A COMPARATIVE PHARMACEUTICO-PHARMACOLOGICAL STUDY OF *LAUHA BHASMA* PREPARED BY *PUTAPAKA* AND *TRIVIDHAPAKA*

Thesis Submitted for the Award of the degree of

DOCTOR OF PHILOSOPHY

in

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DECLARATION

I, hereby declared that the presented work in the thesis entitled "**A Comparative Pharmaceutico-Pharmacological Study of *Lauha Bhasma* Prepared by *Putapaka* and *Trividhapaka*** " in the fulfilment of degree of **Doctor of Philosophy (PhD.)**is outcome of research work carried out by me under the supervision of **Dr Manish Vyas,** working as **Professor,** in the **Department of Ayurvedic Pharmacy, School of Pharmaceutical Sciences, Lovely Professional University, Punjab, India**. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D thesis entitled "**A Comparative Pharmaceutico-Pharmacological Study of *Lauha Bhasma* Prepared by *Putapaka* and *Trividhapaka***" submitted in the fulfillment of the requirement for the award of the degree of **Doctor of Philosophy (Ph.D.)** in the School of **Pharmaceutical Sciences**, is a research work carried out by **Isha Agrawal** under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

Introduction

Lauha Bhasma, a traditional Ayurvedic herbo-mineral preparation, has been widely used for its therapeutic benefits, particularly in treating anemia and related disorders. Despite its historical and clinical significance, there was a lack of standardized preparation methods documented in classical texts. This inconsistency in preparation methods posed challenges in achieving consistent therapeutic outcomes. The literature review revealed that studies focusing on the standardization and characterization of *Lauha Bhasma* were scarce, with limited information on the heating patterns and their impact on the final product. This study aimed to address these gaps by comparing two commonly used preparation methods—*Putapaka* and *Trividpaka*—to establish a standardized process that ensures high-quality and therapeutically effective *Lauha Bhasma*. The objective included were Authentication and standardization of raw materials used for the preparation of *Lauha Bhasma,* development of a standard manufacturing process for *Lauha Bhasma* using *Putapaka* and *Trividpaka* methods, characterization of the prepared *Lauha Bhasma* to evaluate its physico-chemical properties, pharmacological evaluation of the hematinic activity of the prepared *Lauha Bhasma*.

Materials and Methods

Raw Material Authentication and Standardization:

Selection of Raw Materials: High-quality raw materials were sourced from reputed suppliers and authenticated using classical and modern techniques Raw materials were subjected to various tests such as organoleptic evaluation, physico-chemical analysis, and spectroscopic techniques to ensure their purity and quality.

Preparation of *Lauha Bhasma*:

Putapaka Method: The traditional *Putapaka* method involved multiple cycles of calcination (*Putas*) using specific fuels and conditions as described in classical texts.

Trividpaka Method: The *Trividpaka* method involved three distinct phases of heating with controlled temperatures and durations to achieve a finely divided, therapeutically active *Bhasma*. Both methods were meticulously documented, including details on the heating patterns, temperature control, and duration of each phase.

Characterization of Prepared *Lauha Bhasma*: Physico-Chemical Analysis: The prepared *Bhasma* was analyzed for loss on drying, ash value, acid-insoluble ash, and water-soluble ash. Particle Size Analysis: The particle size distribution was determined using techniques such as dynamic light scattering (DLS) and scanning electron microscopy (SEM). Elemental Composition: Energy Dispersive X-ray Analysis (EDAX) was used to quantify the elemental composition, particularly the iron content. Crystallinity and Structural Analysis: X-ray Diffraction (XRD) was employed to assess the crystallinity and phase purity of the *Bhasma*. Functional Group Analysis: Fourier Transform Infrared (FTIR) Spectroscopy was conducted to identify the functional groups and their chemical environment. Thermal Stability: Thermogravimetric Analysis (TGA) was used to evaluate the thermal stability and compositional integrity of the *Bhasma* under various conditions.

Pharmacological Evaluation: Hematologic Studies were conducted on animal models to assess the hematinic activity of the prepared *Lauha Bhasma*. Parameters such as hemoglobin levels, red blood cell count, and other hematological indices were measured. Also, histopathological studies of spleen were conducted to check the splenic wall condition.

Expected Outcomes Establishment of a standardized protocol for the preparation of *Lauha Bhasma* using both *Putapaka* and *Trividpaka* methods.

Enhanced Characterization: Comprehensive characterization of *Lauha Bhasma* to understand the impact of different preparation methods on its physico-chemical properties and therapeutic efficacy.

Improved Therapeutic Efficacy: Identification of the method that yields the most therapeutically effective and safe *Lauha Bhasma*, based on hematinic activity and stability.

This study aimed to fill the existing knowledge gaps by providing a detailed comparison of the *Putapaka* and *Trividpaka* methods for preparing *Lauha Bhasma*. By standardizing the preparation process and characterizing the final product, this research contributed to the field of Ayurvedic pharmaceutics, ensuring consistent quality and enhanced therapeutic outcomes for *Lauha Bhasma*. The findings not only aided

practitioners in selecting the most effective preparation method but also paved the way for further research and development in Ayurvedic medicine.

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CHAPTER 1
INTRODUCTION

Chapter 1.

Introduction

Lauha Bhasma, a unique and intricate form of Ayurvedic medicine. This ancient preparation, deeply rooted in the rich traditions of Indian medicine, has been employed to address a wide range of health concerns, showcasing its remarkable versatility and therapeutic potential. *Lauha Bhasma*, with its complex metallurgical processes and unique chemical composition, holds a significant place in the realm of Ayurvedic medicine. This intricate preparation, deeply intertwined with the principles of traditional Indian healing, offers a fascinating insight into the holistic approach of Ayurveda towards healthcare. *Rasashastra*, is a branch of Ayurveda which mainly deals with the drugsof mineral origin which includes various pharmaceutical processes of *Shodhana, Marana, Jarana, Murchana, Bhavana* and other detail description of metal, minerals, poisonous herbal drugs, animal product used therapeutically in practice of *Ayurveda.*

Traditionally, metals are mostly used in preparations after transforming them into non-metallic forms called as *Bhasma. Bhasma* are unique preparations which means an ash obtained after incineration involves various herbal ingredients and heat to transform metals into nontoxic organometallic form. *Puta* plays an important role in *Bhasmikarana* of metals and minerals. For proper attainment of *Paka* of *Rasadi dhatus* accurate quantity of heat is required which should be neither less nor more.

Lauha Bhasma is traditionally indicated for a range of conditions, particularly those involving deficiencies and systemic imbalances. Anemia, Digestive Disorders, Chronic Fever, Liver Disorders, Skin Conditions, Diabetes and Related Complications, Respiratory health, Menstrual disorders, etc.

The chemical characterization of *Lauha Bhasma* has been a subject of extensive research, as scientists strive to unravel the intricate mechanisms underlying its therapeutic efficacy. Studies have revealed that *Lauha Bhasma* comprises a slightly modified magnetite structure, with a specific ratio of Fe2+ to Fe3+ ions, and an average particle size of approximately 50-100 nm[1]. This unique composition is thought to contribute to the enhanced absorption and utilization of the iron content by the human body, making *Lauha Bhasma* a potentially valuable supplement for individuals with iron deficiencies or related health concerns.

Nowadays, practitioners of Ayurveda extensively utilize metals, minerals, gemstones, and both animal- and plant-based substances for medicinal reasons. However, concerns about their purity and effectiveness often stem from the lack of attention to proper processing methods for these metallic drugs before they are incorporated into treatments.

Rasashasta has revolutionized the field of Ayurveda in current times, providing it with a new approach to healthcare. The attributes of quick efficacy, reduced dosage, extended shelf life, and pleasant taste have increased the popularity of metallic preparations among both patients and pharmaceutical suppliers. Among the different *Rasa Aushadhies* (herbal medicines), *Bhasmas* are predominantly utilized.

By observing all the factors, it has been decided to work on different methods of *Lauha Bhasma*. Two mostly and easily used methods are selected and importance wasgiven on the standardization of method, comparison between the two methods and ultimately animal study was performed to evaluate the therapeutic effectiveness of the *Bhasma*.

The most common nutritional deficiency disorder present throughout the world is iron deficiency but its prevalence is higher in the developing countries. As India is a developing country so most of the population suffer from iron deficiency anemia. Hence, this presents a problem both for the patient and physician. This disease was described many years ago by the name of *Panduroga*, even today has got its place among other diseases. The facts and observations of our ancient Acharyas stand the test of time. Iron being the best remedy for *Panduroga* (iron deficiency anemia), the very same disease has been selected for the comparative animal study of *Lauha Bhasma* with two different methods.

Various procedures have been mentioned in the classical textbook of *Ayurveda* for the *Bhasmikaran* (*Marana*) of *Lauha*. It involves many physical and chemical changes like particle size reduction, biological benefit, changes in the method of preparation etc. In the present study, comparison between two *Lauha Bhasama* one prepared *Trividpaka* and other prepared with *Putapaka* is done.

Importance of present study:

Efficacy of the drugs depends on so many criteria like raw material quality, processing techniques, standardization techniques etc. in today's era, the ancient Indian system of medicines has altered on a very large scale and the quality of the medicine is highly

affected with this. So, in this study a step was taken to standardize the operating procedure of *Lauha Bhasma* with two different methods (one from *Bhanupaka, Sthalipaka* then *Putapaka* and other method is direct *Putapaka*) and also the comparison for the efficacy and quality of the *Bhasma* was done.

CHAPTER 2

REVIEW OF LITERATURE

Chapter 2

Review of Literature

2.1. Lauha

In the *Rigveda*, one of the earliest known texts, the term "*Lauha* " is referenced in the context of rehabilitating the am*Puta*ted limb of *Visphala*, where it is referred to as "*Ayasa*." The first explicit mention of the word "*Lauha* " appears in the *Yajurveda*[2]. The initial documentation of the processing of raw *Lauha* can be found in the *Charaka Samhita*[3], dating back to the 15th century B.C. The detailed *Shodhana* (purification) process of *Lauha* is first recorded in the *Rasarnavam*[4], which also includes the earliest methods for preparing *Lauha Bhasma* (calcined iron). Additionally, *Lauha* plays a significant role in Ayurveda, where it is used to treat various ailments. The ancient Ayurvedic text *Bhavaprakasha* details the medicinal applications of *Lauha Bhasma* in addressing conditions such as anemia, jaundice, and other ailments concerning the blood, digestive, and metabolic systems[5]. The *Rasatarangini*, another seminal text on *Rasashastra*, provides detailed instructions on the preparation of *Lauha Bhasma*, emphasizing the importance of the *Shodhana* (purification) and healing *Marana* (incineration) processes to ensure the safety and efficacy of the final product[6].

2.1.1. Ores of *Lauha* in *Rasa Dravyas*:

Abhraka, *Makshika*, *Vimala*, *Gairika*, *Kasisa* are the major Ores of *Lauha* in ayurveda, apart from this a diverse range of ores and mineral sources of iron are utilized in Ayurveda for the preparation of *Lauha Bhasma*. These include *Shukti Lauha* (iron ore), *Samanya Lauha* (wrought iron), and *Naga Lauha* (cast iron)[7]

Shukti Lauha, which refers to iron-rich sedimentary rocks and deposits, is considered a high-quality source of iron for *Lauha Bhasma* preparation. *Samanya Lauha*, on the other hand, encompasses a broader range of iron-containing minerals and ores, including those found in igneous and metamorphic rocks. *Naga Lauha*, a rare and highly prized form of iron, is believed to possess exceptional therapeutic properties and is used in the preparation of specialized *Lauha Bhasma* formulations.

2.1.2. *Lauha* Extraction (*Sattwapatana*)[8]:

According to ancient texts, the ore is mixed with *Tankana* and vegetable-based substances to create a paste. This mixture is then spread on the inner surface of a *Musha* (crucible). Afterward, high heat is used to fully extract the metal. The traditional

metallurgical process demonstrates the advanced methods practiced in early Indian alchemy. *Tankana*, also called borax, serves as a flux in this procedure by assisting in reducing and purifying the metal. It plays a crucial role in facilitating smelting and improving workability of metals through its fluxing action[9]

2.1.3. Synonyms[10]:

- According to Origin: *Romilasthi, Suram*
- According to Occurrence: *Parvatam, GiriSara*
- According to Structure: *Lauha Sara, Pindam, Teekshna*
- According to Colour: *KalAyasa*, *KrishnaLauha*
- According to Action: *Suraksana*, *Vishaprasuna*, *Ajaram*, *Rudhiram*
- Others: *AyasKanta*, *Uttama*, *Suryam*, *Vahnidam,* etc

2.1.4. Vernacular Names[11]:

Assamese: *Lohalo*

Bengali*: Loha*

English: Iron

Gujrati: *Lodhan*

Hindi: *Loha*

Kannada: *Kabbina*

Kashmiri: *Shastur*

Malayalam: *Irumbu*

Marathi: *Lokhanad*

Oriya: *Luha*

Tamil: *Irumbu*

Telugu: *Demmu, Chumu*

Urdu: *Ain, Loha*

2.1.5. Philisophical origin of *Lauha*:

Lauha is said to have originated from the blood of the Lomils, a type of demon, during the cataclysmic war between the deities and the demons. This mythological account highlights the ancient belief in the divine and mystical origins of metals, imbuing them with significant cultural and spiritual importance.

