Available online on 15.09.2021 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2021 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



Open Access Full Text Article



Review Article

Analytical Methods in Standardization of Bhasmas: A Review

P.G. Dileep Kumar^{1*}, P. Nataraja Yadav², S. Gananadhamu³, K.S. Nataraj⁴¹ Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram - 534202, Andhra Pradesh, India² Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram - 534202, Andhra Pradesh, India³ Professor, Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education & Research, Hyderabad, Telangana, India⁴ Professor, Department of pharmaceutical analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram - 534202, Andhra Pradesh, India

Article Info:



Article History:

Received 18 July 2021
 Reviewed 29 August 2021
 Accepted 01 September 2021
 Published 15 September 2021

Cite this article as:

P.G. DK, P. NY, S. G, K.S. N, Analytical Methods in Standardization of Bhasmas: A Review, Journal of Drug Delivery and Therapeutics. 2021; 11(5):183-192

DOI: <http://dx.doi.org/10.22270/jddt.v11i5.4960>

Abstract

Ayurveda the ancient science well known for its potential therapeutic effects with unique metallic, herbal juices/fruits in the treatment of chronic ailments. A well-known ayurvedic potent preparation, Bhasma is also known as ash which is a metallic base prepared by sophisticated pharmaceutical processes also plays a major role in acute, sub-acute, and chronic diseases. In order to determine its quality and purity to make sure the acceptability, the safety of the formulation standardization is very necessary. In this short review, an attempt has been made to present ancient methods to standardize physical characteristics like Verna, Nisvadutam, Nishchandratvam, Varitara, Unama, Rekhapurmatvam, Slakshmatvam..etc., and chemical characteristics of bhasmas like Apurnabhavta, Niruttha, Amla Pariksha with advanced methods like SEM, TEM, and NPST, along with other natural and ancient analytical techniques.

Keywords: Bhasmas, Standardization, Analytical methods, SEM, TEM.

*Address for Correspondence:

P.G. Dileep Kumar, Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Vishnupur, Bhimavaram - 534202, Andhra Pradesh, India

Introduction:

Ayurveda the science trusted for its longevity¹ and established through thousands of years, since the medicinal plants were primarily used for the preparation of remedial agents by Charaka and Sushruta and first introduced for the use of metallic minerals like - Swarna, Rajat, Tamra, Abhrak, and Makshika, Rasa as medicinal agents by alchemist Nagarjuna at 8 century². It is a science of life or a way of living with the rhythm of nature i.e. connecting the physical, mental, and spiritual connectivity of the human body³. It deals with metals/ non- metals/ herbomineral preparations called as bhasmas⁴. Bhasmas are also defined as drugs based on metal and metal oxides⁵⁻⁶. According to Ayurvedic metallurgy, Bhasmas are unique Ayurvedic metallic preparations¹. Bhasmas(ash) are the products obtained after incineration⁷ of herbals and minerals collectively by the process known as bhasmikanana the process that converts the metals into special chemical compounds with medicinal advantages⁸⁻⁹. Bhasmas are biologically produced metallic nanoparticles obtained by calcination into ash and are taken along with milk, butter, honey, ghee, etc¹⁰. They will be available as nanoparticles and are taken along with milk, butter, honey or ghee; thus making the metals easily assimilable, eliminating their harmful effects and enhancing their biocompatibility¹¹. Nanomaterials and structures possess very unique features like small size when compared with larger bulk materials, allowing as suitable candidates

for novel applications¹². Nanotechnology has been focused to achieve molecular level¹³ interaction at targeted sites with enhancing character to enhance the permeation of active molecule¹⁴. Bioenhancer is a substance which in combination with a drug or nutrient provides more availability of the drug thereby reducing the amount of active molecule that is required¹⁵⁻¹⁶. They were used as a part of the traditional system of Ayurveda¹⁵, an Indian medical system that is still in use in India and elsewhere¹⁷.

¹⁸. Modern-day enhancers are synthetic in nature, and generally enhance the bioavailability by interference with the metabolism of drugs¹⁴. The immunomodulation and ability to target drugs to the site are characteristics of properly prepared organo metallic preparations. Properly prepared bhasma is nontoxic, easily absorbable, adaptable, and digestible in the body¹⁹. The methods of bhasma preparation vary so much for each metal such that bhasma with different colors are produced²⁰. In many cases, the wrong manufacturing and marketing process may lead to the production of inferior-quality products, which reduces the efficacy of products as well as safety parameters. To reduce the variability and to increase the quality of Ayurvedic products, standardization contributes to major way²¹. Standard is the numerical value that quantifies the parameters and thus denotes the quality and purity of material²². These bhasmas are generally prescribed with the several other medicines of Ayurveda²³⁻²⁴. In many disorders

like Kshaya (~Tuberculosis), memory renovators, cures all diseases, suicidal tendencies, increases blood circulation in the brain, osteoporosis, syphilis, sprue, anaemia. Its therapeutic properties like analgesic anticataleptic, anti-anxiety and antidepressant, antioxidant, augmenting effect. Recent studies of gold nanoparticles observed its antiangiogenic properties anti-cancer property²⁵. The ancient application of nanomedicine in the form of ayurvedic bhasma throws light on the safer usage of present nanomedicine for living beings and environment^{23 26}. The present study deals with the preparation and step in the synthesis of bhasma was carried out by the traditional method of heating as well as using electric muffle furnace. The finally synthesized bhasma was then compared with the marketed sample²⁷.

Nanomedicine and Bhasmas:

Bhasmas are said to be biological nanoparticles due to their small particle size less than 100 nm having a very large surface to volume ratio leading to different, novel properties. . Rasashastra advocates some peculiar properties of Bhasma like readily absorbable, adaptable, and assimilable in the body and non-toxic and by this basis may prove to be very effective in medicinal purpose²⁸.

Nanoparticles not only increased the surface area but the nanosize also helped the drugs to reach the target site in the desired time even in smaller doses and the end results were remarkable. They become more palatable with longer shelf life. Preparation of nanoparticles is presented in Fig 1²

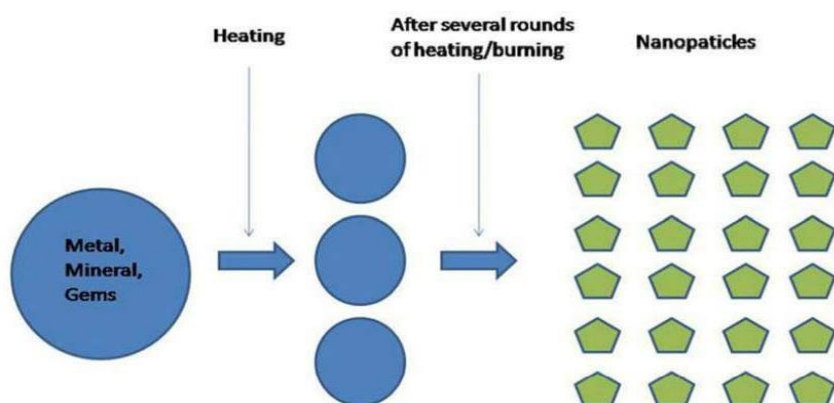


Figure 1: Preparation of Nano particles

Ayurvedic concept of Mardana (trituration) and Bhavana (levigation) to reduce particle size is an ultimate result of these processes of nanostructure Formation by Mechanical Activation Bhasma are nearer to nanocrystalline materials. Nanocrystalline material formation during milling and mechanical alloying was the first suggested²⁹.

