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Characterization of *Tarakeshwara Rasa*: An Ayurvedic herbomineral formulation

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Abstract

Tarakeshwara Rasa (TR) is an Ayurvedic herbomineral compound formulation used in the intervention of *Prameha* vis-à-vis diabetes mellitus. The present study was executed to establish a fingerprint for this unique formulation, which can be adopted by the Ayurvedic pharmacies for drug standardization. TR is a formulation prepared by the trituration of four ingredients *Abhraka Bhasma* (AB), *Loha Bhasma* (LB), *Vanga Bhasma* (VB) and *Rasa Sindhura* (RS) in equal quantities with honey for one day. Each of the ingredients were prepared according to the norms of Ayurvedic classical texts and by employing Electric Muffle Furnace as heating device for incineration. To ensure the proper preparation of *Bhasmas*, standard tests (*Bhasma Pariksha*) were employed. After *Bhasma* complies these tests, TR was prepared and subjected for qualitative analysis, X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) studies. LB, AB, VB, RS and TR were also studied for free metal presence by employing phosphomolybdic acid. Chemical analysis of TR reveals that it contains Fe, Sn, Hg, Al, Mn, Ca and Mg. XRD study indicates that TR contains Fe_2O_3 (maghamite) in major phase and SnO_2 (cassiterite), HgS, SiO_2, HgO in minor phases. SEM study revealed that the compound is an agglomeration of particles. The particle size was in between 0.5 and 2 μ. Free metal detection by phosphomolybdic acid revealed the absence of free metals in the final *Bhasmas*. This is the first study establishing the characterization of *Tarakeshwara Rasa*.

Key words: *Bhasma*, characterization, *rasaushadhi*, scanning electron microscopy, X-ray diffraction

Introduction

Ayurveda employs the usage of *Rasoushadhis* (herbomineral preparations) since centuries for a wide range of maladies. Modern medicine has very less documentation regarding the therapeutic utility of metals and minerals. They are apprehensive about the safety of these compounds. The role of metals in curing ailments was first realized in Ayurveda.[1] Ancient *Acharyas* were conscious about the ill effects of the metals and they were well-versed in transforming them into safe effective medicines. In earlier days, the quality of a medicine was not subjected for critique, but it was based on the sacred trust which existed between the physician and the patient. The technological development and apprehensions of modern science obligated the patients and physicians to be watchful about the quality assurance, safety and efficacy of the medicine. Ayurvedic medicines have no exception in this regard. Hence it is the need of the hour to produce fingerprints for quality medicines. Many researchers have analyzed the metal and mineral-based individual *Bhasmas*. But the independent *Bhasmas* are used occasionally. Hence it is required to develop fingerprints not only for *Bhasmas* but also for the compound formulations. *Tarakeshwara Rasa* (*TR*) is the first line compound *Rasa* formulation used in the treatment of *Prameha* and is well known as "Poor man's *Vasanthakusumakara Rasa*." Literary search of classics reveals that, TR has been mentioned in various classics with different ingredients and different indications[2] i.e., *Prameha*,[3,4] *Mutra Ghata*[5] and *Mutra Krichhra*.[6] *TR* mentioned in *Bhaishajya Ratnavali* is widely used for *Prameha* and is available in the market.[7] Hence this formulation was selected for the study. Earlier few researchers have established the effect of TR in diabetes mellitus clinically.[8,9] But till date no scientific work has been carried out on this formulation with respect to physicochemical characterization, which is essential for drug standardization.

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Materials and Methods

Preparation of research formulation

Vanga Bhasma, *Rasa Sindhura*, *Abhraka Bhasma* and *Loha Bhasma* were taken in equal quantities. These were triturated with honey for 1 day then they were made into tablets. For preparing individual *Bhasmas*, the guidelines were adopted from various classical texts of *Rasa Shastra* and expert opinions[10] [Table 1].