In addition to its mythological roots, the historical and practical applications of *Lauha* were extensive in ancient Indian society. *Lauha* was not only utilized for crafting tools

and weapons but also held a pivotal role in traditional medicine. Ancient texts like the *Charaka Samhita* and *Sushruta Samhita* extensively document the use of *Lauha* in various Ayurvedic treatments. *Lauha Bhasma*, a calcined iron preparation, was particularly valued for its purported health benefits, including enhancing vitality and treating iron deficiency.

2.1.6. Properties[12]:

Table 2.1. *Rasa Panchak* of Iron

Rasa	*Tiktha, Madhura, Kashaya*
Guna	*Ruksha, Guru*
Virya	*Sheeta*
Vipaka	*Madhura*
Doshaghnata	*Kaphapittasamana*

2.1.7. Classification of *Lauha* [13]:

Lauha has been broadly classified into three categories:

- *Munda Lauha*
- *Tikshna Lauha*
- *Kanta Lauha*

2.1.7.1. *Munda Lauha*:

Munda Lauha is further classified into three categories: -

a) *Mridu Munda Lauha*: It is of the best quality. Does not break easily, has glossy surface and melts quickly.

b) *Kuntha Munda Lauha*: Does not expand easily on hammering.

c) *Kadara Munda Lauha*: It is brittle and possesses black surface

2.1.7.2. *Tikshna Lauha*:

a) *Khara Tikshna Lauha*: It is rough and free from hairy lines. It breaks easily on bending and gives silvery luster.

b) *Sara Tikshna Lauha*: It breaks easily by the sides on hammering, has hairy lines, it is originated from pale soil.

c) *Hrinnal Tikshna Lauha*: It is blackish white in color and has beak like hairy lines. It is very difficult to break.

d) *Taravatta Tikshna Lauha*: It is sky color, glossy and shows clear hairy lines on the surface. "T*aravatta*" suggests a particular quality or method of treatment that distinguishes it from other types of iron.

e) *Vajeera Tiskhna Lauha*: It is smooth, glossy and having very fine, clear hairy lines on the surface. It is blue in color and does not rust.

f) *Kala Tikshna Lauha*: The term "*Tikshna Lauha* " translates to "sharp iron," and "*Kala*" denotes its dark or blackened appearance, often resulting from specific forging and treatment processes. It is bluish black in color, heavy, shiny and does not break easily on hammering. It is considered as best *Tikshna Lauha*.

2.1.7.3. *Kanta Lauha*:

a) *Bhramaka KanthaLauha*: It can only move the kinds of iron on attraction.

b) *Chumbaka KanthaLauha*: It is having magnetic like effect, attracts the iron scraps and catch them.

c) *Karshaka KanthaLauha*: It can attract and catch iron pieces.

d) *Dravaka KanthaLauha*: It attracts small pieces of iron and catch them on its surface. It is considered as best *Kantha Lauha*

e) *Romaka Kantha Lauha*: On breaking it attracts its small pieces.

2.1.8. *Shodhana* of *Lauha* [1415]:

To make *Lauha* free from all impurities (*Doshas*), both *Samanya* (general) and *Vishesha* (specific) *Shodhana* methods must be followed. The primary technique for *Lauha Shodhana* is *Nirvapa*, which involves heating the iron until it is red hot and then quenching it in a liquid medium. Commonly, *Triphala Kwatha* is used as this liquid medium due to its potent detoxifying properties[16]. The preparation of *Triphala Kwatha* requires precise steps: 16 Pala of *Triphala* is mixed with eight times its volume in water and then boiled until the liquid is reduced to one-fourth of its original volume. This concentrated decoction is used specifically for the *Shodhana* of 5 Pala of *Lauha* [17]. The process of *Nirvapa* involves repeatedly heating the *Lauha* until red hot and quenching it in the *Triphala Kwatha*, which helps in removing impurities and enhances the metal's beneficial properties.

2.1.8.1. Samanya Shodhana

Samanya Shodhana is a general purification method used for all metals. In this process, the metal is heated until it reaches a red-hot stage and then quenched seven times in

each of the following liquid media: *Til Tail* (sesame oil), *Takra* (buttermilk), *Gomutra* (cow urine), *Arna*l (sour gruel), and *Kulattha Kwatha* (decoction of horse gram). This sequence ensures thorough purification and removal of impurities from the metal[18].

2.1.8.2. Vishesh Shodhana

Vishesh Shodhana is a specialized purification method tailored for a specific metal. This process is typically performed after *Samanya Shodhana* but can also be conducted independently based on the requirements of the purification process. *Vishesh Shodhana* focuses on the unique properties and impurities associated with a particular metal, ensuring its utmost purity and suitability for various applications, especially in Ayurveda[19].

Table2.2. Process and media for the *Vishesh Shodhana* of *Lauha*

No. of Process	Drug and Media	Procedure	Repetition	Reference
1	*Swarasa of Guduchi Hamsapadi, Naktamala, Triphala, Gopalaka, GoRasana, Tumburu Phala*	*Dhalana*	-	*Rasarnavam*
2	*Triphala Jala*	*Dhalana*	-	*Chakraduttah*
3	*Shasha Rakta*	*Lepana and Paritapana*	3 3 7	*Rasendra Chudamani* *Rasa Prakasha* *Sudhakara Rasa* *Ratna Samucchaya*
4	*Saindhava lavana and Triphala Kwatha*	*Lepana and Paritapana*	-	*Rasendra Chudamani* *Rasa Prakasha* *SudhakaraRasa* *Ratna Samucchaya*
5	*Chincha*	*Nirvapa*	-	*Rasendra*

	Phaladala Kwatha			*Chudamani Rasa Ratna Samucchaya*
6	*Triphala Kwatha*	*Nirvapa*	7 7 7 7	*Rasendra Chudamani Rasa Ratna Samucchaya Rasendra Sara Samgraha Ayurveda Prakasha Rasatarangini*
7	*Gomutra*	*Nirvapa*	-	*Rasendra Chudamani*
8	*Sasha Rakta*	*Lepana And Nirvapa*	-	*Ayurved Prakash*
9	*Chincha Patra Swarasa/ Kwatha*	*Nirvapa*	7	*Rasa Ratna Sammuchaya*
10	*Triphala Kwatha In Gomutra*	*Nirvapa*	7	*Rasa Ratna Sammuchaya*

2.1.9. *Lauha Marana*[20]

The *Marana* process is an important procedure in Ayurveda used to convert *Lauha* (iron) into *Bhasma* (a fine powder form). This process, known as *Bhasmikarana*, involves several methods described in classical texts. The primary aim is to make the metal bioavailable and suitable for therapeutic use.

2.1.9.1. *Parada Marita*

Parada Marita involves the use of *Parada* (mercury) and its compounds, such as *Kajjali, Rasasindura*, and *Hingula*, in the *Marana* process to convert metals into *Bhasma*. *Parada* is highly valued for this process due to its exceptional ability to break down particle sizes, facilitating the transformation of the metal into a fine, bioavailable powder. *Parada* possesses unique properties, including *Yogavahi* (enhancing the properties of combined substances), *Sukshmashrotogamitva* (penetrating the smallest bodily channels), and *Jaramrutunashaka* (anti-aging and rejuvenating effects). These properties are imparted to the metal during the *Marana* process, making *Parada Marit Bhasma* particularly potent and effective. The process involves mixing the purified

metal with *Parada* or its compounds, followed by repeated cycles of trituration and controlled heating.

2.1.9.2. *Vanaspati* (herbal drug) *Marita*

Vanaspati (herbal drug) *Marita* involves using herbal drugs in the *Marana* process, which is considered moderately effective. Herbal drugs, which are typically acidic (*Amliya*) and alkaline (*Kshariya*) in nature, aid in the incineration of metals to produce *Bhasma*. During the *Marana* process, these herbal materials interact with the metal, facilitating its transformation. However, some residues from the herbal drugs may remain in the final product, potentially influencing the therapeutic effects of the main drug.

2.1.9.3. *Gandhaka Marita*:

The process includes the use of *Gandhaka* (sulfur) and its compounds, such as *Haratala* (orpiment) and *Manahshila* (realgar), which are toxic in nature. Consequently, this method is considered to fall into the *Kanishtha* (low) category.

2.1.9.4. Ari *Lauha* Marita:

These substances are anti-metals and impart undesirable qualities to the *Bhasma*, making them unsuitable for use in the incineration process[21].

2.1.10. Methods of *Lauha* Marana:

Summary of different methods mentioned in the classical textbook of the *Marana* of *Lauha.* Methods are listed below in Table 2.3.

Table 2.3. Methods of *Marana* of *Lauha*

Sr. No.	Drug And Media for *Bhavana*	Procedure	No. Of *Bhavana*	References
1.	*Hingula + Stanya*	*Putapaka*	-	*Rasarnava*
2.	*Triphala Kwath*	*Bhanupaka*	7	*RT, RSS*
3.	*Triphala Kwath/Hastikarna /Satamulika/ Bhringaraj-Rasa*	*Sthalipaka*	7	*RT, RSS*
4.	*Triphala Kwath*	*Putapaka*	10 -1000	*RT, RSS*
5.	*Hingula* And *Jambira Rasa*	*Nirvapa*	-	*RSS*
6.	*Triphala Kwath* Prepared by *Gomutra*	*Putapaka*	21	*RSS*
7.	*Triphala Kwath*	*Putapaka*	4	*RSS*

8.	*Oudan* And *Triphala Kwath*	*Putapaka*	5	*RSS*
9.	*Guda, Gandhaka* and *TriphalaKwath*	*Putapaka*	20-30	*RSS*
10.	*Gandhaka* And *Kumari Vari*	*Niragnipaka*	-	*RSS*
11.	*Suta* And *Gandhaka*	*Putapaka*	20	*RSS*
12.	*Hingula-1* Part and *Nari Stanya* and *Hingula-1/20* Part and *Triphala Kashaya, Jambira Rasa* and *Arnal*	*Puapaka*	2+38	*RSS*
13.	*Parada Bhasma-* ¼ Part or *Makshika / Gandhaka / Parada - 1 Part, Shasha Rakta, Kshar, AmlaDravya*	*Puapaka*	-	*RSS*
14.	*Suddha Parada* – 1 Part *Gandhaka* - 2 Part and *Kumari Swarasa*	*Niragni Paka*	3 days	*RSS, AP*
15.	*Makshika Bhasma-* ¼ Part,*Nimbuka Vara*	*Putapaka*	3	*RT*

2.1.11. *Amritikarana* of *Lauha* [22]:

Amritikarana is a process used to eliminate any remaining *Doshas* (impurities) in *Bhasma* after the *Marana* process. This purification step is also applied to *Lauha Bhasma* to remove any residual impurities and enhance its therapeutic properties. In the *Amritikarana* process, *Lauha Bhasma* is mixed with an equal or double quantity of *Triphala Kwatha* and cooked over mild heat. This gentle heating ensures that the *Bhasma* is thoroughly purified, and any remaining impurities are eradicated.

To prepare *Lauha Bhasma*, equal amounts of *Lauha Bhasma*, *Goghrut* (cow ghee), and *Gandhak* (sulfur) are taken and mixed in a *Khalva Yantra* (a traditional grinding apparatus). The mixture is then subjected to *Bhavana* with *Kumari Swaras* (aloe vera juice), where it is triturated until it dries completely. Once dried, the resultant *Churna* (powder) is placed in a *Sarav* (earthen container), and the process of *Samutikaran* (sealing) is performed to ensure that impurities can not occur[23].

Following this, the sealed container is subjected to *GajaPuta* (a specific high-temperature heating method). This method involves placing the container in a large pit filled with cow dung cakes and igniting it to provide consistent and intense heat. This

step is crucial as it transforms the mixture into *Niruthita Bhasma*, ensuring that the final product is finely powdered, detoxified, and ready for therapeutic use[24].

2.1.12. Pharmacological action of *Lauha Bhasma* [25]:

Lauha Bhasma, a potent Ayurvedic formulation derived from iron, exhibits multifaceted pharmacological actions that underscore its therapeutic significance. As *Kantijanana,* it rejuvenates the skin, promoting a radiant complexion and overall skin health. Its *Raktajanana* property aids in the production of red blood cells and hemoglobin, addressing conditions like anemia and enhancing systemic oxygenation. *Tridoshonmulana* ensures equilibrium among *Vata, Pitta*, and *Kapha Doshas*, fostering holistic well-being and vitality. Recognized as *Vrishya*, *Lauha Bhasma* supports reproductive health, enhancing libido and fertility. Its *Rasayana* quality promotes longevity and vitality, rejuvenating body tissues and fortifying the immune system. Moreover, *Lauha Bhasma* 's *Sarva-Vyadhi-Hara* nature makes it effective against a broad spectrum of ailments, ranging from weakness and fatigue to various systemic disorders.

2.1.13. Therapeutic indications of *Lauha Bhasma* [26]:

Lauha Bhasma finds its indications in various ailments such as *Raktapitta, Kasa, Shwasa, Vali, Palita, Shula, Amlapitta, Mutrakriccha, Ekangavata, Pandu, Kamala*, *Kusta,* and more. Specific *Anupana* (adjuvant) and *Sahapana* (supportive therapy) accompany its administration for optimal therapeutic outcomes.

2.1.14. Dose[27]:

1/4th to 2 *Ratti* / day

2.1.15. Adverse effect:[28]

a. ***Lauha* Dosha:** *Guruta, Dridhata, Utkleda, Kashmala (Glani), Dahakarita, Ashmadosha* and *Durgandhata*.

b. ***Ashuddha Lauha*:** *Shandatwa, Kustha, Hridroga, Shula, Ashmari*, many other diseases even death.