Bhasmas Classification²¹:

1. Metal-based Bhasma
2. Mineral-based Bhasma
3. Herbal Bhasma

Preparation of Bhasma²¹:

Bhasmikaran the process of making bio incompatible to biocompatible products. The objectives of samskara are:

1. To eliminate harmful matters.
2. To modification of undesirable physical properties.
3. To convert undesired characteristics to desired.
4. To enhance the therapeutic action of drugs.

Steps of Bhasmikaran

1. **Shodhan:** Shodhan (Purification) means purifying by removing the unwanted parts from raw materials and making the product suitable for further process.

Ayurveda classifies shodhan process as:

- a. General:** The sheets of metals are heated till red hot and are successively dipped into liquids like oil, buttermilk, cow's urine, etc. for seven times.
 - b. Specific:** For some metals like Jasad, the molten mass is poured in cow's milk 21 times.
2. **Maran:** Maran (Powdering) a change is brought about in

the chemical form or state of the metal in presence of a) mercury b) plants and c) sulphur to lose its metallic and physical character as form suitable for administration.

3. **Chalan:** Chalan (Stirring) is carried out during heating either with an iron rod or stick made from a specific plant (like neem) to enhance the therapeutic effect by phytoconstituents of plant stick.
4. **Dhavan:** Dhavan (Washing) water washes are given to remove the excess amounts of agents and water-soluble constituents used in previous stages which may adversely affect the final product quality.
5. **Galan:** Galan (Filtering) through a fine cloth or through sieves of suitable mesh the product is then sifted to separate residual material of larger size.
6. **Puttan:** Puttan (Heating or ignition). The key step in bhasmas manufacturing a special earthen pot, Sharav is used for the process. It has two parts of soccer shape, used for direct heating of the material. Uniform and faster heating is possible due to its shallowness and the other part is used as a lid for placing it in an inverted position.

Jarana (polling) is performed For metals having a low melting point (lead, tin, and zinc), between Shodhana and Bhavana procedure, where metals are melted and mixed with some plant drugs powders and are rubbed by an iron ladle with the inner surface of pot resulting complete powder form².

Cycles of Inceneration²⁹

Incineration was done in Bhavana and incineration. Bhasmas were trituated with a decoction of Vasa for three hours until the liquid gets completely absorbed. With the help of some

round-shaped facilitates the pellets in round shapes were prepared.

The overview of the preparation of bhasmas are presented in Fig 2³⁰

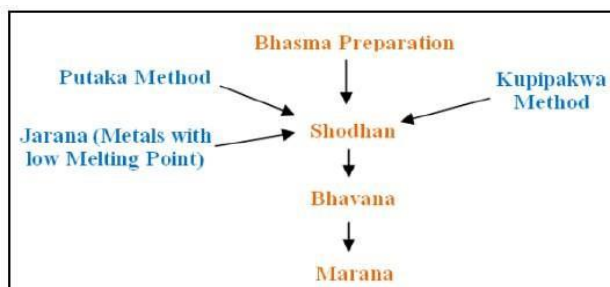


Figure 2: Preparation of Bhasmas

Bhasmas as Multi-elemental Cocktail³¹:

Bhasmas based on calcium, iron, zinc, mercury, silver, arsenic, copper, tin, and gemstones is analyzed for elements including C, H, N, and S contents. In addition to the major constituent element found at % level, several other essential elements (Na, K, Ca, Mg, V, Mn, Fe, Cu, and Zn) have also been found in $\mu\text{g/g}$ amounts and ultra-trace (ng/g) amounts of Au and Co.

Bhasmas Importance²¹:

1. Bhasma is potent in small doses.
2. Provides easily absorbed and usable calcium
3. Maintains optimum alkalinity for optimum health
4. Cleanse the kidneys, intestines, and liver
5. Maintains healthier teeth and stronger bones
6. Alleviate depression and insomnia.

Pharmacological evaluation³²:

For pharmacological evaluation, the samples are tested for specific pharmacological activity using animal models. Animals are selected and treated according to GLP guidelines. By inducing some Specific pathological conditions are produced. Specific activity studies like hepatoprotective, antihyperlipidemic study, and toxicological (acute and

chronic toxicity study) and histopathological studies of Bhasma are included here. Some laboratory studies like antimicrobial, antifungal study also carried out for ensuring the quality and therapeutic efficacy of bhasmas.

Bhasmas in Therapeutics:

1. Nutritional anemia in nonpregnant adolescent girls can be improved by a daily dose of Sootshekhar Rasa (250 mg) plus Sitopaladi Churna (400 mg)³³.
2. Kukkutanda twak bhasma on clinical study reveals statistically significant improvement in Swetapradara, an important gynecological disorder³⁴.
3. 'Swarna Bhasma' has shown some response in the treatment of solid tumor³⁵, and some herbominerals preparations were effective towards leukemia³⁶ ³⁷and antioxidant/restorative effects against global and focal models of ischemia (stroke).
4. Nāga bhasma (calx of lead) being a potent metallic formulation is indicated for Prameha (diabetes) treatment³⁸ and with 44 formulations of Naga Bhasma were developed, and herbs along animal-based products enhanced, to prevent diabetic complications, and reduce side effects³⁹.
5. Randomized controlled study of iron deficiency anemia patients shown effective results with Kasisa Bhasma treatment⁴⁰.
6. Many metallic preparations had antibacterial activity. For example, Rajata (Ag) Bhasma nanoparticles could suppress *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*⁴¹.
7. Yashada Bhasma (Zinc calx) could inhibit *Propionibacterium acne* and suppresses acne induced inflammation⁴².
8. Tamra (Cu) Bhasma is effective in inhibiting the growth of gram-negative (*P. aeruginosa*, *K. pneumoniae*) and gram-positive (*S. aureus*) bacteria⁴³.

Uses of some of the marketed products are presented in Fig 3².