For the preparation of *Rasa Sindhura*, *Kumari Bhavita Kajjali* (black sulfide of mercury, wet-triturated with fresh juice of aloe) was taken in a beer bottle wrapped by seven layers of clay and cloth. It was subjected to mild (250°C) moderate (250-450°C) and severe (650°C) heat through vertical electric muffle furnace. Total duration of heating was nine hours. *Rasa Sindhura* thus obtained was collected in the form of sublimate at the neck of the bottle.

Characterization of *Tarakeshwara Rasa*

Characterization with Ayurvedic parameters

The final *Bhasmas* were analyzed for quality control as per the Ayurvedic texts and then subjected for the preparation of TR.

a. Physical characterization

i. *Varna* (Color): Well-prepared *Bhasmas* possess a specific color as per the classics. In the present study the following were the colors of the *Bhasmas*, *Abhraka Bhasma*- brick red,[11] *Vanga Bhasma*- white (conch shell),[12] *Loha Bhasma*- ripened *Jamoon* fruit (purple),[13] *Rasa Sindhura* -*Sindhura Varna* (orange red) TR - light brick red.

ii. *Nischandratvam* (lusterless): Metal loses its metallic luster after proper incineration. This was examined under sunlight.[14] No luster was found in any of the *Bhasmas*.

iii. *Rekhapurnata* (finger lines test): The particles were so fine that when a pinch of *Bhasma* was taken between index finger and thumb, all the particles entered the furrows of the fingers.[14]

iv. *Varitara* (floating on water): Small quantity of each *Bhasma* was sprinkled on still water and it floated for some time on water.[14]

v. *Unama*: On each *Varitara Bhasma* rice grain was kept, it floated on the water.[14]

Thus three *Bhasmas* passed all the five tests for physical characterization.

b. Chemical characterization

vi. *Apunarbhava*: It is the inability of *Bhasmas* to revert to its original form. *Bhasmas* were mixed with *Mitra panchaka*, triturated, and subjected for *Puta*. After cooling no metallic particles were detected.[14]

vii. *Niruttha*: It is the inability to regain its alloy forming metallic property. If the *Bhasma* is subjected to *Puta* and kept along with silver foil, the weight of silver foil did not increase.

All three *Bhasmas* passed these two tests.

Characterization of TR by using modern parameters

i. Qualitative analysis: Qualitative analysis[15] was carried out in the Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical sciences, Banaras Hindu University, Varanasi.

For digestion of sample, one gram of sample was added with 20 ml of HCl+20 ml of HNO_3+4 ml of H_2O_2 in a closed vessel device using temperature control microwave heating at 200°C for 45 minutes then after cooling vessel device, solution is filtered and washed by ionized water. The insoluble portion was discarded and the solution was taken for the analysis.

ii. X-ray diffraction: XRD Study was carried out in the Department of Metallurgy, Institute of Technology, Banaras Hindu University, Varanasi.

The sample of TR was grinded well in agate mortar and desiccated well prior to the experiment, as any sort of moisture will give improper results.

The samples of the study were mounted on the sample holder of a commercial high resolution X-ray power Diffractometer fitted with a curved crystal monochromator. This diffractometer operates on "Bragg- Brentano geometry". An 18 Kw rotating anode generator was used as a source of X-ray. The XRD data was collected[16] [Figure 1, Table 2].

Table 1: Pharmaceutical preparation of *Tarakeshwara Rasa*

Ingredient	*Shodhana*	Pre incineration processes	*Putapaka* (EMF method)			
			Bhavana Dravya	Peak temperature (°C)	Duration of peak temperature	Number of incineration cycles
Vanga (Tin metal)	*Dhalana* in *Churnodaka* and *Nirgundi Swarasa* mixed with *Haridra Churna* both 7 times	*Jarana* with *Apamarga Panchanga Churna*	*Kumari Swarasa* (aloe juice)	500	1 hr	5
Krishna Vajra Abhraka (Biotite)	*Nirvapa* in *Triphala* decoction 7 times	*Dhanyabhrakeekarana* (special method of milling)	*Kasamarda Swarasa* (*Cassia oxidentalis* fresh leaf juice)	800	1 hr	40
Tikshna Loha (iron)	*Nirvapa* each 7 times in sesame oil, butter milk, cows urine, sour gruel and decoction of horse gram	1. *Bhanu Paka* (sun drying) with *Triphala Kwatha* 2. *Sthali Paka* (boiling) with *Triphala Kwatha*	*Triphala Kwatha*	650	1 hr	20