2.1.16. Suitable *Lauha* for processing:

The fragments of *Tikshna Lauha* collected during the crafting of weapons such as swords, or *Lauha Patra*, which are iron leaves made by hammering, serve as suitable sources for the preparation of *Lauha Bhasma*.

2.1.17. Modern concept of *Lauha* (IRON):

Around 1500 B.C., iron was in widespread use in both India and Egypt. The renowned iron pillar located in Delhi, known for its remarkable resistance to rust, is believed to have been erected around 300 A.D., providing evidence of the advanced iron smelting techniques prevalent in ancient India. The introduction of blast furnaces for iron extraction occurred later, with Germany adopting the technology around the mid-14th century A.D., followed by Great Britain in approximately 1500 A.D. This technological advancement marked significant milestones in the history of metallurgy, transforming iron production methods and contributing to the industrial progress of these regions[29].

2.2. Physical properties:

Iron, characterized by its firmness and grayish-white hue, exhibits a high density and melting point coupled with a low atomic volume. It possesses magnetic attributes and showcases catalytic properties[30].

Table 2.4. Physical Properties of Iron

Atomic number	26
Atomic weight	55.85
Density (g./mL)	7.86
Atomic volume (mL)	7.1
Melting Point (°c)	1539
Boiling Point (°c)	2450
Red Hematite	Fe_2O_3,
Limonite	$2Fe_2O_3$, $3H_2O$
Magnetite	Fe_3O_4
Siderite	$FeCO_3$
Iron Pyrites	FeS_2

2.2.1. Extraction of iron from ores:

Extraction of Iron from its oxide and carbonate ores is done by reduction with coke in a blast furnace[31]. The process is carried out in two steps:

a. Preliminary roasting or calcination:

The ore is calcined with a small amount of coal in heaps, resulting in the conversion of ferrous oxide to ferric oxide. The roasted mass obtained from this process contains only ferric oxide, Fe_2O_3.

b. Smelting or reduction in the blast furnace:

The roasted ore is mixed with coke and limestone (flux) and charged into the blast furnace, where the ferric oxide is reduced to metallic iron.

Fe2O3 + 3C = 2Fe + 3CO.

2.2.2. Allotropic forms of iron:

Pure iron exists in three forms: α-iron, β-iron, and δ-iron. α-Iron is stable below 912°C, is soft and magnetic, and has a body-centered cubic (BCC) lattice structure. β-Iron, stable between 912°C and 1400°C, is nonmagnetic and has a face-centered cubic (FCC) lattice structure. δ-Iron, stable above 1400°C, also has a body-centered cubic lattice like α-iron but remains nonmagnetic due to the high temperature[32].

2.2.3. Chemical properties of iron[33]:

Iron is stable in dry air; it rusts in moist air. It is oxidized at a red-hot state by air or steam to ferrosol ferric oxide, Fe_3O_4.

$$3Fe + 4H_2O = Fe_3O_4 + 4H_2$$

It combines directly, when heated, with carbon, Sulphur, chlorine, yielding the carbide Fe_3C, the ferrous sulphide FeS, and the ferric chloride, $FeCl_3$ respectively.

It dissolves readily in dilute hydrochloric or sulphuric acid. It is not attacked by alkalis. Iron does not readily form any amalgam with mercury.

2.2.4. Source of iron[34]:

All animal food: Meat, liver, egg, etc. excepting milk and butter.

Vegetables: Peas, lentils, green leaves, spinach, fruits

2.2.5. Requirement of iron[35]:

The Table below describes the daily requirement and absorption of Iron in human body.

Table 2.5. Requirement of iron

		Iron Requirement (mg)	Dietary Iron(mg)	Required Absorption (%)
1	Men	0.9 (0.6-1.2)	15	6 (4-8)
2	Menstruating Women	1.3 (0.7-2.5)	10	13 (7-25)
3	Pregnant Women	2.5 (2.0-5.0)	10	25 (20-50)

2.2.6. Absorption of iron:

Iron is primarily absorbed throughout the gastrointestinal tract, with a significant amount being absorbed in the upper part of the small intestine, particularly in the duodenum[36].

2.2.7. Storage of iron:

Approximately 21.5% of the body's total iron is stored as storage iron, primarily in the form of ferritin and hemosiderin, and is found in the bone marrow, liver, and spleen[37].

2.2.8. Iron adverse effect:

Iron bound to transferrin is non-toxic. However, in cases of overdose, free iron that surpasses the binding capacity of transferrin and elevated ferritin levels can cause tissue damage and lead to the release of vasoactive substances such as serotonin and histamine[38].

2.3. *Triphala*:

Triphala, an integral part of traditional Indian medicine, is a renowned polyherbal formulation composed of three fruits: *Amalaki (Emblica officinalis), Bibhitaki (Terminalia bellirica), and Haritaki (Terminalia chebula)*. Known by various synonyms such as *Vara*, *Phalatrikam*, and *Sresthatamam*, *Triphala* is rich in antioxidants and offers a multitude of health benefits. Widely prescribed in Ayurveda, it is effective in balancing all three doshas (*Vata, Pitta*, and *Kapha*), enhancing digestive capacity, acting as a rejuvenative (*Rasayana*), and serving as an aphrodisiac (*Vrisya*). According to the Ayurvedic Formulary of India (AFI), *Triphala* is prepared by combining equal parts of the ground dry fruits, known as myrobalans. This consistent 1:1:1 ratio ensures a balanced therapeutic effect.

Triphala is a highly versatile Ayurvedic drug extensively used for a wide range of purposes due to its diverse therapeutic activities. This renowned formulation is composed of three myrobalans: *Terminalia chebula (Haritaki*), *Terminalia bellerica (Bibhitaki*), and *Emblica officinalis (Amalaki)*. As one of the most commonly used Ayurvedic preparations, *Triphala* typically consists of equal proportions of the pericarps of these three fruits[39].

The balanced combination of these fruits ensures a synergistic effect, enhancing the overall efficacy of the formulation. *Haritaki* is known for its laxative and astringent properties, *Bibhitaki* for its rejuvenative and detoxifying qualities, and *Amalaki* for its

high vitamin C content and antioxidant capabilities. This unique blend not only balances all three doshas (*Vata, Pitta*, and *Kapha*) but also supports digestive health, detoxification, and rejuvenation[40].

2.3.1. Preparation of *Triphala Kwatha*:

Type of procedure: *Kwatha* (boiling)

Equipment: Gas stove, lighter, stainless-steel vessel (2), measuring mug, clothes, spatula, weighing machine.

Ingredients: *Triphala* (coarse powder): 2 Kg, Water: 16 L

Procedure: Water was taken in a big stainless-steel vessel and coarse powder of *Triphala* was poured into the vessel. Both was mixed and boiled till the liquid part is reduced to ¼ th i.e., 4 L, it was strained by clothes and used for *Shodhana*.

Precaution: *Triphala* should be in the form of coarse powder.

2.3.2. Ayurvedic Pharmacodynamic of *Triphala*[41]:

Table 2.6 Properties of *Triphala*

Rasa	*Kasaya*
Guna	*Ruksha, Sara*
Virya	*Anusna*
Vipaka	*Madhura*
Doshaghnata	*Tridoshasamaka*
Karma	*Chaksusys, Dipana, Vrishya, Prameha, Kustha,Vishamajwarnashaka, Medohara*

2.3.3. Therapeutic uses:

Triphala is widely utilized in Ayurveda for its broad spectrum of therapeutic benefits. It acts as an effective laxative, particularly in cases of chronic constipation, and is also used for colon cleansing, aiding digestion, and improving food assimilation. Additionally, *Triphala* is beneficial in managing cardiovascular diseases, high blood pressure, reducing serum cholesterol levels, and enhancing liver function. It is effective in treating inflammation of the large intestine, ulcerative colitis, and various digestive disorders. Renowned as a rejuvenator and tonic, *Triphala* supports overall health, including hair health, digestive health, and acts as a purgative. It is also known to treat

eye diseases, heal ulcers, and address skin disorders, obesity, diabetes, blood impurities, and fever[42].

In terms of its preparation, *Triphala* is traditionally made using a ratio of 1:1:1, combining equal parts of *Terminalia chebula (Haritaki*), *Terminalia bellerica (Bibhitaki), and Emblica officinalis (Amalaki*)[43]. However, some formulations suggest varying the proportions, such as a 1:2:4 ratio, where one part of *Haritaki*, two parts of *Bibhitaki*, and four parts of *Amalaki* are mixed. This variation can be tailored to specific therapeutic needs, as each fruit contributes distinct properties: *Haritaki* is a potent detoxifier and laxative, *Bibhitaki* offers rejuvenating and detoxifying effects, while *Amalaki* is rich in antioxidants and supports immune function[44]. In Ayurvedic practice, *Triphala* is revered for its ability to balance the three *Doshas* (*Vata, Pitta*, and *Kapha*), making it a versatile and essential component of holistic health regimens[45].

It is also used as *Shodhana* and *Bhavana Dravya* in many formulations.

2.3.4. Traditional uses of *Triphala*:

In Ayurvedic practice, *Triphala* is extensively utilized for its remarkable therapeutic benefits, particularly in addressing gastric disorders such as indigestion, poor food assimilation, colon cleansing, constipation, and as a tonifier for the gastrointestinal tract and colon. It is also highly recommended for cardiovascular health, helping to manage high blood pressure, reduce serum cholesterol levels, and support overall heart function[46]. Additionally, *Triphala* is beneficial for ophthalmic issues, liver dysfunction, and inflammation and complications of the large intestine. Its use as a blood purifier is well-documented, and it is known to enhance mental faculties.

Beyond these applications, *Triphala* is reported to possess significant anti-inflammatory, analgesic, anti-arthritic, hypoglycemic, and anti-aging properties. In Ayurvedic texts, *Triphala* is often prescribed to balance the three *doshas* (*Vata, Pitta*, and *Kapha*), promoting holistic health and wellness. The formulation, which traditionally combines equal parts of *Terminalia chebula (Haritaki*), *Terminalia bellerica (Bibhitaki), and Emblica officinalis (Amalaki)*, is celebrated for its ability to detoxify and rejuvenate the body. Its antioxidant-rich composition supports immune function and protects against oxidative stress[47]. As a *Rasayana* (rejuvenative), *Triphala* not only aids in maintaining digestive health but also contributes to longevity and vitality, making it a cornerstone of Ayurvedic medicine.

The different properties and the characters of the various ingredients of the drug are as mentioned below:

2.3.5. *Haritaki*[48]:

Latin name - *Terminalia chebula Linn.*

Family - *Combretaceae*

Classical name - *Haritaki*

Sanskrit synonyms - *Haritaki, Pathya, Abhaya, Avyatha,Vayastha, Haimavati, Shiva*

Hindi name - *Harre, Harad*

English name - *Chebulic Myrobalan*

***Swaroopa* (Habit) -** A moderate sized / large deciduous tree

Habitat - Found in MP, W. Bengal, Karnataka and Maharashtra in India, Burma

Types - Seven types of namely *Vijaya, Rohini, Putana, Amrita, Abhaya, Jivanti and Chetaki*

Ayurvedic Pharmacodynamics:

***Rasa* -** *Pancharasa (Kashaya predominance, Lavan Sahita)*

***Guna* -** *Laghu, Ruksha*

***Virya* -** *Ushna*

***Vipaka* -** *Madhura*

***Prabhava* -** *Tridoshahara*

***Dosha karma* -** Mainly *Kapha Pitta Samaka*

Parts used - *Fruits*

Chemical Composition:

Fruit contains tannin up to 30 %, chebulic acid and gallic acid and some purgative constituents of the nature of Anthraquinone.

Therapeutic Uses:

The fruit is a prominent herbal drug extensively used in the Indian system of medicine and is a common component in numerous formulations. It is highly beneficial for a wide range of ailments, including asthma, sore throat, excessive thirst, vomiting, eye diseases, and conditions affecting the heart and bladder. Additionally, it is effective in treating strangury (painful urination), urinary discharges, ascites (fluid accumulation in the abdomen), biliousness (excess bile), inflammation, bleeding piles, typhoid, constipation, anemia, elephantiasis, and delirium. The ripe fruit is known for its

purgative, tonic, and carminative properties, and it strengthens the brain, eyes, and gums. Conversely, the unripe fruit is astringent and particularly useful in managing dysentery and diarrhea. In Ayurvedic practice, the fruit is highly valued for its versatility and potency. The ripe fruit's purgative properties make it an effective remedy for constipation, while its tonic qualities contribute to overall vitality and wellness. As a carminative, it helps in relieving flatulence and digestive discomfort. The unripe fruit's astringent nature makes it beneficial in treating gastrointestinal disorders, such as diarrhea and dysentery, by reducing bowel movements and inflammation. Moreover, this fruit is often incorporated into Ayurvedic formulations aimed at detoxification and rejuvenation, reflecting its broad spectrum of therapeutic applications. Its regular use is believed to enhance physical and mental health, making it a staple in traditional Ayurvedic medicine.

2.3.6. *Bhibhitaki*[49]:

Latin name - *Terminalia bellerica Roxb.*

Family - *Combretaceae*

Classical name – *Vibhitaka*

Sanskrit synonyms - *Aksha, Kaliphala, Bhutavasa,Kalidruma, Karnaphala*

Hindi name - *Bahera, Baherha*

English name - *Belleric Myrobalan*

***Swaroopa* (Habit) -** A large deciduous tree

Habitat - Throughout the deciduous forests of India and Burma

Ayurvedic Pharmacodynamics

***Rasa* -** *Kashaya*

***Guna* -** *Laghu, Ruksha*

***Virya* -** *Ushna*

***Vipaka* -** *Madhura*

***Prabhava* -** *Tridoshagna*

***Dosha Karma* -** *Kapha hara*

Parts used - Fruit

Chemical Composition:

Fruit contains 17 % tannin and Gallo-tannic acid (coloring matter) and resin. Seeds contain greenish yellow oil.