Name	Ingredients	Uses
<i>Navrattankalp amrit ras</i>	Calcined ash of expensive gems, minerals like ruby, sapphire, emerald, cat's eye stone, pearl, coral, silver, gold, iron, zinc	Cancers of all types, anemia, complication of diabetes
<i>Heerak Bhasma</i>	Diamond	Useful in cancers, immunity disorders, crippling rheumatoid arthritis, bone marrow depression
<i>Tsrailokya chintamani ras</i>	Diamond, gold, silver, iron	Severe respiratory tract infection, marrow depression, ovarian cysts, uterine fibroids
<i>Swarna basant malti ras</i>	Gold, <i>piper-nigrum</i> , white pear powder	Tonsillitis, fevers, cough, bronchitis, decreased immunity, cancers, autoimmune disorders
<i>Kamdudha ras</i>	Ochre, <i>Tinospora cordifolia</i> , mica (calcined)	Hyperacidity, headache, fever, blood pressure
<i>Vasant kusumakar ras</i>	Gold, silver, coral	Complications of diabetes, neuropathy, general weakness
<i>Kumar kalian ras</i>	Gold, iron, mica, copper pyrite, red sulfide of mercury	General debility in children, fever, respiratory tract infections
<i>Tamra Bhasma</i>	Copper, mercury, sulfur	Anemia, jaundice, digestive disturbance, abdominal disorders
<i>Loha Bhasma</i>	Iron, cinnabar	Enlargement of liver, anemia, jaundice
<i>Vaikrant Bhasma</i>	Manganese, sulfur (Tourmaline)	Diabetes, can be used in place of diamond ash in case of poor patients
<i>Loknath ras</i>	Mercury, sulfur, conch shell	Diarrhea, respiratory disorders, immunity disorders, cancers, ovarian cysts

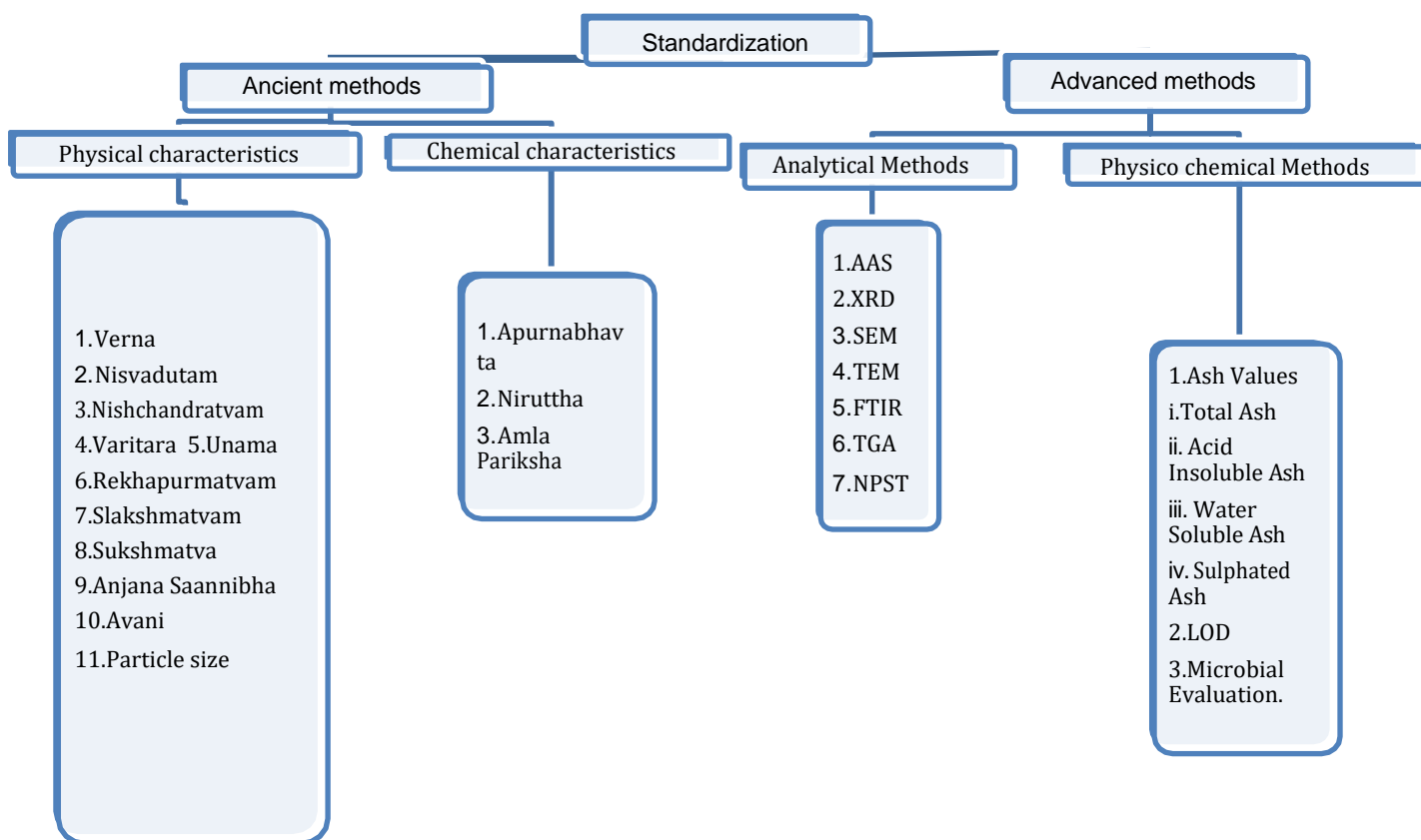
Figure 3: Marketed products and their uses.

Toxicity Issues of Bhasmas

In spite of presence of toxic metal components either in trace amounts Ayurveda is widely used in India⁴⁴. Inferior quality and lack of authentication along with non-availability of standards in raw materials manufacturers of Ayurvedic medicines now facing more number of problems. Intentionally or unintentionally Use of an inferior grade of raw material, adulteration, leads to the production of inferior quality products including deviations in standard manufacturing practice. Because of the widespread use of Ayurvedic medicines, it has become necessary to lay down stringent parameters to ensure batch to batch consistency and reproducibility⁴⁵.

Standardization

A measurement for ensuring the quality to describe all measures, during the manufacturing process and leading to a reproducible quality. In herbals, it places a major role from birth to clinical applications of a plant. Adjusting the herbal drug preparation constituent or a group of substances with therapeutic activity with the excipients being added either in combined or mixed forms. As there are many factors influencing bioefficacy in addition to reproducible therapeutic result standardization is not an easy task. Care should be taken right from beginning to end of the process to obtain a desired quality of the herbal formulation required⁴⁶⁴⁷. The methods under standardization are represented in the flow chart ²¹



Need of Standardization ²¹:

Bhasma cannot be considered scientifically valid if the drug tested has not been authenticated, characterized and standardized ⁴⁸systematic characterization of the samples, utilizing complementary analytical techniques studies were done on nanoparticles for its quality and efficacy which resulted eventually in the structural, morphological, chemical and elemental characterization of the sample. Prediction of the possible model of gold and iron bhasmas and its structure that of a core-shell nanostructure as possible².

Methods of Standardization ²¹:

Advanced methods of analysis:

Physicochemical Methods for Analysis of Bhasma²¹:

1. Ash Value

I To determine the quality and to establish the identity of

it in addition to the purity of a crude drug

II Ash contains inorganic radicals like magnesium, potassium, carbonates, silicates of sodium, phosphates, carbonates, etc. Quantitative determination of the radicals present as ash and their values helps in the standardization of particular crude drugs.

III To determine foreign inorganic impurity matter present.