iii. Detection of free metal: Phosphomolybdic acid was used as a reagent for the detection of free metal.[17]

Preparation of reagent: Two grams of phosphomolybdic acid was accurately weighed and transferred to a clean volumetric flask (100 ml). Fifty milliliters of distilled water was poured into the flask and the acid was dissolved by warming on the water bath. The volume of the solution was made up to 100 ml by addition of acetone. The color of the solution should be bright yellow. In case the solution shows a dull green color, addition of a drop or two of nitric acid is necessary.

Procedure: Four clean sample tubes were taken for tin and iron and the prepared *Bhasmas* were labeled. Fifty milligrams of each sample was weighed and transferred into appropriate sample tubes and 1 ml of phosphomolybdic acid reagent was added in each of the sample tubes. All the sample tubes were shaken well for 1 minute and then allowed to stand for 5 minutes. The color change if any was noted.

iv. Scanning electron microscope: SEM was performed in the Department of Physics, Faculty of Science, BHU, Varanasi.

One of the basic features of SEM is their excellent depth of focus due to which it provides a three dimensional view of the surface. Hence, the specimen surface does not require any elaborate surface preparation and even a fractured surface can also be studied at sufficiently high magnification i.e., up to 5000 magnification. For the present study SEM 840 A (JEOL - Japan electronics optical limited) was used for taking the micrographs. It is a state of the art high resolution SEM. Its maximum limit of magnification is 3 lakh.[18]

Method

In this study a small pellet of sample of TR was kept in desiccator for drying. After complete desiccation the sample was subjected for carbon coating and then was placed over the specimen holder and observed under the microscope at 500, 1000, 1500×. Micrographs were taken with the inbuilt camera.

Observations and Results

In qualitative analysis TR showed the presence of Sn, Hg, Fe, Ca, Al, Mn and Mg. Detection of free metal by using phosphomolybdic acid reagent shown that there is no color change observed with final *Bhasmas* of *Lauha*, *Vanga* and *Abhraka* but tin and iron showed immediate color change from yellow to blue. There were no free metals present in the *Bhasmas* selected. In SEM pictures [Figures 2 and 3] of TR, individual particles were visualized clearly. The horizontal line in the right corner of the micrograph corresponds to 10 and 5-μ length. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the *Bhasma* particle was calculated. The SEM study pictures show that the particles were homogenously mixed. The particle size ranges from <1 to 40 μ and the bigger particles were agglomeration of smaller particles itself. The smaller particles had a size less than 0.5 μ which shows high dispersion.

Discussion

Lack of standardization is considered as the biggest pitfall in the growth and or differentially as per the individual requirement dissemination of Ayurveda.[19] Previously, the *Bhasmas* were being prepared on small scale by the Ayurvedic physicians themselves, but now they are manufactured on large scale in pharmaceutical houses. This new approach has created several troubles, as the uses of new appliances have not