Therapeutic Uses:

The bark is highly beneficial for treating asthma and leukoderma. The fruit is known for its digestible, laxative, and anthelmintic properties, making it effective for managing bronchitis, sore throat, biliousness, inflammation, and various diseases affecting the eyes, nose, heart, and urinary bladder. The oil extracted from the fruit is an excellent application for hair care, promoting hair health and strength. Additionally, the fine powder of the fruit can be dusted on fresh cuts and wounds to arrest bleeding, serving as a potent astringent and styptic agent.

The fruit of the Beleric myrobalan, along with Emblic (*Amla*) and Chebulic (*Haritaki*) myrobalans, forms the cornerstone of the renowned Ayurvedic formulation known as *Triphala*. *Triphala* is celebrated for its extensive therapeutic properties, including its ability to balance the three *Doshas*, detoxify the body, and promote overall health. The inclusion of Beleric myrobalan in this trio enhances the formulation's effectiveness in digestive health, immune support, and rejuvenation. The fruit's diverse medicinal properties make it a valuable ingredient in a wide range of Ayurvedic treatments, addressing conditions from gastrointestinal disorders to respiratory issues and beyond. Its comprehensive benefits underscore its importance in traditional Ayurvedic medicine, contributing to the holistic approach of maintaining and restoring health.

2.3.7. *Amalaki*[50]:

Latin name - *Emblica officinalis Gartn*.

Family - *Euphorbiaceae*

Classical name - *Amalaki, Dhatri*

Hindi name - *Awala, Amla, Aonla*

Sanskrit synonyns - *Amalaki, Dhatri, Vyastha*

English name - *Indian gooseberry*

***Swaroopa* (Habit) -** A medium sized tree

Habitat - Found throughout India; often planted in gardens and cultivated also in small and large scale

Ayurvedic Pharmacodynamics:

***Rasa* -** *Pancharasa (Amla predominance and Lavana Sahita)*

***Guna* -** *Laghu, Ruksha, Sita*

***Virya* -** *Sita*

Vipaka - *Madhura*

Prabhava - *Rasayan*

Dosha Karma - *Tridoshhara, Pittasamaka* (mainly)

Parts used - Fruit

Chemical Composition:

Fruit is a well-known rich source of Vitamin C. Seeds contain fixed oil, phosphatides and an essential oil. Fruits, barks and leaves are rich in tannins.

Therapeutic Uses:

The fruits are the most valuable part of the plant, utilized medicinally in various forms to treat a wide range of diseases. Rich in Vitamin C and other essential nutrients, these fruits are among the most popular and highly esteemed remedies in the indigenous system of medicine. They are employed in the treatment of anemia, hyperacidity, peptic ulcers, dyspepsia, anorexia, diarrhea, dysentery, hemorrhage, eye inflammations, bladder irritability, leucorrhea, spermatophore issues, epistaxis (nosebleeds), menorrhagia (heavy menstrual bleeding), jaundice, weak memory, nervine debility, edema, and liver conditions.

In addition to their therapeutic uses, the juice of fresh fruit is administered as a tonic, refrigerant, and antiscorbutic. It is also valued for its diuretic, laxative, and anti-bilious properties, making it a versatile remedy for a variety of health issues. The high Vitamin C content boosts the immune system and enhances overall vitality. In Ayurveda, these fruits are often recommended for their rejuvenating and restorative effects, helping to balance the doshas and promote holistic health. Their wide application in treating digestive disorders, blood-related conditions, and inflammatory issues underscores their importance in traditional medicine. The fruits' ability to support both physical and mental health further highlights their indispensable role in maintaining overall well-being.

2.3.8. *Bhaat* (Boiled rice)

Boiled rice, or *Bhaat*, is an essential component in the Marana process of preparing *Lauha Bhasma*. The preparation involves mixing one part rice with two parts water and heating this mixture until it reaches a semi-solid consistency. This semi-solid *Bhaat* is then used in the Bhavana process, an integral step in the *Bhasmikarana* (incineration) of *Lauha*.

In Ayurvedic practice, the Bhavana process entails repeatedly grinding the substance with specific liquids to enhance its potency and ensure thorough integration of properties. In the case of *Lauha Bhasma*, using *Bhaat* for *Bhavana* helps to break down the particles, ensuring a finer and more bioavailable final product. This meticulous process is crucial as it not only aids in detoxifying the iron but also imparts additional therapeutic properties to the *Bhasma*. The semi-solid consistency of the rice mixture facilitates effective grinding and incorporation of the liquid medium, promoting the transformation of the raw metal into a highly beneficial form.

2.4. *Shodhana*[51]:

Shodhana is a critical process in Ayurvedic medicine involving the purification and detoxification of substances to eliminate physical and chemical impurities as well as toxic materials. This process ensures that the substances are safe and effective for further processing and therapeutic use[52]. *Shodhana* employs various methods, including *Swedana* (sudation or steaming) and *Mardana* (grinding or triturating), utilizing specific herbal or mineral drugs to achieve purification.

The *Shodhana* process not only eliminates contaminants but also enhances the therapeutic properties of substances. By removing physical and chemical impurities through traditional pharmaceutical techniques, the efficacy and safety of Ayurvedic preparations are significantly improved. For example, metals and minerals subjected to *Shodhana* become more bioavailable and less toxic, making them suitable for medicinal use. The detailed procedures involve treating substances with various herbal decoctions, oils, or other mediums, which break down harmful compounds and enhance beneficial properties. *Shodhana* is not limited to metals and minerals; it is also applied to herbs and other organic materials to ensure they are free from harmful effects and to potentiate their medicinal qualities. This meticulous process underscores the depth of Ayurvedic pharmacology, highlighting the importance of purification in achieving holistic health benefits. By combining these ancient techniques with modern scientific understanding, Ayurveda continues to provide safe and effective remedies for a wide range of health conditions[53].

2.4.1. Type of *Shodhana*:

Shodhana process is grossly subdivided into two major categories as follows:

• ***Samanya Shodhana*:** It is used as general procedure for *Shodhana* of all drugs of a particular group, in other words these drugs should be purified individually through the same *Shodhana* procedure. e.g., *Samanya Shodhana* of *Dhatu*.

• **Vishesh *Shodhana*:** It is used as specific procedure for particular drug material individually not for a group. It should be applied after *Samanya Shodhana*. e.g., Vishesha *Shodhana* of *Lauha* in *Triphala Kwatha.*

2.4.2. Methods of *Shodhana*:

1. *Abhisheka* (sprinkling)
2. *Achushana* (absorption)
3. *Atapa / Agni Shoshana* (drying)
4. *Bharjana* (frying or roasting)
5. *Bhavana* (levigation)
6. *Dhalana* (melting and quenching)
7. *Galana* (melting and straining)
8. *Mardana* (trituration)
9. *Nimajjana* (dipping)
10. *Nirjalikarana* (evaporation of water)
11. *Nirvapa* (heating and quenching)
12. *Parishravana* (straining)
13. *Patana* (sublimation)
14. *Prakshalana* (washing)
15. *Prithakikarana* (separation)
16. *Swedana* (boiling under liquid bath):
17. *Vilayana* (elutriation)

2.4.3. Role of media[54]:

The choice of media plays an important role in the *Shodhana* process. In some cases, the media acts as a solvent, dissolving the material to facilitate the separation of insoluble impurities, as seen in the *Shodhana* of *Guggulu* and *Navasadara*. In other instances, the media helps to eliminate toxic chemical substances from the drug. It's noteworthy that specific media are used for the *Shodhana* of particular substances, such as *Triphala Kwath* for *Lauha* and *Gomutra* for *Vatsanabha*. This specificity ensures that

the purification process is tailored to the unique properties and requirements of each substance.

The media also aids in the physical transformation of certain metals and minerals. For example, in the *Nirvapa* process, repeated heating and quenching in a liquid medium cause metals and minerals to become brittle, break, and reduce in size. This mechanical transformation is essential for further processing and enhancing the bioavailability and efficacy of the final product. Additionally, the choice of media can enhance the therapeutic properties of the substance being purified. For instance, using herbal decoctions not only purifies but also imparts additional medicinal qualities to the substance.

The use of specific media for different substances in *Shodhana* reflects the depth of knowledge and precision in Ayurvedic pharmacology. By carefully selecting the appropriate media, Ayurveda ensures that the purification process not only removes impurities but also optimizes the therapeutic potential of the substances. This meticulous approach to purification is a testament to the sophistication and efficacy of traditional Ayurvedic practices, ensuring that the final products are safe, potent, and beneficial for a wide range of health conditions.

2.4.4. Necessity of *Shodhana*:

- Elimination of physical and chemical impurities, which are not desired
- Eradication or minimization of toxicity of the material.
- Transformation of the hard and non-homogeneous material to soft, brittle, ductile and homogeneous material
- Induction of desired quantities.
- Potentiation of therapeutic efficacy of the drug.
- Converting the material in desired form.

2.5. *Bhawana*:

Bhawana is a vital process in Ayurvedic medicine where the material is fully submerged in a prescribed liquid and triturated until it reaches a dry state. This wet grinding technique involves grinding the materials with a specific liquid medium for a designated period, which aids in easy absorption and further processing of the substance. The *Bhawana* process enhances the therapeutic properties of the material by

allowing the liquid medium to penetrate deeply, thus facilitating better assimilation of the active ingredients[55].

This technique is particularly significant in the preparation of various Ayurvedic formulations, where the choice of liquid media—such as herbal decoctions, juices, or other medicinal liquids—can impart additional therapeutic qualities to the substance. By meticulously grinding the material with the liquid, *Bhawana* ensures a uniform and fine consistency, which is crucial for the efficacy of the final product. Moreover, this process can also help in detoxifying the material, removing any residual impurities, and enhancing its bioavailability. The repeated grinding and drying cycles not only purify but also potentiate the material, making it more effective for its intended therapeutic use. This demonstrates the depth of traditional Ayurvedic practices and their emphasis on precision and thoroughness in the preparation of medicinal substances.

2.5.1. Necessity of *Bhawana*:

• To bring minute particles of the material in contact with the liquid media.
• Impregnation of properties of the media to the material.
• Transformation of the coarse powder to finer state.
• To facilitate the material for further processing.
• Leads to unique and suitable physico-chemical changes.
• To potentiate the efficacy of the material.

2.6. *Marana*:

Marana is a pivotal process in Ayurvedic medicine that transforms purified metals and minerals into *Bhasma* through a series of levigation and incineration steps. This procedure is essential for converting these substances into an acceptable and bioavailable form suitable for therapeutic use. The Marana process involves meticulous grinding (levigation) of the material with specific herbal juices or other liquids, followed by repeated cycles of heating (incineration) at high temperatures[56].

During Marana, the metals and minerals undergo significant physical and chemical changes, rendering them more assimilable and enhancing their medicinal properties. This transformation is critical because it reduces the particle size of the substances, increasing their surface area and making them more easily absorbed by the body. Additionally, the process helps in detoxifying the metals and minerals, ensuring that any remaining impurities are eliminated.

2.6.1. Necessity of *Marana*:

- Reduction in particle size.
- Elimination of certain unwanted elements.
- Transformation into suitable compound form.
- To make the material non-irritant to the G.I. tract.
- To increase potency and quality of the material.
- Conversion into absorbable, adaptable and assimilable form.
- To make the material suitable for therapeutic uses.

2.6.2. Procedure of *Marana*[57]:

***Putapaka* Method:**

Shodhit drug is mixed with *Maraka Dravya* and levigated with particular liquid media till doughy mass formed. Pellets were prepared from doughy mass and kept in *Sharava* after drying. Another *Sharava* is placed over it and junction is sealed with mud smeared cloth. This *Sharava SamPuta* is subjected to *Puta* for incineration.

***Kupipakwa* Method[58]:**

Shodhit drug is levigated with *Bhavana Dravya* for certain period. Then *Kachakupi* was prepared by applying mud smeared cloth. *Kachakupi* placed in *Valukayantra* for heating for certain time period, after self-Cooling bottle was broken and prepared drug is collected from bottle.

***Niragni* Method:**

Shodhit drug is mixed with *Kajjali* and it is subjected to levigation for certain period. Then dough placed in copper saucer, covered by specific leaves and placed in sunlight to dry.

After this it is covered with another saucer which is sealed by mud smeared cloth. This *Sharava SamPuta* is kept in *Dhanyarashi* for specific period.

2.6.3. *Chakrikakarana*:

Chakrikakarana is a process in which small, flat, smooth pellets are made following the completion of the *Bhavana* process. This step ensures that the material is evenly prepared for subsequent procedures. The creation of these uniform pellets is important for achieving homogenous heating during the *Puta*, or incineration, process.

2.6.4. Importance of *Chakrikakarana*:

By forming these consistent pellets, *Chakrikakarana* facilitates even exposure to heat, which is essential for the effective transformation of the material into *Bhasma*. This uniform heating ensures that the incineration process is thorough, allowing for the complete conversion of the substance into a fine, bioavailable ash. The *Chakrikakarana* process exemplifies the meticulous attention to detail in Ayurvedic medicine, aimed at optimizing the quality and efficacy of the final medicinal product.