At temperature 450°C or above Carbon and organic matter is converted to ash which is present in the drug. It mostly contains silica, silicates, phosphates, and carbonates. Total ash value can be used to study water-soluble and acid-insoluble ash.

Acid Insoluble Ash: Process of removing many inorganic salts where mainly silica is obtained in the residue by treating with dil. Hydrochloric acid (HCl)

Water Soluble Ash: separating the water-soluble material by drying to yield water-soluble ash. Many water-soluble salts contributing to total ash content are removed.

Sulfated Ash: This process includes converting of all oxides and carbonates to sulfates by subjecting crude drug to incineration at a temperature of about 600°C with dil. Sulphuric acid before ignition.

2. Loss on Drying:

Measurement of the amount of water content and other volatile material in the sample by drying or heating. The process includes weighing of crude drug about 2 gm in nickel or silica crucible or in dry heat oven at 110°C till the constant weight is obtained. The difference in the weight before and after drying or heating gives the loss on drying.

3. Microbial Evaluation:

The microbial evaluation includes the determination of values of the total viable aerobic count, total Enterobacteriaceae, and total fungal count, test for specific pathogen: E. coli, Salmonella spp., S. aureus, and Pseudomonas aeruginosa⁴⁹.

Characteristics of Bhasma

A. Physical Characteristics (preliminary tests):

- i. Verna:** A specific color is mentioned for each bhasma⁵⁰. Like red or pale and white colors. Which is based on parent material⁴. Improper preparation of bhasma can be tested by testing the adulterations.
- ii. Nisvadutam:** By placing a pinch of bhasma except for some metallic preparations on the tongue and tasting that whether the taste is to be tasteless is performed here. The bhasmas prepared by Proper incineration should be of particular taste²². Transformation to compounds of specific taste from metallic taste is indicated⁴⁷.
- iii. Nishchandratvam:** Prior to therapeutic application bhasmas should be lusterless. Luster is the character that is lost by metal after proper incineration. To conduct this test Bhasma is seen under bright sunlight by taking it in a petri dish, to see whether the luster is present or not; Further incineration is needed if the luster is present²¹.
- iv. Varitara:** Lightness and fineness should be the

characteristic of bhasma. Based on the law of surface tension this test is performed by sprinkling the small amount of bhasmas over the stagnant cold water in a beaker. Light and fine bhasmas which are prepared by proper incineration will flow on water²¹.

- v. Unama Test:** Test is performed by placing a grain on the floated layer of bhasma. Bhasma is considered as excellent when it floats but not when it sinks. It is a further test performed in the continuation of varitara test⁴⁹.
- vi. Rekhapurnatvam:** For easy absorbing and assimilation in the body the bhasmas should be of smaller size. Here, bhasma is rubbed by spreading it in between the index finger and thumb and it should be so fine so that it gets easily into the cervixes and lines of the fingers and should not get washed out from the finger lines²¹.
- vii. Slakshnatvam:** Tactile sensation produced by bhasma when we touch with fingertips is tested. Without producing any irritation to the mucous membrane of GIT Tactile sensation can be properly absorbed and assimilated into the body²¹.
- viii. Sukshmatva:** Precision with varitara and rekhapurnatvam, fineness of bhasma preparation is known as Sukshmatva. Bhasma must be fine so that it will be absorbed properly in the body²¹.
- ix. Anjana Sannibha:** Anjana (collyrium) is the smoothness character of bhasmas when applied. Properly incinerated bhasma should be smooth without producing any irritation to the mucous membrane of the GIT²¹.
- x. Avami:** After administration bhasmas should not produce any nausea or vomiting sensation²¹.
- xi. Particle Size:** Bhasma which is properly incinerated should be in churna (powder) form. Size of bhasma particles should be like pollen grains of Pandanus odoratissimus flower (ketaki rajah)²¹.

The various techniques and their observations are presented in **Table 1**⁵¹ and various stages of vanga bhasma are shown in **Fig 4**²⁷

Techniques	Observations
Rekhapurnatvam	To observe whether the bhasma has washed out from the cleavages after it enters the fingers.
Varitaratavam	To observe whether the particles of bhasmas are floating in the surface of the water or not
Avami	whether bhasmas are producing any vomiting or nausea
Nischandratva	To observe whether the luster is present to the bhasmas or not
Amla pariksha	To observe whether the change of colour of curd or lemon when they were added

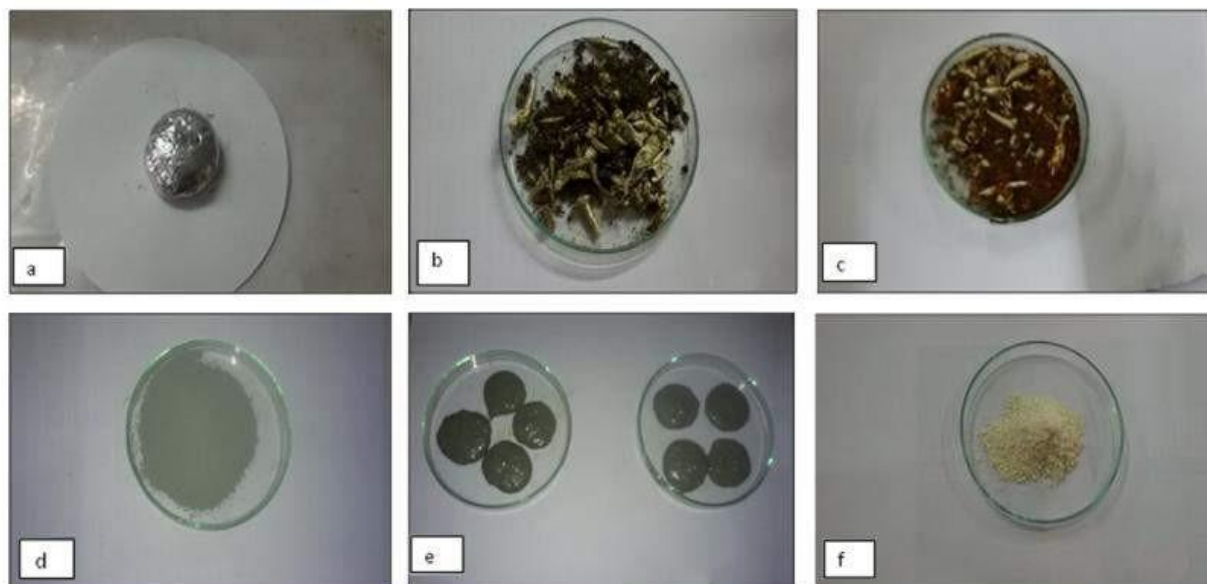


Figure 4: Vanga Bhasma images (a) after Melting (b) after Samanya Shodhan (c) after vishesh shodhan (d) after Jaran process (e) after bhavan (f) final product.