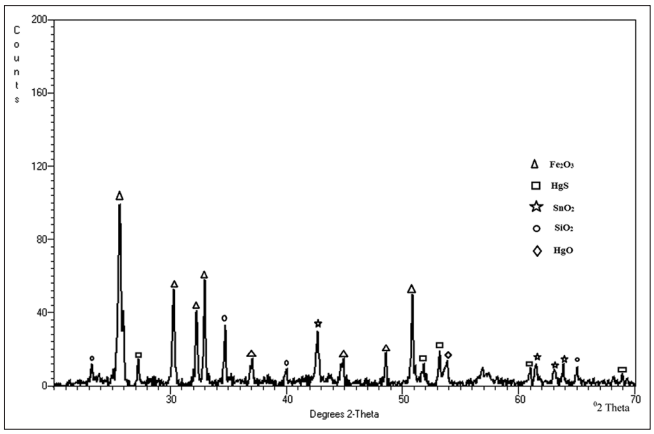

Figure 1: X-ray diffraction pattern of *Tarakeshwara Rasa*

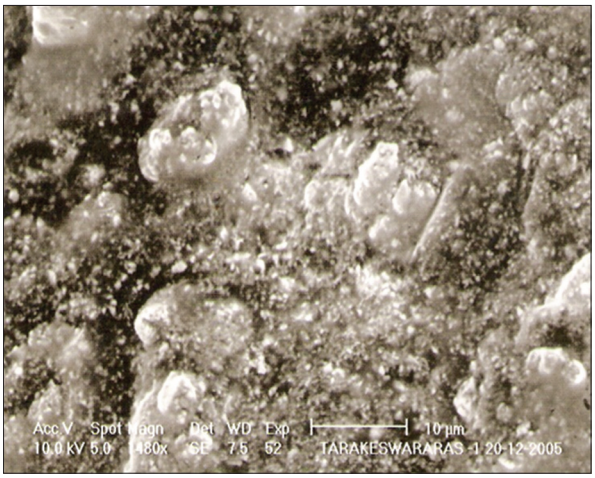

Figure 2: Scanning electron microscopy of *Tarakeshwara Rasa* *(upto 10 μ magnification)*

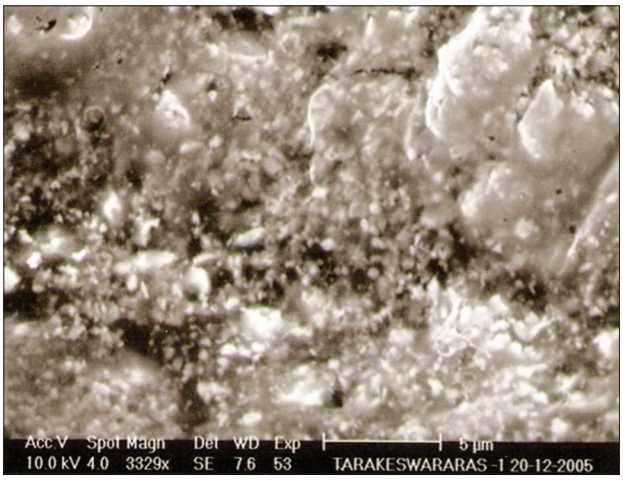

Figure 3: Scanning electron microscopy of *Tarakeshwara Rasa* (upto 5 μ magnification)

Table 2: The standard diffraction data from JCPDS for the compounds of the study

Compounds	d Å	Intensity	h k l
Fe_2O_3	3.469	100	-
	3.427	40	-
	2.946	54	-
	2.772	41	-
	2.713	59	-
	2.421	19	-
	2.017	14	-
	1.872	18	-
	1.793	51	-
	1.616	10	-
HgS	3.269	15	-
	1.762	12	-
	1.719	19	-
SnO_2	1.432	10	-
	2.115	30	2 1 0
	1.517	10	0 0 2
	1.505	11	3 1 0
	1.471	8	2 2 1
SiO_2	3.826	11	-
	2.579	33	-
	2.215	9	3 1 2
	1.456	12	6 0 1
HgO	1.701	13	4 0 0