2.6.5. *Putapaka*:

It is a process of giving a specific amount of heat (*Puta*) in a particular atmosphere to maintain the uniform temperature.

2.6.6. Importance of *Putapaka*:

- To provide a particular temperature pattern (no less or more heating).
- To reduce particle size.
- To facilitate proper incineration.
- To provide a suitable atmosphere for desirable chemical reactions.
- To make the material ductile, smooth and homogeneous.
- To potentiate the material for therapeutic purposes.
- To make the material absorbable, adaptable and assimilable form

2.7. Procedure of *Putapaka*:

A specific *Puta* should be chosen for each material; for example, *GajaPuta* is ideal for *Lauha Marana*. To begin, the *Puta* is filled two-thirds full with cow dung cakes, then the *Saravasampata* containing the material is placed on top. The remaining one-third of the *Puta* is filled with more cow dung cakes, completely covering the *Saravasampata*. Once the cow dung cakes are ignited, they burn until completion. After allowing the *Puta* to cool naturally, the *Saravasampata* is removed, opened, and the pellets inside are collected and ground into powder[59].

2.8. Concept of Nano technology

Nanotechnology involves the manipulation of matter on an atomic, molecular, and supramolecular scale. Due to the wide variety of research and applications within this field, terms like "nanotechnologies" and "nano-scale technologies" are often used to emphasize its diverse scope. This includes advancements in materials science, electronics, medicine, and energy, where the common factor is the nanoscale dimension of the materials involved. Nanotechnology holds the potential to revolutionize

industries by enabling the creation of stronger materials, targeted drug delivery systems, highly efficient energy storage solutions, and advanced electronic devices. Its interdisciplinary nature bridges physics, chemistry, biology, and engineering, driving innovation and opening new frontiers in science and technology.

2.8.1. Applications of nano technology:

The different fields that find potential applications of nanotechnology are as follows:

a) Health and Medicine
b) Electronics
c) Transportation
d) Energy and Environment
e) Space exploration
f) Agriculture
g) Textiles
h) Food and Beverage
i) Cosmetics
j) Construction

2.8.2. Nanotechnology in Ayurveda in relation with *Rasa Shastra*:

The Ayurvedic system of medicine, one of the oldest traditional systems in India, incorporates a unique branch known as *Rasa Shastra*, which focuses on the use of metal and mineral formulations called *Bhasma*. These *Bhasmas* are traditionally prepared using meticulous processes to ensure their therapeutic efficacy. Recent advancements have revealed that the particles in *Bhasma* are often nanoscale, typically around 10-15 nm in diameter, making them an early form of nanomedicine. This insight bridges ancient Ayurvedic practices with modern nanotechnology, highlighting the potential of *Bhasma* as a sophisticated drug delivery system. The integration of nanotechnology with Ayurveda opens new avenues for drug design, enhancing the bioavailability and therapeutic potential of traditional herbal and metallic preparations. By aligning ancient wisdom with contemporary scientific approaches, Ayurvedic nanomedicine offers promising opportunities for developing advanced treatments in various medical fields.

2.8.2.1. *Bhasma* and its Correlation with Nanotechnology

Bhasma refers to fine powders prepared through traditional Ayurvedic processes by burning or calcining various metals and minerals. These powders, made from metals

like gold, silver, copper, and zinc, are processed in such a way that they are thought to reach the nanoscale. Recent studies suggest that *Bhasma* exhibits properties comparable to engineered nanomaterials, which has opened up a new frontier in understanding ancient medicine through a scientific lens.

1. Size and Surface Properties

Scientific studies using advanced techniques, such as Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), have shown that many types of *Bhasma* indeed have particles in the nanometer range. The small particle size and increased surface area are critical to the *Bhasma's* effectiveness, allowing better absorption and interaction at the cellular level—properties that modern nanomaterials also exploit for targeted drug delivery and improved bioavailability.

2. Bioavailability and Cellular Interaction

Due to their nanoscale size, *Bhasma* can permeate cellular membranes more effectively, enhancing their bioavailability and potentially allowing them to reach target sites within the body more efficiently. This aspect of *Bhasma* aligns with the concept of nanocarriers in drug delivery systems, where nanoparticles are engineered to deliver drugs to specific locations in the body, minimizing side effects and increasing therapeutic efficacy.

3. Stability and Biocompatibility

Traditional Ayurvedic techniques are known to impart stability and a biocompatible structure to *Bhasmas*, often coating or modifying the particle surface during the manufacturing process. This coating is thought to make *Bhasma* safer by reducing potential toxicity—a concern in both ancient and modern nanomedicine. Engineered nanomaterials similarly undergo surface modifications to improve compatibility with biological tissues and minimize potential toxicity.

4. Catalytic and Therapeutic Properties

The catalytic activity of nanoscale particles is higher due to the increased surface area, which may explain why *Bhasmas* are used as catalysts in biochemical reactions within the body. Some *Bhasmas*, like *Swarna Bhasma* (gold ash), exhibit antioxidant properties that protect cells from oxidative stress, a feature also noted in certain gold-based nanoparticles. Thus, *Bhasma* could be considered a form of natural nanomedicine that Ayurvedic practitioners have used for centuries.

5. **Modern Analytical Verification**

Analytical techniques, such as X-ray diffraction (XRD), Energy-Dispersive X-ray Spectroscopy (EDS), and Fourier-transform infrared spectroscopy (FTIR), confirm the composition and nanostructure of *Bhasma*. This scientific validation helps bridge the understanding between traditional Ayurvedic practices and contemporary nanotechnology, revealing that *Bhasma's* therapeutic properties might stem from its nanoscale features.

CHAPTER 3

AIMS AND OBJECTIVES

Chapter 3.

Aims and Objectives

3.1. Research Gap

The objective of this study is to systematically evaluate and establish a standardized method for the preparation of *Lauha Bhasma*, addressing the lack of consistency and clarity in the current preparation methods documented in classical Ayurvedic texts. Given the therapeutic importance of *Lauha Bhasma* in the treatment of various health conditions, this research aims to identify an optimal preparation protocol by comparing classical methods, including purification and calcination steps, to assess their pharmacological efficacy and safety. Additionally, the study will investigate the heating patterns and temperature controls during the *Bhasma* preparation process, which are crucial yet underexplored factors influencing the quality and bioavailability of the final product. This analysis intends to fill critical gaps in existing literature and provide scientifically validated guidelines for producing high-quality *Lauha Bhasma* with reliable therapeutic outcomes.

In the present study, two different methods which are commonly used are taken keeping in mind the following objectives.

3.2. Objectives

- Authentication and standardization of raw materials used for the preparation of *Putapaka* and *Trividpaka Bhasma.*
- Preparation of standard manufacturing process of *Lauha Bhasma* prepared by *Putapaka* and *Trividpaka*.
- Characterization of prepared Loha *Bhasma* by *Putapaka* nad *Trividpaka*
- Pharmacological evaluation of prepared *Lauha Bhasma* by *Putapaka* and *Trividpaka* on hematinic activity.

CHAPTER 4

RESEARCH METHODOLOGY

Chapter 4.

Research Methodology

4.1. Selection of Procedure

In this research, two distinct methods have been selected for the preparation of *Lauha Bhasma*: *Trividha Paka* and *Putapaka*. The *Trividha Paka* method, a classical Ayurvedic technique, involves three stages of heating and processing, ensuring thorough transformation and detoxification of raw materials. The *Putapaka* method, on the other hand, is a sophisticated procedure that entails multiple cycles of incineration and quenching, aimed at achieving a fine, bioavailable form of the *Bhasma*.

The choice of these two methods is pivotal as each offers unique advantages and challenges that influence the final product's quality. *Bhasma* was prepared by these two methods by controlling and characterizing each step.

From Procurement of raw material to the testing of final product each step is taken into consideration.

4.2. Procurement of the raw material:

Appropriate quality of raw drug as mentioned in the classical text was collected as per the requirement, details are mentioned in chapter 5. List of raw material required in the present study is listed below in the table:

Table: 4.1. Raw material required in the present study

S.No.	Material Required
1.	*Haritaki*
2.	*Bhibhitaki*
3.	*Amalaki*
4.	Iron (*Lauha*)

4.3. Authentication of raw material[60]:

The authentication of raw materials is a critical aspect of modern manufacturing and production processes, as it ensures the quality, safety, and consistency of the final products. Accurate identification and verification of raw materials are essential in various aspects, particularly in the cosmetic, pharmaceutical and dietary supplement sectors, where the use of adulterated or incorrect materials can have serious

consequences. Raw material authentication was done based on various analytical parameters.

4.3.1. Collection of Raw material:

The required quantity of the Raw Material was collected from Shri Ayurveda Seva Sadan with authentication. And iron (*Lauha*) was purchased from the authorized seller. Details are mentioned in the next chapter.

4.3.2. Organoleptic Study:

The collected samples were first observed for its colour, odour, taste and texture.

4.4. Physicochemical Analysis of raw herbal sample[61]:

Physicochemical analysis of raw herbal sample includes individual testing of all herbal plant, then the analysis of *Kwatha* prepared. Procedure for physicochemical analysis is described below.

4.4.1. Foreign Matter:

100g of each sample was spread evenly on a stainless-steel tray. Visible foreign matter was identified without the aid of magnification. The remaining portion of the sample was then weighed, and the percentage of foreign matter was determined using the formula: -

$$\textbf{Foreign matter} = \frac{\textbf{Weight of foreign matter}}{\textbf{Weight of drug}} \times 100$$

4.4.2. Loss on drying:

The loss on drying procedure involved weighing of 5-10gm of the raw material, then drying it at a 105 ºC for 5 hours to evaporate the moisture. After drying, the sample was cooled in a desiccator and then re-weighed. The difference in weight before and after drying noted. The process was repeated till the weight stopped changing. Then the petri dish was allowed for self-cooling and from the weight loss the percentage for LOD was calculated.

4.4.3. Total ash:

The sample weighing 2.5 grams was combusted in a crucible at 450°C for a duration of 5 hours. Subsequently, the crucible was allowed to cool on a shelf before being placed in a vacuum desiccator. The weight of the resulting ash was determined and used to calculate the percentage yield of ash.

$$Total\ Ash = \frac{weight\ of\ ash}{weight\ of\ sample} x\ 100$$

4.4.4. Acid insoluble extractive:

The ash produced from the described procedure was mixed with 25 ml of diluted hydrochloric acid and heated for 5 minutes. After that, the mixture was filtered using filter paper designed to leave behind no residue. The liquid obtained through filtration was washed with hot water to eliminate chlorides, then heated until a consistent weight was reached. The proportion of ash that is insoluble in acid was subsequently calculated by measuring the weight of the resulting ash.

$$Acid\ insoluble\ ash = \frac{weight\ of\ residue\ x\ volume\ made}{weight\ os\ sample\ x\ volume\ taken} x\ 100$$

4.4.5. Alcohol soluble extractive:

5 gm of the coarse powder sample were placed in a sealed conical flask with 100 mL of alcohol. The flask was shaken several times over a period of 6 hours and then left to settle for 18 hours. Subsequently, it was filtered using filter paper. A volume of 25 mL from the filtrate was evaporated in a china dish, and the residue's weight was used to determine the proportion.

$$Alcohol\ soluble\ extractive\ value = \frac{weight\ of\ residue\ x\ volume\ made}{weight\ of\ sample\ x\ volume\ taken} x\ 100$$

4.4.6. Water soluble extractive:

A quantity of 5 grams of the coarse powder sample was added to a sealed conical flask with 100 mL of water. The flask was shaken intermittently for a period of 6 hours and then left still for 18 hours. Subsequently, it underwent filtration using filter paper. A volume of 25 cubic centimeters from the filtered solution was placed in a ceramic dish and permitted to evaporate, after which the proportion was calculated by weighing the remaining residue.

$$Water\ soluble\ extractive\ value = \frac{weight\ of\ residue\ x\ Volume\ made}{weight\ of\ sample\ x\ volume\ taken} x\ 100$$

4.4.7. Qualitative analysis:

4.4.7.1. Test for Alkaloids: Mayer's test: Take 3 to 4 drops of 1M HCl and treat it with an aqueous extract to acidify it. The prepared sample is then treated with 3–5 drops

of Mayer's regent. White or yellowish-coloured turbidity or precipitates show the presence of alkaloids.

4.4.7.2. Dragendorff's test: Each extract was individually dissolved in diluted hydrochloric acid and then filtered. Dragendorff's reagent, a solution of potassium bismuth iodide, was subsequently added to the filtrates. The formation of a crimson precipitate indicates the presence of alkaloids.

4.4.7.3. Wagner's Test: Wagner's reagent when treated with the filtrates. The presence of a reddish or brownish precipitate shows the presence of alkaloids.

4.4.7.4. Test for Tannin: Boil the specific amount of extract and then filter it. The prepared sample filtrate was treated with ferric chloride. If greenish-black precipitates appeared, it indicated the presence of tannins.

4.4.7.5. Test for Phenol (Ferric Chloride Test): The extract sample was treated with ferric chloride. If bluish-black precipitates appeared, it indicated the presence of tannins. phenols.

4.4.7.6. Test for Saponins: 2 ml of water shaken with 0.5 g of sample extract if gave persistence of foam make for 10 min. or more shows the presence of saponins.

4.4.7.7. Test for Proteins: A few drops of concentrated nitric acid were applied to the extracts. The development of a yellow hue signifies the existence of proteins.

4.4.7.8. Test for Glycosides: The sample is extracted with chloroform as a solvent, and the remaining solvent is evaporated to dry it. Add glacial acetic acid with a trace amount of ferric chloride, 0.4 ml only. Take the prepared sample into a test tube, and by the side of the tube, carefully add 0.5 ml of concentrated sulphuric acid. The acetic acid layer gave it a blue colour.