B. Chemical Characteristics²²:

i. Apurnabhavta:

Incapability to retain its original metallic form. Equal quantity of mitrapanchaka the seeds of (*Abrus precatorius*, ghee, jaggery, borax, and honey) are sealed in sarava samputa, heated with a similar grade of heat and allowed for self-cooling after self-cooling particular products are observed with Lustrous showing the presence of free metal which are inactive after incineration.

ii. Niruttha:

Test of inability to regain metallic form from metallic bhasma. In this test bhasma by fixing a weight of silver leaf in sarava samputa and heating with a similar grade of heat after allowing to self-cooling the weight of silver is taken and improper incinerated bhasma indicates the increase in silver weight.

iii. Amla pariksha:

A little amount of curd in Petri dish and some amount of lemon juice in a test tube and mixed with a pinch of bhasma and seen for color change. No color change indicates the well- prepared bhasmas.

Imperative need for a scientific approach in standardization and characterization is needed and it includes the following steps⁵²:

- Elemental analysis with physical standardization of raw material and finished products.
- Oxidation state and their association with acidic radicals of metals in the finished product.
- Prominent metallic component along with pharmacokinetics of bhasma using metallic extraction from tissues or tracer techniques.
- Study of metallic accumulation in different organs and tissues.
- Acute and chronic toxicity study with heat shock protein expression.

- Normal physiological and antioxidant parameters of bhasmas and their effects.
- Study of therapeutic responses of bhasmas at the cellular and molecular level to recommended diseases.
- The role of bhasmas in body immunomodulation and physiology of gastrointestinal tract (GI) (site of jataragani) and as drug carriers.
- The limits of heavy metals like lead(Pb) about 10.0ppm, cadmium(Cd) 0.30ppm, mercury(Hg) 1.00ppm, arsenic(As) 10.0ppm are permissible.

Detection of Nanoparticles in Bhasma²⁵:

Various methodologies are used to test NPs like environmental electron microscopy, Transmission electron microscopy (TEM), energy dispersive x-ray analysis (EDAX), inductively coupled plasma (ICP), atomic absorption spectroscopy (AAS), gel electrophoresis (GE), enzyme expression, etc.

Nano Particles testing in Bhasmas involves the following steps²⁵:

- To estimate the presence of NPs in the test sample;
- To identify whether the chemical composition is homogeneous;
- To know the nature of NPs whether they are in crystalline or amorphous;
- To know the defects in the sample; and
- To check the bioactivity of the sample.

Modern Advanced Analytical Methods for Analysis of Bhasma²¹:

a. Atomic Absorption Spectrophotometry:

In this technique quantitative analysis of elements especially metals is done and the process includes subjection of sample to the flame using a nebulizer, light is emitted at a specific wavelength when any inorganic

matter present in the sample gets excited by the flame and is directly proportional to their concentration⁵³.

b. X-ray Diffraction:

XRD is a technique through which the spatial arrangement of structural units of a substance in its crystalline state is known by applying Bragg's law. By applying Bragg's law and with the help of wavelength the distance between the set of atomic planes is determined and in the form of the heterogeneous solid mixture and crystallographic structures, the results are obtained⁵⁴.

The XRD Pictorial presentation of Muktha bhasma is presented in Fig 5²⁸

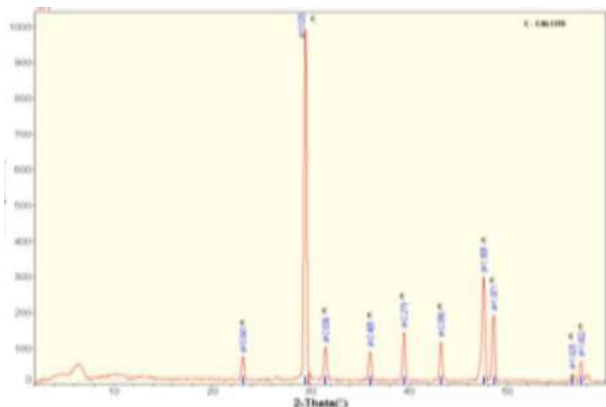


Figure 5: XRD of Muktha Bhasma

c. Scanning Electron Microscopy:

SEM is a technique where a focussed scanned electron microbeam is used to produce images of the sample, at both bottom and top with necessary cross-sections and sample preparations. The topographic nature of the specimen is emphasized and with this high magnification microscope, surface morphology is examined²¹. The pictorial presentation of SEM of Rajatha bhasma at various magnifications are presented in Fig 6⁵⁵ and Fig 7⁵⁵ and vanga bhasma at Fig 8²⁷