been standardized with respect to the quality of these *Bhasma* preparations. For standardized *Bhasma* preparations, there is a need for scientific approach, which may be defined as 1. Physical standardization and elemental analysis of raw materials and final products. 2. Determination of oxidation state of metals and association of these metals with acidic radicals in the final product.[20] Along with these parameters for quality assurance of *Bhasmas*, process standardization is also essential. Preparation of *Bhasma* is very elaborative, time, and labor consuming. After the purification methods (*Shodhana*), pre-incineration methods, converting metal and minerals into suitable form for incineration i.e., powder form (*Dhanyabhrakeekarana* and *Jarana*), the individual drugs were triturated with herbal juices and incinerated. The impregnated herbal coat on the surface of the metal or mineral and trituration helps in particle size reduction. Method of preparation of *Bhasma* is similar to the modern day surfactant-mediated nano-particle metal oxide production.[21] Classical texts of Rasa Shastra have explained tests (*Bhasma Parikshas*) to identify the accuracy of *Bhasma* preparation. *Bhasma Parikshas* are nothing but characterization of *Bhasmas* with minimal tools. They may be classified into nondestructive (*Nischandratvam, Rekhapurnata, Varitara,* and *Unama*) and destructive (*Apunarbhava* and *Niruttha*).

Qualitative analysis of TR reveals the presence of Sn, Fe, Al, Mn, Ca and Mg. The other metal presence in the final *Bhasma* may be due to adulteration or usage of earthen casseroles during preparation or the herbs and methods used throughout the pharmaceutical study. The role of these elements in the characterization is uncertain.

In XRD pilot study, it was found that if honey is used for trituration, it showed amorphous nature of TR. Hence, the fine powder of TR without addition of honey was subjected to XRD studies. This study confirms the presence of ingredients of TR. Ten Fe_2O_3 (maghamite) peaks, four SnO_2 peaks, four SiO_2 peaks, four HgS peaks and one HgO peak were observed in the study. These peaks indicate the presence of respective *Bhasmas* and *Rasa Sindhura*. The XRD pattern of TR shows the presence of Fe_2O_3 (maghamite) as the major phase, which is marked to the corresponding peak of each phase and remaining peaks of SnO_2, SiO_2, HgS and HgO were minor phases. The presence of Fe_2O_3 (maghamite) in *Loha Bhasma*,[22] SiO_2 peaks along with Fe_2O_3 peaks in *Abhraka Bhasma*,[23] the traces of HgO along with major phase of HgS in *Rasa Sindhura*[24] were reported by the earlier scholars. The present study also supports these findings.

The scanning micrographs obtained from the various surface regions of the sample clearly depicts the particle size which ranges from <5 to ~ 40 μ. Bigger particles mimic the agglomeration of the smaller particles, which are of the size <5 μ. More interestingly various crystallites in different shapes namely rod (5-10 μ length, ~2-4 μ diameter), elliptical (~4 μ length), Cubical (~1 μ) and rectangular (length ~ 10 μ, width 1-4 μ) were embedded in the lumps and hence bigger particles (~40 μ) were obtained. Unlike the bigger particles, these shaped particles had smooth surface features. It is evident from the well-defined XRD peaks, that these particles were crystalline. Distribution of smaller particles (<1 μ) shows high dispersion throughout the surfaces and their typified surface features are favorable to consider that they are crystalline. Some bigger crystalline particles may be of *Rasa Sindhura*. It shows that even after triturating for one day, the particles of *Rasa Sindhura* were still bigger. Remaining all particles are of 0.5-2 μ size. Hence a conclusion can be drawn that all the *Bhasmas* were of the size less than 2 μ in TR. The earlier SEM studies on Swarna Makshika *Bhasma* - particle size 1-2 μm,[25] Swarna *Bhasma* -particle size 56-57 nm,[26] 669-717 nm,[27] Naga *Bhasma* -particle size 60 nm[28] and Yashada *Bhasma* has identifiable fraction of particles in nano size.[29] Particle size distribution of LB - the particle size range of 2.55-40.9 μm was found in *Sahastraputi Lauha Bhasma* and 5.1-51.6 μm in *Satputi Lauha Bhasma*.[30] Hence a logical conclusion can be drawn, that the *Bhasmas* possess significant percentage of particles in nanosize, but microsized particles are also present. So *Bhasmas* have nano to micro particles. Particle size depends on the nature of drug and number of calcination cycles.