4.4.8. Test for Heavy metal[62]

The heavy metal test is intended to assess the amount of metallic contaminants. The sample was investigated for the presence of Lead, Cadmium, Mercury, and Arsenic. No heavy metal was detected in the sample.

4.4.9. Microbial and Pathogen test

4.4.9.1. Total microbial plate count: Petri dishes with a diameter of 9-10 cm were utilized for bacteria culture. To one dish added a mixture of 1 ml of the pre-treated herbal material and about 15 ml of liquefied casein-soybean digest agar at a temperature not exceeding 45 °C. Alternatively, spread the material on the surface of the solidified

medium in a Petri dish. Material is diluted to obtain an expected colony count of not more than 300. Two dishes were prepared using the same dilution, inverted, and incubated at 30–35 °C for 48–72 hours unless a more reliable count was obtained in a shorter period. The number of colonies formed was counted and the results were calculated using the plate with the largest number of colonies, up to a maximum of 300.

4.4.9.2. Pathogen test:

Pre-treated material was homogenized appropriately and incubated at 30–37 °C for a length of time sufficient for the revivification of the bacteria, but not sufficient for the multiplication of the organisms (usually 2–5 hours). Shake the container, aliquots transferred equivalent to 1 g or 1 ml of the homogenized material to 100 ml of Enterobacteriaceae enrichment broth Mossel and incubated at 35–37 °C for 18–48 hours. Subculture on a plate with violet-red bile agar with glucose and lactose was prepared. Incubate at 35–37 °C for 18–48 hours. The material passes the test as no growth of colonies of the pathogen was detected on the plate.

4.4.9.2.1. *Escherichia coli*: Quantity of the homogenized material was transferred in lactose broth, prepared, and incubated as described above, and containing 1 g or 1 ml of the material being examined, to 100 ml of MacConkey broth and incubated at 43–45 °C for 18–24 hours. Subculture was prepared on a plate with MacConkey agar and incubated at 43–45 °C for 18–24 hours. The growth of red, generally non-mucoid colonies of Gram-negative rods, sometimes surrounded by a reddish zone of precipitation, indicates the possible presence of E. coli. This may be confirmed by the formation of indole at 43.5–44.5 °C or by other biochemical reactions. The material passes the test as no such colonies were detected.

4.4.9.2.2. *Staphylococcus aureus*: The solution was inoculated into a Soybean casein digest medium, and suspension thus obtained containing 1 g or of the material being examined. Mix and incubate at 35–37 °C for 24–48 hours. Subculture on a suitable medium Baird-Parker agar was prepared. Incubated at 35–37 °C for 24–48 hours. The material passes the test as no growth of microorganisms was detected.

4.4.9.2.3. *Pseudomonas aeruginosa*: The solution was inoculated into a Soybean casein digest medium, and suspension thus obtained containing 1 g or of the material being examined. *S*ubculture was prepared on a plate of cetrimide agar and incubated at

35–37 °C for 24–48 hours. As no growth of microorganisms was detected, so the material passes the test.

4.4.9.2.4. *Salmonella spp.*: The solution was incubated, and a suspension of the pre-treated material was made at 35-37 °C for 5-24 hours, as needed for enrichment. Following that, 10 ml of the enrichment culture was added to 100 ml of tetrathionate bile brilliant green broth and incubated at 42-43 °C for 18-24 hours. Subcultures were then made on at least two of the three agar media listed below: deoxycholate citrate agar; xylose, lysine, deoxycholate agar; and brilliant green agar, and incubated at 35-37 °C for 24-48 hours. Because no microbial growth was detected, the item passed the test.

4.4.10. High-Performance Thin Layer Chromatography (HPTLC)[63]:

4.4.10.1. Test Solution:

10 g coarsely powdered sample soaked in 100 ml of methanol. 6 hours of shaking is given and 18 hours stand-by then filtered through Whatman filter paper No.1, dried, and Made 10% solutions.

4.4.10.2. Standard Solution:

Dissolve 2 mg of each Gallic acid in 10 ml of methanol separately.

4.4.10.3. Solvent System:

Mobile Phase for *Haritaki, Bhibhitaki* and *Amalaki*: Toluene (5): Ethyl acetate (3.5): Formic acid (0.5)

4.4.10.4. Procedure:

5µL solution was applied as bands by linomat applicator on a pre-coated Aluminium plate with silica gel 60 of 0.2mm thickness.

4.4.10.5. Visualization:

The plate was developed in the mobile phase. The plate was dried and visualized in UV 254 & 366 nm.

4.5. Preparation of *Triphala Kwatha:* [64]

16 *Pala (750gm)* of *Triphala Yavkuta* was taken and boiled with 8 times water (6lit.). Till it was reduced to one forth, the *Kwatha* was filtered and used for further process. Detail of the process is mentioned in Chapter 5.

4.6. Analytical parameters of *Triphala Kwatha*:

Triphala Kwatha prepared was tested for the following parameters:

4.6.1. Organoleptic parameters:

Colour, odour and taste for *Kwatha* was analysed before further analysis.

4.6.2. pH:

To check the pH of a decoction, a digital pH meter was used. The steps involved are as follows: The decoction was prepared as usual by boiling the ingredients in water for a set period of time. After cooling to room temperature. pH meter was calibrated properly, the pH probe was dipped into the decoction and reading was taken after a stabilized reading.

4.6.3. Specific gravity:

Specific gravity is a key parameter used to assess the density of the liquid. The specific gravity of the *Kwatha* was determined by the following process. Picometer was taken and empty weight of the picometer is noted down. Then the picometer was filled with the *Kwatha* prepared and weighed again. Then again empty the picometer and fill it with water up to the calibration mark. Weighed the water filled picometer and calculated the specific gravity using the given formula:

$$\textbf{Specific gravity} = \frac{\textbf{Wt. of Kwatha with pycometer} - \textbf{Wt. of empty pycometer}}{\textbf{Wt. of dist. water with pycometer} - \textbf{Wt. of empty pycomete}}$$

4.6.4. Total solid content:

The determination of the total solid content in *Kwatha*. The following procedure outlines the method for determining the total solid content: Weighed an empty, clean, and dry evaporating dish. Recorded its weight as W1. Accurately weighed about 5-10 g of the test sample into the evaporating dish. The weight of the dish and sample was noted as W2. Placed the dish containing the sample in a hot air oven maintained at 105°C and dried it to constant weight. Cooled the dish in a desiccator and weighed it. Record the weight as W3. The total solid content was calculated using the formula:

$$\textbf{Total Solid Content}\ (\%) = \frac{\textbf{W3} - \textbf{W1}}{\textbf{W2} - \textbf{W1}} \times \textbf{100}$$

Where: - W1 = weight of empty evaporating dish - W2 = weight of dish + sample before drying - W3 = weight of dish + sample after drying

4.6.5. Viscosity:

To check the viscosity of a sample, a viscometer was used to measure the resistance of the material to flow. The procedure involved filling the viscometer with the sample allowed to flow through under the force of gravity. The time taken for the sample to flow through the viscometer was recorded, and the viscosity is then calculated using the formula. This method provides valuable information about the flow properties and consistency of the material, which is essential for various industrial applications and product development.

4.6.6. Qualitative analysis:

4.6.6.1. Test for Alkaloids: Mayer's test: Take 3 to 4 drops of 1M HCl and treat it with an aqueous extract to acidify it. The prepared sample is then treated with 3–5 drops of Mayer's regent. White or yellowish-coloured turbidity or precipitates show the presence of alkaloids.

4.6.6.2. Dragendorff's test: Each extract was individually dissolved in diluted hydrochloric acid and then filtered. Dragendorff's reagent, a solution of potassium bismuth iodide, was subsequently added to the filtrates. The formation of a crimson precipitate indicates the presence of alkaloids.

4.6.6.3. Wagner's Test: Wagner's reagent when treated with the filtrates. The presence of a reddish or brownish precipitate shows the presence of alkaloids.

4.6.6.4. Test for Tannin: Boil the specific amount of extract and then filter it. The prepared sample filtrate was treated with ferric chloride. If greenish-black precipitates appeared, it indicated the presence of tannins.

4.6.6.5. Test for Phenol (Ferric Chloride Test): The extract sample was treated with ferric chloride. If bluish-black precipitates appeared, it indicated the presence of tannins. phenols.

4.6.6.6. Test for Saponins: 2 ml of water shaken with 0.5 g of sample extract if gave persistence of foam make for 10 min. or more shows the presence of saponins.

4.6.6.7. Test for Proteins: A few drops of concentrated nitric acid were applied to the extracts. The development of a yellow hue signifies the existence of proteins.

4.6.6.8. Test for Glycosides: The sample was extracted with chloroform as a solvent, and the remaining solvent was evaporated to dry it. Glacial acetic acid was added with a trace amount of ferric chloride, 0.4 ml only. The prepared sample was taken into a

test tube, and by the side of the tube, carefully added 0.5 ml of concentrated sulphuric acid. The acetic acid layer gave it a blue colour.

4.6.7. Test for heavy/toxic metals:

The aim of the heavy metal test was to evaluate the levels of metallic impurities. The sample was analyzed for the existence of Lead, Cadmium, Mercury, and Arsenic.

4.6.8. Microbial contamination and Pathogen test:

Petri dishes measuring 9-10 cm in diameter were employed for bacterial culture. One dish received a combination of 1 ml of the pre-treated herbal substance and approximately 15 ml of liquefied casein-soybean digest agar not exceeding a temperature of 45 °C. Alternatively, the material was evenly spread on the surface of the solidified medium in a Petri dish. The material was diluted to achieve an expected colony count not exceeding 300. Two dishes with the same dilution were prepared, inverted, and then placed in an incubator at a temperature range of 30–35 °C for 48–72 hours unless more reliable results were obtained sooner. The number of colonies formed was counted, and calculations were based on the plate with the largest number up to a maximum limit of 300 colonies.

4.7. Analytical specifications of *Lauha* [65]:

Elemental analysis for raw *Lauha* was performed to ensure the percentage of elements present in the *Lauha*. Results are mentioned in the next chapter.

4.8. Pharmaceutical Processing

4.8.1. Preparation of *Lauha Bhasma*:

Preparation of *Lauha Bhasma* was done with two methods i.e., *Trividpaka* and *Putapaka*. The steps involved in the process of *Lauha Bhasma* preparation are:

4.8.1.1. *Lauha Shodhana*[66,67]:

The process of *Shodhana* was done with two methods: first with *Samanya Shodhana* then *Vishesh Shodhana*

- ***Samanya Shodhana*:** *Samanya Shodhana* is process of purification which is considered as the common method for all type metal.

- **Liquid media for *Samanya Shodhana*[68,69]:**
- ***Taila***: - Marketed *Taila* was used for the process.

- ***Takra*****:** - *Takra* was prepared by adding equal quantity of water to curd (*Dadhi*) and diluting the same by churning.
- ***Kanji*****:** - *Kanji* was prepared by placing rice along with small quantity of white radish cutted into pieces were placed in an earthen pot and 4 times of water was added. The mixture was kept for fermentation for 3 weeks. The prepared sour mixture was used for *Shodhana*.
- ***Kulatha Kwatha***: - *Kulatha Kwatha* was prepared by adding 16 times of water to *Kulatha* and then reducing to 1/4th.
- ***Go-Mutra***: - Fresh *Go-Mutra* was collected at the time of *Shodhana*
- **Process of *Samanya Shodhana*:** For *Samanya Shodhana Taila, Takra, Go-mutra, Kullatha Kwatha* and *Kanji* was taken and *Aashudha Lauha* was taken and in each liquid media red hot *Lauha* was quenched 7 times, each time fresh liquid media was taken. After the completion of *Shodhana* process, *Lauha* was subjected for the *Vishesh Shodhan* process.
- ***Vishesh Shodhan*****:** *Vishesh Shodhana* is different for different method.
- **Preparation of Liquid media:**16 Pala (750gm) *Triphala Yavkuta* was taken and boiled with 8 times water (6lit.). *Kwatha* was filtered after it got reduced to one fourth.
- **Process of *Vishesh Shodhana*:** *Samnya Shodhit Lauha* was taken and then heated till red hot and quenched (*Nirvapa*) into the above prepared liquid media (*Triphala Kwatha*), the process is repeated for seven times. After the completion of *Vishesh Shodhana* the *Lauha* was used for further process.

4.8.2. *Lauha Marana*:

4.8.2.1. Method 1 *Trividpaka* method: It involves the following steps: - *Bhanupaka, Sthalipaka* and *Putapak.*

4.8.2.1.1. *Bhanupaka* of *Lauha* [70]

Ingredients

Shuddha Lauha (purified iron), *Triphala Kwatha* (decoction of three myrobalan).

Process:

*Lauha i*s mixed with *Triphala Kwatha* and was exposed to roasting under sun rays. The process was repeated seven times consuming fresh *Triphala* in each repetition.

4.8.2.1.2. *Sthalipaka* of *Lauha* [71]

Ingredients

Bhanupakwa Lauha and *Triphala Kwatha*

Process

Lauha lumps obtained after *Bhanupaka* was taken in a stainless-steel vessel and was heated over fire. *Triphala Kwatha* was added to it and the material was given intense heat till all the water gets evaporated.