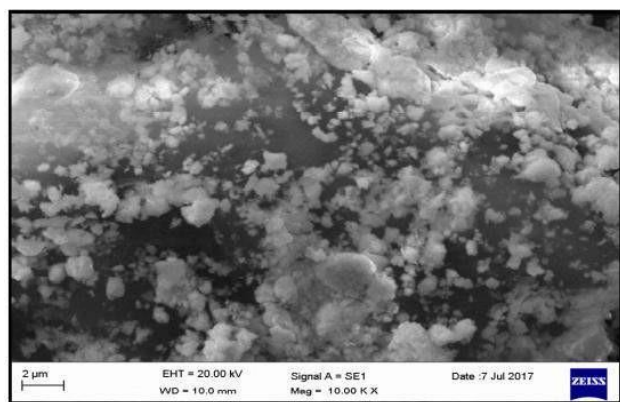


Figure 6: SEM of Rajatha bhasma at Mag 10 kx

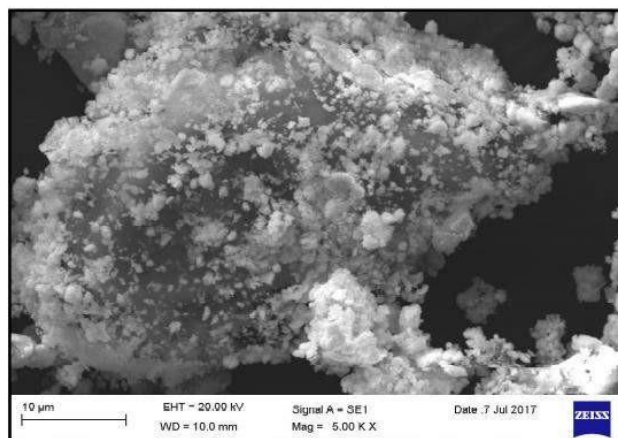


Figure 7: SEM of Rajatha bhasma at Mag 5 kx

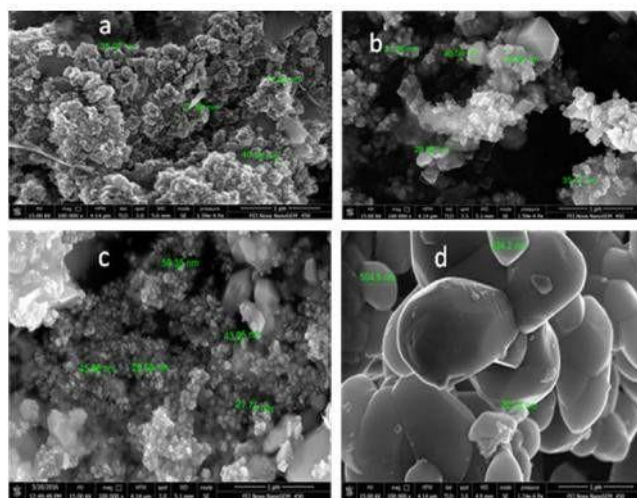
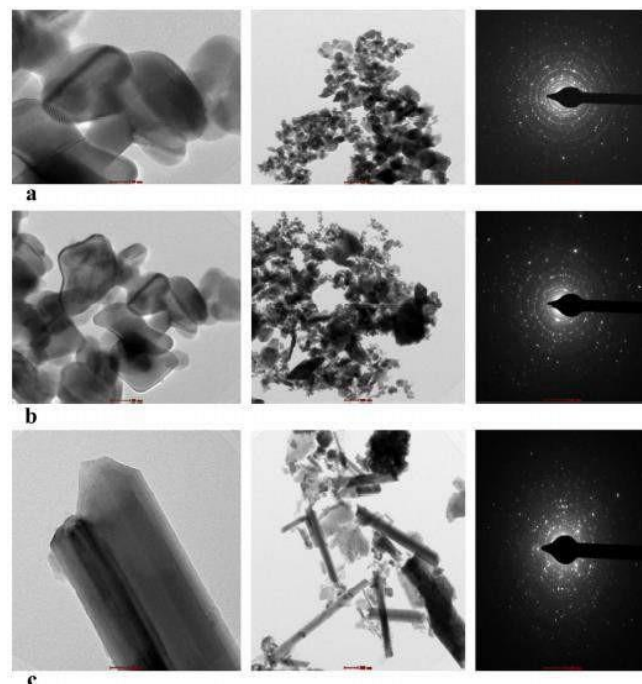


Figure 8 SEM of Vanga bhasma



d. Transmission Electron Microscopy:

In this microscopic technique, an image is formed when a beam of electrons is transmitted through an ultra-thin specimen and gets interacted with the electrons transmitted through the specimen. The imaging device magnifies and focuses on the image. The magnifying device contains layers such as fluorescent screen, on a layer of photographic film⁵⁶.

The TEM images of vanga bhasma are presented in **Fig 9**²⁷

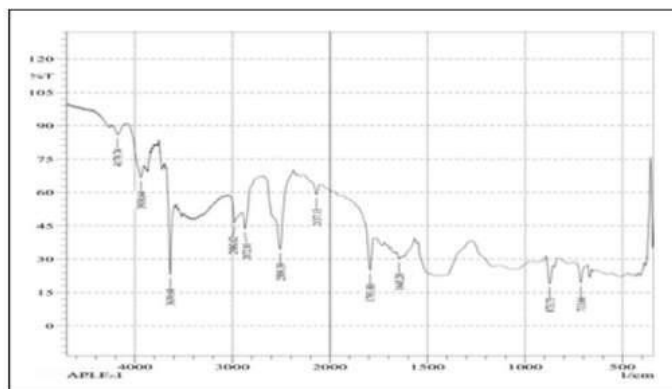
- a. TEM image after maran with the traditional method of heating

- b. TEM image after maran with electric muffle furnace

- c. TEM image of commercial Vanga Bhasma

e. Fourier Transform Infrared:

FTIR technique is based on the simple fact that the molecules in the chemical substance get vibrated and give rise to close-packed absorption bands known as IR absorption spectrum which gets extended over a wavelength range and various bands in the IR Spectrum will get corresponded to the functional groups of the chemical structure of our specimen or sample⁵⁷. It is also used to establish the unknown structure of a compound and functional group analysis⁵⁸.



The FTIR image of Muktha bhasma is presented in **Fig 10**²⁸.

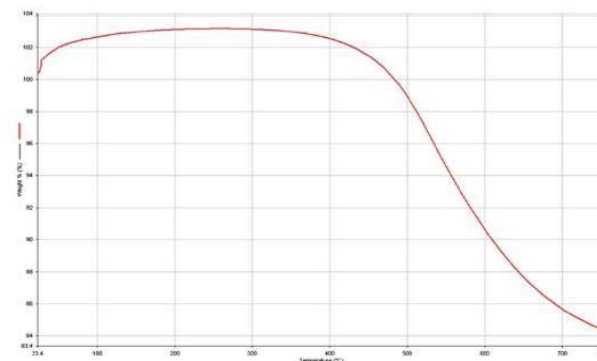
f. Thermo Gravimetric Analysis:

TGA Technique records the change in the weight of the substance when the substance is allowed for the heated or cooled environment at a controlled rate and recorded as the function of time or temperature. Plotting a graph between the changes in weight versus temperature of time are represented in the results. It is used in the testing of purity of samples and referred to as TGA curve⁵⁷.

The TGA of Rajatha bhasma is presented in the following **Fig 11**⁵⁹.

intervals is known as phased spot test. For Quality assessment of bhasmas as per their standards, this article is helpful⁶⁰.

NPST of Muktha Bhasma is mentioned in the following **Fig 12**²⁸.



e. Nimburi Phased Spot Test:

This test includes the appearance of a spot from a clear solution of a substance is placed on any of the chemically reacting papers like potassium ferrocyanide paper and by the study of this spot with the series of the pattern at three successive phases spreading over with three different



NPST at 1stPhase

NPST at 2ndPhase

NPST at 3rd Phase

These studies will provide knowledge regarding their mechanism of action and for the safety behind the use of bhasmas.

Conclusion²¹:

Ayurveda constituting Herbo-mineral formulations of bhasma as ingredients are as superior to yesterday, in view of increasing demand for the use of bhasma, standardization of their raw material, preparation process, and the end product is need to be done. In spite of being as complex materials, both ancient and advanced methods for standardization of bhasmas will assure in building confidence in the use of such products for medicinal purposes by ensuring safety, efficacy, and batch to batch uniformity of the product. Additional requirements are needed to develop characterization and standardization of bhasmas by comparing them with nano preparations of powdered dosage forms to emphasis and enhance the usage of bhasmas. In this review, the attempt has been made to enhance the bhasmas importance step forward.

Acknowledgement:

The authors would like to thank Sanjoy Kumar Pal, school of Animal & Range Sciences, Haramaya University, Dire Dawa, Ethiopia, Avani Parrek and Nitu Bhatnaga Department of Chemistry, Manipal University Jaipur and SirishaMukkavalli Biomedical Engineering and Biotechnology Program, University of Massachusetts Dartmouth, Dartmouth, MA 02747, USA. In addition authors would like to thank Department of Pharmaceutical Analysis and the Project Director, Shri Vishnu College of Pharmacy-Andhrapradesh.