The interaction of biological systems with particles depends on various factors such as nature of particles (size, shape, composition, etc), cell type and environment. Other factors governing the interaction are yet to be completely understood.[29] Thus it can be concluded that *Bhasmas* possess a micro to nano-sized particles.

Free metals exert their toxic effects by combining with one or more reactive groups (ligands) essential for normal physiological functions. Hence, free metal presence is not desirable in final *Bhasma*. The phosphomolybdic acid test is useful for primitive screening of *Bhasma*.

Conclusion

TR is a red-colored compound formulation used in the

intervention of *Prameha*. Chemical analysis of TR reveals the presence of iron, tin, mercury, manganese, magnesium and calcium. The XRD studies indicate Fe_2O_3 in major phase and SnO_2, HgS, SiO_2, HgO as minor phases. SEM studies reveals that majority of particles were found to be in between 0.5 and 2 μ. Analysis with phosphomolybdic acid confirmed the absence of free metal in the prepared *Bhasmas*. Free metal absence is essential in the safety of *Bhasma*, as elemental form of metal can produce oxidative stress. This study can serve the need for the characterization of TR. This study can be a direction for establishing the fingerprint of, *Tarakeshwara Rasa* a herbomineral compound formulation.

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हिन्दी सारांश

आयुर्वेदिक वानस्पतिक–खनिज योग तारकेश्वर रस का मानकीकरण

विरुपाक्ष गुप्ता के. एल., नीरज कुमार

तारकेश्वर रस (ता.र.) एक विशेष वानस्पतिक–खनिज योग है जो प्रमेह (डाइबिटिज मेलाइटस) के लिए प्रयोग किया जाता है । वर्तमान अध्ययन इस योग के फिंगर प्रिन्ट स्थापित करने के लिए निष्पादित किया गया है, जिसे आयुर्वेदिक फार्मेसियों द्वारा औषध मानकीकरण के लिए अपनाया जा सकता है । ता.र. योग के निर्माण के लिए अभ्रक भस्म (अ.भ.), लौह भस्म (लौ.भ.), वंग भस्म (वं.भ.) और रससिन्दूर (र.सि.) इन चारों घटक का सम प्रमाण में मधु के साथ १ दिन मर्दन करते हैं। प्रत्येक घटक का आयुर्वेदिक ग्रन्थों के मापदंड के अनुसार निर्माण किया गया है तथा भस्मीकरण के लिए इलेक्ट्रिक मफल फर्नेस का प्रयोग किया गया है । भस्मों का उचित निर्माण सुनिश्चित करने के लिए मानक परीक्षा (भस्म परीक्षा) का उपयोग किया है । भस्मों की परीक्षा के बाद, ता.र. का निर्माण किया गया एवं उसे गुणात्मक विश्लेषण, एक्स–रे डिफरेक्सन (XRD) और Scanning Electron Microscopy (SEM) के लिए भेजा गया । Phosphomolybdic acid के उपयोग द्वारा लौ.भ., अ.भ., र.सि. और ता.र. में फ्रि मेटल की उपस्थिति का भी अध्ययन किया गया । ता.र. के रसायनिक विश्लेषण में Fe, Sn, Hg, Al, Mn, Ca एवं Mg पाया गया । XRD परीक्षण से पता चला कि ता.र. में मुख्यतः Fe_2O_3 (मेघामाइट) होता है एवं $Sn0_2$ (केसीटेराइट), HgS, SiO_2, HgO कम मात्रा में होते हैं । SEM के द्वारा पता चला कि यह योग कणों का सम्मिश्रण है । Phosphomolybdic Acid द्वारा परीक्षण करने पर सभी भस्मों में Free metal अनुपस्थित पाया गया । वानस्पतिक खनिज योग तारकेश्वर रस के लक्षणों को स्थापित करने वाला यह पहला अध्ययन है ।