4.8.2.1.3. *Putapaka* of *Lauha*[72]

Ingredient

Sthalipakwa Lauha, and *Triphala Kwatha* - Q.S

Method of preparation

Sthalipakwa Lauha was taken. To it an equal quantity of *Triphala* made in decoction form was added. The mixture was levigated for about 4 hours. The material was mixed thoroughly in between the process. After complete levigation, *Chakrikas* were prepared and dried properly and weighed. Then *Chakrika's* were kept in an earthen basin (*Sharava*) and was covered by another basin making a *SaravasamPuta* of it. The *SamPuta* formed was closed properly with the help of *Kapadmitti* and dried. The *SamPuta* was subjected to *GajaPuta (Puta* process was carried out through classical method using *Uplaas*). After attaining the desired temperature, the heat continued for one more hour as maintenance. After that it was left as it is for self-cooling and was taken out next day. This whole process was repeated 12 times, since after that all the *Bhasma* criteria were passed and it was considered that the *Bhasma* is prepared. After completing all *Puta*, the *Bhasma* prepared was triturated into fine powder and further testing was done.

4.8.2.2. Method 2 *Putapaka* method[73]: This method involves only *Putapaka*.

Preparation of Liquid media: 16 *Pala (750gm) Triphala Yavkuta* was taken and boiled with 8 times water (6lit.). Filter the *Kwatha* when it is reduced to one forth.

Preparation of rice (*Bhaat):* Take one part rice and cook with two times of water till it attain *Bhaat* like consistency.

Process of *Marana:* *Shudha Lauha Churna* was taken and subjected for *Mardana* with *Triphala Kwatha and Bhaat* till is attain a dough like consistency. After that *Chakrikakaran* was done, after that dry *Charkrika* in sun light and *Sarava SamPuta*

was done. After that it was subjected to *GajaPuta* (*Puta* process was carried out through classical method only i.e., using *Uplaas*). The process is repeated till the *Bhasma* is prepared properly. The *Bhasma* obtained was of *Rakta Varna*[74].

4.9. Evaluation of prepared *Lauha Bhasma:*

Evaluation of *Bhasma* was done on the basis of two characters:

4.9.1. Classical evaluation parameters[75]:

Classical evaluation parameters include the following parameters:

Rekhapurnatava: This test indicates the fineness of the *Bhasma.* In this test small amount of *Bhasma* was taken in between the index finger and thumb, rubbed gently and observed that whether, the *Bhasma* particles is filling in the furrows of the fingertips or not. Particle size should be so small that it should take place in the lines of the finger, if not present then the *Bhasma* need more *Puta*.

Nishchandratva: In this parameter a small amount of *Bhasma* was taken and observed under sunlight to check the presence of shiny particles in the *Bhasma*. The presence indicates that the *Bhasma* is not prepared properly, and the lusterless *Bhasma* indicates that *Bhasma* is prepared.

Varitaratva: This parameter is to check the weight of the *Bhasma*. The weight of *Bhasma* should be so light that the *Bhasma* should float on the surface of water. For this test water was taken in a beaker and allowed to stagnant, then sprinkle small amount of *Bhasma* on the surface of water, the *Bhasma* should not settle down, it should float on water.

Unnaman: This test is performed in continuation with the *Varitaratva* test, in this test a piece of rice is kept on the floating *Bhasma* and as a result the *Bhasma* should not settle down, it should float on the surface of water with rice also. It indicates that the weight of the *Bhasma* is so light that it could not break the surface tension of water.

Nirdhoom: The *Bhasma* prepared should not procedure any smoke when kept on fire. Sprinkle the *Lauha Bhasma* on red hot coal. It should not emit smoke.

4.9.2. Modern evaluation parameters[76]:

4.9.2.1. Physico-chemical analysis[77]

Organoleptic characters: *Bhasma* was tested for its Organoleptic characteristics. Color, odor, taste, touch was observed before further analysis.

Loss on drying: The loss on drying procedure involves weighing a sample of the raw material, then drying it at a specified temperature for a specific period to evaporate the moisture. After drying, the sample is cooled in a desiccator and then reweighed. The difference in weight before and after drying is used to calculate the percentage of moisture content in the raw material. 1gm of the sample is weighed in a previously weighed petri dish and dried in an oven at 110°C till the weight stops changing. Then the petri dish was allowed for self-cooling and from the weight loss the percentage for LOD was calculated.

Loss on drying is a common test used to determine the moisture content of *Bhasma*. This procedure is crucial in ensuring the quality and consistency of the final product.

Ash value: In addition to the loss on drying procedure, another crucial test for raw material authentication is the determination of total ash content. Total ash content refers to the residue left behind after heating the raw material to complete combustion. This test is essential for assessing the purity and inorganic content.

The procedure for determining total ash involves carefully weighing 2.5 gm of sample of *Bhasma* in a crucible and incinerating it at a specific temperature of 450°C to completely burn off the organic components. The remaining ash is then cooled and weighed to calculate the percentage of total ash content.

$$\textbf{Total Ash} = \frac{\textbf{Weight of ash}}{\textbf{Weight of sample}} \times 100$$

Acid-insoluble ash: The acid insoluble ash test helps in evaluating the amount of impurities and contaminants in the raw material that are resistant to acid treatment. This is crucial for assessing the purity and overall quality of the *Bhasma*.

The procedure for determining acid insoluble ash involves taking 2.5ml of diluted hydrochloric acid and combining it with the prepared ash material and subjecting it for boiling for 5 minutes. The remaining insoluble matter is then filtered, washed with hot water to remove chloride, dried, ignited till constant weight is achieved, and weighed to calculate the acid insoluble ash content.

$$\textbf{Acid insoluble ash} = \frac{\textbf{Weight of reside x Volume made}}{\textbf{Weight of sample x Volume taken}} x100$$

Water-soluble ash: The water-soluble extractive test is essential for evaluating the amount of soluble matter in the *Bhasma* that can be extracted using water.

The procedure for determining water soluble extractive involves taking a 5 gm of *Bhasma* sample in a conical flask containing 100ml of distilled water and subjecting it for 6hrs continuous shaking and then leave overnight undisturbed. The extracted solution is then filtered, and the filtrate is evaporated to dryness. The residue is then weighed to calculate the percentage of water-soluble extractive content in the raw material.

$$\textbf{Water solube extractive value} = \frac{\textbf{Weight of residue x Volume made}}{\textbf{Weight of sample x Volume taken}} \textbf{x100}$$

4.9.2.2. Semi-Quantitative analysis: Semi-quantitative analysis includes the evaluations of the percentage of compound present in the *Bhasma*. This analysis was done by FP analysis method in Varsha Balluni lab, Mumbai. By this analysis the presence of compound was confirmed.

4.9.2.3. Sophisticated analysis: In addition to the conventional tests like loss on drying, total ash, acid insoluble ash, and water-soluble extractive procedures, sophisticated analytical techniques play a crucial role in *Bhasma* authentication. Four major analytical techniques commonly used for raw material authentication are Fourier Transform Infrared Spectroscopy, Field Emission Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy mapping, X-ray Diffraction analysis, and Thermogravimetric Analysis.

FTIR Analysis: Fourier Transform Infrared Spectroscopy (FTIR) is a powerful analytical technique used to identify the functional groups present in the *Lauha Bhasma*. By analyzing the characteristic absorption bands in the infrared region, FTIR can provide valuable information about the chemical composition and structure of the material. This technique is particularly useful for verifying the authenticity of plant extracts and organic materials, as it can distinguish between different species and detect the presence of contaminants or adulterants.

FTIR analysis was done in Central Instrumentation Facility, LPU. The sample was analyzed in the spectrometer and the results are mentioned in the next chapter.

FESEM-EDAX Analysis: FESEM-EDAX mapping is a high-resolution imaging technique combined with elemental analysis that allows for the detailed examination of the surface morphology and elemental composition of *Lauha Bhasma*. This analysis

can help in identifying any irregularities, impurities, or foreign particles present in the material, providing valuable insights into its quality and authenticity. FESEM is a powerful tool for determining the size and morphology of materials at high resolution. By using FESEM, valuable insights into particle size distribution, surface roughness, and porosity, which are crucial factors in understanding material properties and performance.

FESEM- EDAX analysis was done in Central Instrumentation Facility, LPU.

XRD Analysis: XRD analysis is used to determine the crystalline structure and phase composition of raw materials. By analyzing the diffraction pattern of X-rays interacting with the material's crystal lattice, XRD can identify the presence of different crystalline phases and polymorphs. This information is essential for verifying the identity of mineral-based raw materials and detecting any changes in crystalline form due to adulteration or processing.

XRD analysis was also performed in Central Instrumentation Facility, LPU. The results are mentioned in next chapter.

Thermogravimetric analysis: TGA is a thermal analysis technique used to study the thermal decomposition and stability of *Lauha Bhasma*. By subjecting the material to controlled temperature changes and monitoring its weight loss as a function of temperature, TGA can provide insights into its thermal behavior, decomposition kinetics, and purity. This information is crucial for assessing the stability and quality of *Lauha Bhasma*, particularly in industries where thermal stability is a critical parameter.

TGA analysis was also performed in Central Instrumentation Facility, LPU. The sample was kept in the analyzer and analyzed at the temperature 1000 °C. The results are given in the next chapter.

4.9.3. Animal Study[78]:

4.9.3.1. Induction of anemia in animals: Anemia was induced in rats by using phenyl hydrazine. The solution was prepared by dissolving phenyl hydrazine (50mg / ml) in absolute alcohol and diluting with distilled water. Dose of 10mg/kg was administered by oral route for 8 days[79].

4.9.3.2. Treatment Model: Different group of animals are categorized for the treatment as mentioned in the table below. The blood sample were collected on 0^{th}, 3^{rd},7^{th} and 15^{th} day for examination[80].

Table 4.2. Detail of the animal, treatment and dose with administration

Group	Treatment	Dose and route	No. of animals
Group 1: Vehicle treated control animals	Distilled water with 1 ml absolute alcohol for 15 days	50 ml distilled water + 1 ml absolute alcohol; oral route	6
Group 2: Phenyl hydrazine treated animals	Phenyl hydrazine 10mg/kg for 3 days	10mg/kg (50 mg/ml solution in absolute alcohol and diluting with distilled water); oral route	6
Group 3: Standard treated animal	Phenyl hydrazine 10mg/kg for 3 days + Ferrous Sulphate (std) for 15 days	10mg/kg +50mg/kg; oral route	6
Group 4: *Lauha Bhasma* (*Trividhpaka*)	Phenyl hydrazine 10mg/kg for 3 days + *Lauha Bhasma* for 15 days	22 mg/kg; oral route	6
Group 5: *Lauha Bhasma* (Trividhpaka) + *Triphala Churna*	Phenyl hydrazine 10mg/kg for 3 days +*Lauha Bhasma* for 15 days+ *Triphala Kwatha*	22 mg/kg; oral route	6
Group 6: *Lauha Bhasma (Putapaka)* treated group	Phenyl hydrazine 10mg/kg for 3 days +*Lauha Bhasma* for 15 days	10mg/kg +6 mg/kg; oral route	6

Group 7: *Lauha Bhasma (Putapaka)* treated group + *Triphala Churna*	Phenyl hydrazine 10mg/kg for 3 days +*Lauha Bhasma* for 15 days+ *Triphala Kwatha*	10mg/kg +6 mg/kg; oral route	6

4.9.3.3. Evaluation Parameters:

4.9.3.3.1. Blood Parameters: - The various blood parameters like red blood cell count (RBC), hemoglobin (HB) concentration, packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and white blood cells (WBC) count were analyzed for evaluating hematinic potential of both the *Lauha Bhasma* formulations. Blood was collected (1-2 ml) from the retro-orbital plexus, under slight chloroform anesthesia, in copper vials with EDTA solution. The blood was collected on day 0 before phenyl hydrazine administration, and then on 7^{th}, 14^{th} and 15^{th} days after phenyl hydrazine administration and formulation treatment. All the parameters were determined by blood test examination in laboratory.

4.9.3.3.2. Histological examination: - On 15^{th} day rats were sacrificed by cervical dislocation and spleen was collected for histopathological study. Tissues w fixed at 10% neutral-buffered formalin solution embedded in paraffin and used for histopathological examination. 4-5 µm tissue section was taken on a glass slide coated with albumin. The hematoxylin-stained section was stained with eosin for two minutes and quickly passed through ascending grades of alcohol, cleaned with xylene and mounted in Canada Balsam. The stained section was examined under a photomicroscope and photographed. The samples were sent to pathologists to determine histopathological changes.

CHAPTER 5

RESULT AND DISCUSSION

Chapter 5.

Result and Discussion

5.1. Collection of Drug:

The required quantity of *Triphala* was Procured from the company Shri Ayurveda Seva Sadan, Firozabad, U.P.

5.2. Authentication of Sample:

Triphala (*Haritaki, Bhibhiatki* and *Amalaki*) were authenticated and verified in Shri Ayurved Seva Seva Sadan the certificate number is SASS/231/21-23.