References:

- Jie Liu, Feng Zhang, Velagapudi Ravikanth, Olumayokun A. Olajide, Cen li, and Li-Xin Wei. Chemical Compositions of Metals in Bhasmas and Tibetan Zuotai are a Major Determinant of Their Therapeutic Effects and Toxicity. Evidence-based complementary and Alternative Medicine, 2019; 4:1-13. <https://doi.org/10.1155/2019/1697804>
- Sanjoy Kumar Pal. The Ayurvedic Bhasma: The Ancient Science of Nanomedicine. Recent Patents on Nanomedicine, 2015; 5(1). <https://doi.org/10.2174/1877912305666150417233945>
- Singh RK, Kumar S, Aman AK, Karim SM, Kumar S, Manoranjan Kar. Study on physical properties of Ayurvedic nanocrystalline Tamra Bhasma by employing modern scientific tools. Journal of Ayurveda and Integrative Medicine, 2019; 10(2):88-93. <https://doi.org/10.1016/j.jaim.2017.06.012>
- Pal D, Sahu CK, Haldar A, Bhasma: The ancient Indian nanomedicine. Journal of Advanced Pharmaceutical Technology and Research, 2014; 5:4-12. <https://doi.org/10.4103/2231-4040.126980>
- Thakur RS, Gupta LN, Kumar N. Standard manufacturing procedure of Teekshna Lauha Bhasma. Journal of Ayurveda and Integrative Medicine, 2016; 7:100e8. <https://doi.org/10.1016/j.jaim.2015.08.003>
- Manikantan N. Ayurveda simplified body-mind matrix. Sri Sri Publication Trust; 2012.
- Vadhre GP, Pathan AR, Singhai AK. Characterization of indigenous Traditional medicine Muktashukti Bhasma. Indian Journal of Traditional Knowledge, 2013; 12(3):483-488.
- Tripathi YB, Singh VP, Sharma GMK, Sinha RK, Singh D. X-rays diffraction and microscopic analysis of tamra bhasma: An Ayurvedic metallic preparation. Indian Journal of Traditional Knowledge, 2003; 2:107-117.
- Wadekar MP, Patel RK, Preparation and characterization of a copper based Indian traditional drug: Tamra bhasma, Journal of Pharmaceutical and Biomedical Analysis, 2005; 39:951-955. <https://doi.org/10.1016/j.jpba.2005.06.015>
- Rasheed A, Naik M, Pillanayil K, Haneefa M, Pillai R, Kumar A, Azeem AK. Formulation, Characterization and comparative evaluation of Trivanga bhasma: A herbo-mineral Indian traditional medicine. Pakistan journal of pharmaceutical sciences, 2014; 27(4):793-800.
- Tripathi YB, Singh VP, Role of Tamra bhasma, an Ayurvedic preparation, in the management of lipid peroxidation in liver of albino rats, Indian Journal of Experimental Biology, 1996; 34:66-70.
- Conde J, Dias JT, Grazú V, Moros M, Baptista PV, de la Fuente JM. Revisiting 30 years of biofunctionalization and surface chemistry of inorganic nanoparticles for nanomedicine. Frontiers in Chemistry, 2014; 2:48. <https://doi.org/10.3389/fchem.2014.00048>
- Ouvinha de Oliveira R, de Santa Maria LC, Barratt G. Nanomedicine and its applications to the treatment of prostate cancer. Annales Pharmaceutiques Francaises-journal-Elsevier 2014; 72 (5):303-316. <https://doi.org/10.1016/j.pharma.2014.04.006>
- Benet L, Chi.Y.W, Screening method for the identification of bioenhancers through the inhibition of p-glycoprotein transport in the gut of a mammal. Patent US 5567592A, 1994.
- Atal N, Bedi K, Bioenhancers: revolutionary concept to market, Journal of Ayurveda Integrative Medicine, 2010; 1-96. <https://doi.org/10.4103/0975-9476.65073>
- Kesarwani. K, Gupta. R, Bioavailability enhancers of herbal origin: an overview, Asian Pacific Journal of Tropical Biomedicine, 2013; 3(4):253-266. [https://doi.org/10.1016/S2221-1691\(13\)60060-X](https://doi.org/10.1016/S2221-1691(13)60060-X)
- Berra. J.L, Molho. R. Ayurveda in Argentina and other Latin American countries. Journal of Ayurveda Integrative Medicine, 2010; 1(3):223-224. <https://doi.org/10.4103/0975-9476.72614>
- Upadhyay. D, Ayurveda takes firm roots in Russia. Russia and India report May 23. Available from, 2013.
- Singh SK, Gautam DN, Kumar M, Rai SB. Synthesis, characterization and histopathological study of lead based Indian traditional drug: Naga bhasma. Indian Journal of Pharmaceutical Science, 2010; 72(1):24e30. <https://doi.org/10.4103/0250-474X.62232>
- Sagar Bhanu PS, Zafar R, Panwar R, Herbal drug standardization, The Indian Pharmacist, 2005; 4(35):19-22.
- Vakte M, Pawar S, Pande V. Standardization of Ancient Nanomedicine: Bhasma, Researchgate, 2015.
- Mishra A, Mishra AK, Tiwari OP, Jha S. In-house preparation and characterization of an Ayurvedic bhasma: Praval bhasma. Journal of Ayurveda Integrative Medicine, 2014; 12:52-58. [https://doi.org/10.1016/S2095-4964\(14\)60005-4](https://doi.org/10.1016/S2095-4964(14)60005-4)
- Pal D, Sahu CK, Haldar A. Bhasma: the ancient Indian nanomedicine. Journal of Advanced Pharmaceutical Technology and Research. 2014; 5(1):4e12. <https://doi.org/10.4103/2231-4040.126980>
- Chaudhari YC, Nariya MB, Galib R, Prajapati PK. Acute and sub chronic toxicity study of Tamra Bhasma (incinerated copper) prepared with and without amritikarana. Journal of Ayurveda Integrative Medicine, 2016; 7:23e9. <https://doi.org/10.1016/j.jaim.2015.11.001>
- Khedekar SB, Patgiri B, Prajapati PK. Pharmaceutical Standardization of Swarna Bhasma (Incinerated Gold) by Adopting Traditional Method. Annals Ayurvedic Medicine, 2015; 4(3-4):83-94.
- Chaudhary A. Ayurvedic bhasma: nanomedicine of ancient India its global contemporary prospective. Journal of Biomedical Nanotechnology, 2011; 7(2):68e9. <https://doi.org/10.1166/jbn.2011.1205>
- Kale B, Rajurkar N, Synthesis and characterization of Vanga bhasma. Journal of Ayurveda and Integrative medicine, 2019; 10(2):111-118. <https://doi.org/10.1016/j.jaim.2017.05.003>

