSION

- pH of Tarakeswara rasa is 6.94.
- XRD data reveals a Tin rich Astrophyllite form of Tarakeswara rasa.
- XRF data suggests concentration of Mercury and Iron in the sample
- EDXRF findings includes Hg, S, Fe, Sn, K, Mg, Mn, Cu, Zn, Ti, Rb, Sr, Ni, V, Cr and Ba as elements in Tarakeswara Rasa.
- PSA study explores the mean particle diameter as 8.04 microns. 5% of the sample shows particles less than 0.1microns and 15% of particles are below 1 micron.

Declaration by Authors

Ethical Approval: Not required

Acknowledgement: R. Kumar- Professor & Head of the Department- Nuclear & Radioanalytical chemistry section, fuel chemistry division, Materials chemistry & Metal fuel cycle group IGCAR, Kalpakkam

T.V. Sreeni – Professor, Department of Rasashastra and Bhaisajya kalpana, Government Ayurveda College, Thiruvananthapuram

Jacob. M. Titus – Professor (retired), Department of Rasashastra and Bhaisajya kalpana, Government Ayurveda College, Thiruvananthapuram

Source of Funding: None

Conflict of Interest: The author declares no conflict of interest.

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How to cite this article: Krishnaveni Rengarajan. Analytical study (XRD, XRF, EDXRF & PSA) on Tarakeswara Rasa - a Herbo-Mineral preparation. *Int J Health Sci Res.* 2023; 13(8):27-34.
DOI: *https://doi.org/10.52403/ijhsr.20230805*

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