28. Utikar. M.C, Pentewar. R.S, Thonte. S.S., Bhosale. P.H and Narhare. M.T. Standardization, Formulation Development and Characterization of Antiulcer Drug: Mukta Bhasma. International Journal of Pharmaceutical Sciences and Research, 2019; 11(1):183-191.
29. Rajput DS, Gokarn RA, Shukla VJ, Patgiri BJ. Pharmaceutical Standardization of Naga Bhasma (Incinerated Lead) Prepared By Using Herbal Media. Ayurpharma- International Journal of Ayurveda and Allied Sciences, 2013; 2(7):212- 223. <https://doi.org/10.4103/2278-344X.126691>
30. Pareek A, Bhatnagar N. Revisiting Ancient Therapeutic Potential of Ayurvedic Bhasma. International Journal of Pharmaceutical Sciences and Research, 2018; 5: 3150- 3165.
31. Kumar A, Nair A G C, Reddy A V R & Garg A N, Unique ayurvedic metallic-herbal preparations- chemical characterization, Biol Trace Element Res, 2007; 109: 231-254. <https://doi.org/10.1385/BTER:109:3:231>
32. Bondar SS, Wododkar. D.S, Gupta. S.L. A Critical Review on Standardization of Bhasma: Ayurvedic and Modern View. World journal of Pharmacy and Pharmaceutical Sciences, 2019; 8(4):261-274.
33. Prakash VB, Prakash S, Sharma R, Pal SK. Sustainable effect of Ayurvedic formulations in the treatment of nutritional anaemia in adolescent students. J Altern Complement Med 2010; 16 (2):205- 211. <https://doi.org/10.1089/acm.2008.0573>
34. Panda G, Mohapatra KB. Clinical effect of Kukkutanda Twak Bhasma in the management of Swetapradara. AYU 2011; 32 (3):370-374. <https://doi.org/10.4103/0974-8520.93917>
35. Das S, Das MC, Paul R. Swarna Bhasma in cancer: A prospective clinical study. AYU 2012; 33:365-7. <https://doi.org/10.4103/0974-8520.108823>
36. Pal SK. A review on an Ayurvedic approach for cancer treatment developed by Vaidya Balendu Prakash. IJIMS 2014; 1 (6):1-11.
37. Prakash VB, Parikh PV, Pal SK. Herbo-mineral Ayurvedic treatment in a high risk acute promyelocytic leukemia patient with second relapse: 12 years follow up. J Ayurveda Integr Med 2010; 1(3):215-8. <https://doi.org/10.4103/0975-9476.72618>
38. Sarkar. P.K, Das. S, and Prajapati P.K, "Ancient concept of metal pharmacology based on Ayurvedic literature," Ancient Science of Life, 2010; 29(4): 1.
39. Rajput. D, Patgiri. D, Galib. R, and Prajapati. P. Anti-diabetic formulations of Naga bhasma (lead calx): A brief review. Ancient Science of Life, 2013; 33(1):52. <https://doi.org/10.4103/0257-7941.134609>
40. Tubaki. B, Benni. J, Rao. N, and Prasad. U.R. Effect of Ayurveda Medications (Kasisa Bhasma and Dhatri Avaleha) on Iron Deficiency Anaemia: A Randomized Controlled Study. Ancient Science of Life, 2016; 36(1):48. <https://doi.org/10.4103/0257-7941.195406>
41. Sharma. R, Bhatt. A, and Takur. M, Physicochemical characterization and antibacterial activity of Rajata Bhasma and silver nanoparticle. AYU, 2016; 37(1):71. https://doi.org/10.4103/ayu.AYU_167_15
42. Varma S.R, Shamsia. S, Tiyyagarajan. O.S, Vidyashankar. S, and Patki. P.S, Yashada bhasma (Zinc calx) and Tankana (Borax) inhibit Propionibacterium acne and suppresses acne induced inflammation in vitro. International Journal of Cosmetic Science, 2014; 36(4):361-368. <https://doi.org/10.1111/ics.12134>
43. Singh. R K, Kuma. S, Aman. A K, Karim. M.S, Kumar. S, and Kar. M. Study on physical properties of Ayurvedic nanocrystalline Tamra Bhasma by employing modern scientific tools. Journal of Ayurveda and Integrative Medicine, 2018. <https://doi.org/10.1016/j.jaim.2017.06.012>
44. Uttara J, Vandana M. Standardization and evaluation of safety of herbo-mineral formulation Articulin® forte tablets. Res Pharmacy 2012; 2 (1):14-17.
45. Joshi U, Mhasakar V. Standardization and evaluation of safety of herbomineral formulation Articulin® forte tablets. Res Pharmacy 2012; 2 (1):14-17.
46. Shrikumar S, Maheshwari U, Sughanti A, Ravi TK. WHO guidelines for herbal drug standardization, 2006.
47. Shinde. Application of Quality Control Principles to Herbal Drugs. International Journal of Phytomedicine, 2009; 1:4-8. <https://doi.org/10.5138/ijpm.2009.0975.0185.05786>
48. Mahajan M, Khurana RK, Singh H, et al. An overview of current applications of nanotechnology in biomedical research: A patent Survey. Recent Pat Nanomed 2014; 4: 46-56. <https://doi.org/10.2174/1877912304666140722181100>
49. Arun Sudha. Standardization of Metal-Based Herbal Medicines. American Journal of Infectious Diseases, 2009; 5(3): 193-199. <https://doi.org/10.3844/ajidsp.2009.193.199>
50. Prashanta Kumar Sarkar, Anand Kumar Chaudhary. Ayurvedic Bhasma: The most ancient application of nanomedicine. J Sci Ind Res, 2010; 69: 901-905.
51. Senthil Kumar. C, moorti. C, Prabu PC, Benoto Jonson B, Venkatnarayan R. Standardization of anti-arthritis herbo-mineral preparation. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2011; 2(3):679.
52. Arun Rasheed, Anvesh Marri, Madhu Naik. M. Standardization of Bhasma-importance and prospects. Journal of Pharmacy Research, 2011; 4(6):1931-1933.
53. Jadhao Ujwala Ashokrao, Ingole Rajesh Kundlikrao. Standardization of saptamruta loha. Int Res J Pharm, 2014; 5(10):773-777. <https://doi.org/10.7897/2230-8407.0510157>
54. Sharma. B K. Instrumental methods of chemical analysis. Ed 21st. Goel Publishing House; Meerut, 2002; pp. 252-356.
55. Durga Bhavani M, Mahesh Raju, Sridurga. CH, Venkata Subbaiah K. Analytical Standardization of Rajita Bhasma. International Journal of Research in AYUSH and Pharmaceutical Sciences, 2018; 2(3):229-238.
56. Douglas A Skoog, Stanley R Crouch, F James Holler. Principles of Instrumental analysis. Cengage Learning India Pvt. Ltd. New Delhi, 2007; pp. 668-674.
57. Gurdeep R Chatwal, Sham K Anand. Instrumental methods of chemical analysis (analytical chemistry) Himalaya publishing house, Delhi, 2007; pp. 2.702-2.720.
58. Sharma. Y R. Organic Spectroscopy, principles and chemical applications. S Chand and company ltd. New Delhi, 2007; pp. 68-154.
59. Sirisha Mukkavalli, Vijay Chalivendrab, Bal Ram Singh. Physico-chemical analysis of herbally prepared silver nanoparticles and its potential as a drug bioenhancer. OpenNano, 2017; 2:19-27. <https://doi.org/10.1016/j.onano.2017.01.001>
60. Rajendraprasad M L, Shruti Shekhar, Subramanya A R. Pharmaceutical and analytical study on loha bhasma. Int J Ayurvedic Medicine, 2010; 1(1): 47-59. <https://doi.org/10.47552/ijam.v1i1.3>