5.3. Organoleptic Characteristics of *Triphala*:

Table 5.1. Organoleptic Characteristics of *Triphala*

S.No.	Characteristics	Sample		
		Haritaki	***Bhibhitaki***	***Amalaki***
1.	**Colour**	Brownish	Grayish Brown	Grayish Brown
2.	**Odour**	Indistinct	Indistinct	Distinct
3.	**Taste**	Astringent	Astringent	Sour & Astringent

5.4. Quality control Parameters of *Haritaki*

5.4.1. Common name: *Haritaki*

5.4.2. Botanical Name: *Terminalia chuebula* Retz.

5.4.3. Part Used: Fruit

5.4.4. Physicochemical parameters: Dried Sample of *Haritaki* was used to perform the physicochemical parameters.

Table 5.2. Results of Physico-chemical parameters of *Haritaki*

Parameters	Batches						Std. Div.	Standard
	I	II	III	IV	V	VI		
Foreign Matter (%)	0	0	0	0	0	0	0±0.0	NMT 1%
Loss on Drying (105°C)	8.97	9.2	9.2	8.8	7.9	8.8	8.81±2.1	-
Total Ash (%w/w)	2	2.7	2.1	1.9	2.6	2.4	2.2±0.4	NMT 5%

Acid Insoluble Ash (%w/w)	0.35	0.4	0.36	0.42	0.32	0.3	0.35±0.1	NMT 5%
Alcohol Soluble extractive (%w/w)	42.2	42.2	48.2	46.5	50.0	51.3	39.7±0.1	NLT 40%
Water Soluble extractive (%w/w)	59.8	62.2	58.9	63.5	58.7	61.3	60.68±0.9	NLT 60%

The Physico-chemical parameters shows that foreign matter was not present in *Haritaki.* Also, the result for other parameters were within the API standard limit i.e., LOD 8.81±2.1%, Total Ash 2.2±0.4%, Acid insoluble ash 0.35±0.1%, Alcohol soluble extractive 39.7±0.1%, Water soluble extractive 60.68±0.9% respectively, as shown in Table 5.2.

5.4.5. Phytochemical analysis of *Haritaki:*

Aqueous solution of *Haritaki* was prepared and was tested for Phytochemical analysis. Results are shown below in the Table 5.3.

Table 5.3. Phytochemical analysis of *Haritaki*

Compound	Test Performed	Result (Batch)					
		I	II	III	IV	V	VI
Alkaloids	Dragendroff's reagent	+	+	+	+	+	+
Proteins and Amino acids	Millon's reagent	+	+	+	+	+	+
	Ninhydrin reagent	+	+	+	+	+	+
Carbohydrates	Molisch's reagent	+	+	+	+	+	+
	Fehling solution	+	+	+	+	+	+
	Reducing Sugar test	+	+	+	+	+	+
Saponins	Foam Test	+	+	+	+	+	+
Glycosides	Molisch's test	+	+	+	+	+	+

The result for phytochemical analysis shows that all the 6 batches show positive result for various phytochemicals like Alkaloids, Proteins, Amino acids, Carbohydrates, Saponins and Glycosides. * '+'- Positive.

5.4.6. Heavy metal and Microbial Limit Test

Table 5.4. Heavy metal and microbial limit test of *Haritaki*

S.No.	Test Parameters	Results	Specifications
1	**Lead (Pb) ppm**	1.12	<10.0
2	**Arsenic (As) ppm**	<0.50	<3.0
3	**Cadmium (Cd) ppm**	0.04	<0.3
4	**Mercury (Hg) ppm**	<0.03	1.0

As shown in the Table 5.3 the sample of *Haritaki* has very less amount of Heavy metal i.e., 1.12 ppm of Lead, <0.50 ppm As, 0.04 ppm Cd, <0.03 ppm Hg which are under prescribed standard limit and are present in very trace amount.

Table 5.5. Microbial test of *Haritaki*

S.No.	Test Parameters	Results	Specifications
1	**Total bacterial count (cfu/g)**	80	10^5
2	**Yeat and mould count (cfu/g)**	20	10^3
3	**E. Coli**	Absent	Should be absent
4	**S. Aureus**	Absent	Should be absent
5	**P. Aeruginosa**	Absent	Should be absent
6	**Salmonella sp.**	Absent	Should be absent

The result of the total bacterial count, total yeast and mould count shows that 80 cfu/g and 20 cfu/g while E. Coli, S. Aureus, P. Aeruginosa, Salmonella sp. were absent, which are as per the standard specifications.

5.5. Quality control parameters of *Bhibhitaki*

5.5.1. Common name: *Bhibhitaki*

5.5.2. Botanical Name: *Terminalia belerica* (Gaertn.) Roxb.

5.5.3. Part Used: Fruit

5.5.4. Physicochemical parameters: Dried Sample of *Bhibhitaki* was used to perform the physicochemical parameters.

Table 5.6. Results of Physico-chemical parameters of *Bhibhitaki*

Parameters	Batches						Std. Div.	Standard
	I	II	III	IV	V	VI		
Foreign Matter (%)	2	1	3	0	3	2	1.8±0.0	NMT 2%
Loss on Drying (105°C)	8.65	7.8	8.9	8.2	8.63	7.8	8.05±2.1	-
Total Ash (%w/w)	4.4	4.3	3.9	4.5	4.4	4.43	4.17±0.4	NMT 7%
Acid Insoluble Ash (%w/w)	0.6	0.63	0.59	0.66	0.62	0.63	0.6±0.1	NMT 1%
Alcohol Soluble extractive (%w/w)	7.2	7.3	6.9	8.0	7.7	8.1	7.53±0.1	NLT 8%
Water Soluble extractive (%w/w)	49.9	46.2	39.9	35.6	36.6	41.2	49.9±0.9	NLT 35%

The Physico-chemical parameters shows that foreign matter was not present in *Haritaki.* Also, the result for other parameters were within the API standard limit i.e., LOD 8.81±2.1%, Total Ash 2.2±0.4%, Acid insoluble ash 0.35±0.1%, Alcohol soluble extractive 39.7±0.1%, Water soluble extractive 60.68±0.9% respectively.

5.5.5. Phytochemical analysis of *Bhibhitaki*

Table 5.7. Phytochemical analysis of *Bhibhitaki*

Compound	Test Performed	Observation	Result (Batch)					
			I	II	III	IV	V	VI
Alkaloids	Dragendroff's reagent	A reddish-brown precipitate	+	+	+	+	+	+
Tannin	Neutral $FeCl_3$	Buff color ppt	+	+	+	+	+	+
			+	+	+	+	+	+
Amino acids	Ninhydrin Test	Production of deep blue color	+	+	+	+	+	+
			+	+	+	+	+	+
			+	+	+	+	+	+
Saponins	Foam Test	Formation of 2 cm thick layer of foam	+	-	-	-	+	-
Glycosides	Molisch's test	A rose-pink to blood red coloured sol	+	+	+	+	+	+
Phenolic compound	$FeCl_3$ test	Green Colour	+	+	+	+	+	+

The result for phytochemical analysis shows that all the 6 batches show positive results for various phytochemicals like Alkaloids, Tannins, Amino acids, Phenolic compounds, and Glycosides. * '+'- Positive while Saponins shows negative result is more of the batches * '-' Negative.

5.5.6. Heavy metal and Microbial Limit Test

Table 5.8. Heavy metal and microbial limit test of *Bhibhitaki*

S.No.	Test Parameters	Results	Specifications
1	**Lead (Pb) ppm**	0.21	<10.0
2	**Arsenic (As) ppm**	<0.50	<3.0
3	**Cadmium (Cd) ppm**	0.12	<0.3
4	**Mercury (Hg) ppm**	<0.13	1.0

The sample of *Bhibhitaki* has very less amount of Heavy metal i.e., 0.21 ppm of Lead, <0.50ppm As, 0.12ppm Cd, <0.13ppm Hg which are under prescribed limit and are present in very trace amount as shown in the Table 5.6.

Table 5.9. Heavy metal and microbial limit test of *Bhibhitaki*

S.No.	Test Parameters	Results	Specifications
1	**Total bacterial count (cfu/g)**	20000	10^5
2	**Yeat and mould count (cfu/g)**	8000	10^3
3	**E.Coli**	Absent	Should be absent
4	**S. Aureus**	Absent	Should be absent
5	**P. Aeruginosa**	Absent	Should be absent
6	**Salmonella sp.**	Absent	Should be absent

The result of the total bacterial count, total yeast and mould count shows that 20000 and 8000, while E. Coli, S. Aureus, P. Aeruginosa, Salmonella sp. were absent, which are as pre the standard specifications.

5.6. Quality control parameters of *Amalaki*

5.6.1. Common name: *Amalaki*

5.6.2. Botanical Name: *Emblica officinalis* Gaertn.

5.6.3. Part Used: Fruit

5.6.4. Physicochemical parameters: Dried Sample of *Amalaki* was used to perform the physicochemical parameters.

Table 5.10. Results of Physico-chemical parameters of *Amalaki*

Parameters	Batch						Std. Div.	Standard
	I	II	III	IV	V	VI		
Foreign Matter (%)	0	0	0	0	0	0	0±0.0	NMT 3%
Loss on Drying (105°C)	9.15	8.20	8.70	9.20	8.75	8.53	8.75±2.1	-
Total Ash (%w/w)	3.1	2.1	3.4	3.6	3.8	3.3	3.21±0.4	NMT 7%

Acid Insoluble Ash (%w/w)	0.74	0.62	0.69	0.72	0.77	0.63	0.69±0.1	NMT 2%
Alcohol Soluble extractive (%w/w)	50.95	52.62	49.82	46.30	50.82	53.10	50.60±0.1	NLT 40%
Water Soluble extractive (%w/w)	48.04	46.28	45.09	53.20	50.60	53.90	49.51±0.9	NLT 50%

The Physico-chemical parameters show that foreign matter was not present in *Amalaki.* Also, the result for other parameters were within the API standard limit i.e., LOD 8.75±2.1%, Total Ash 3.21±0.4%, Acid insoluble ash 0.69±0.1%, Alcohol soluble extractive 50.60±0.1%, Water soluble extractive 49.51±0.9% respectively.

5.6.5. Phytochemical analysis of *Amalaki*

Table 5.11. Phytochemical analysis of *Amalaki*

Compound	**Test Performed**	**Result (Batch)**					
		I	**II**	**III**	**IV**	**V**	**VI**
Alkaloids	Mayer's reagent	-	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-	-
Carbohydrates	Molisch's reagent	+	+	+	+	+	+
Tannin	Neutral $FeCl_3$	+	+	+	+	+	+
Glycosides	Keller-Killani test	+	+	+	+	+	+

The result for phytochemical analysis shows that all the 6 batches show positive results for various phytochemicals like Carbohydrates, Tannins and Glycosides. * '+'- Positive, and for Alkaloids and Saponins the result was negative *'-' Negative.

5.6.6. Heavy metal and microbial limit test

Table 5.12. Heavy metal and microbial limit test of *Amalaki*

S.No.	Test Parameters	Results	Specifications
1	**Lead (Pb) ppm**	0.25	<10.0
2	**Arsenic (As) ppm**	<0.50	<3.0
3	**Cadmium (Cd) ppm**	0.08	<0.3
4	**Mercury (Hg) ppm**	<0.13	1.0

The sample of *Amalaki* has very less amount of Heavy metal i.e., 0.25 ppm of Lead, <0.50ppm As, 0.08ppm Cd, <0.13ppm Hg which are under prescribed limit and are present in very trace amount.

Table 5.13. Heavy metal and microbial limit test of *Amalaki*

S.No.	Test Parameters	Results	Specifications
1	**Total bacterial count (cfu/g)**	14000	10^5
2	**Yeat and mould count (cfu/g)**	28000	10^3
3	**E. Coli**	Absent	Should be absent
4	**S. Aureus**	Absent	Should be absent
5	**P. Aeruginosa**	Absent	Should be absent
6	**Salmonella sp.**	Absent	Should be absent

The result of the total bacterial count, total yeast and mould count shows that 14000 and 28000 while E. Coli, S.Aureus, P.Aeruginosa, Salmonella sp. were absent, which are as pre the standard specifications.

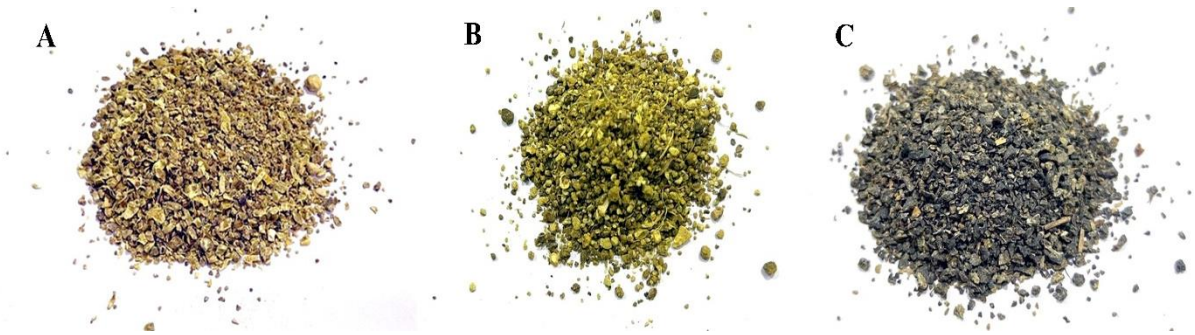


Fig. 5.1. (A) *Haritaki* coarse powder (B) *Bhibhitaki* coarse powder (C) *Amalaki* coarse powde

5.7. HPTLC analysis of *Haritaki, Bhibhitaki* and *Amalaki*

Table 5.14. ***HPTLC analysis of Haritaki, Bhibhitaki and Amalaki***

S.No.	Solvent system	Rf value of standard (Gallic acid)	Rf value of *Amalaki* (Gallic acid)	Rf value of *Bhibhitaki* (Gallic acid)	Rf value of *Haritaki* (Gallic acid)
	Toluene:	-	0.07	0.06	0.32
	Ethyl acetate:	-	0.38	0.28	0.42
	Formic acid	0.51	0.54	0.53	0.52
	(5:3.5:0.5)	-	-	0.66	0.66

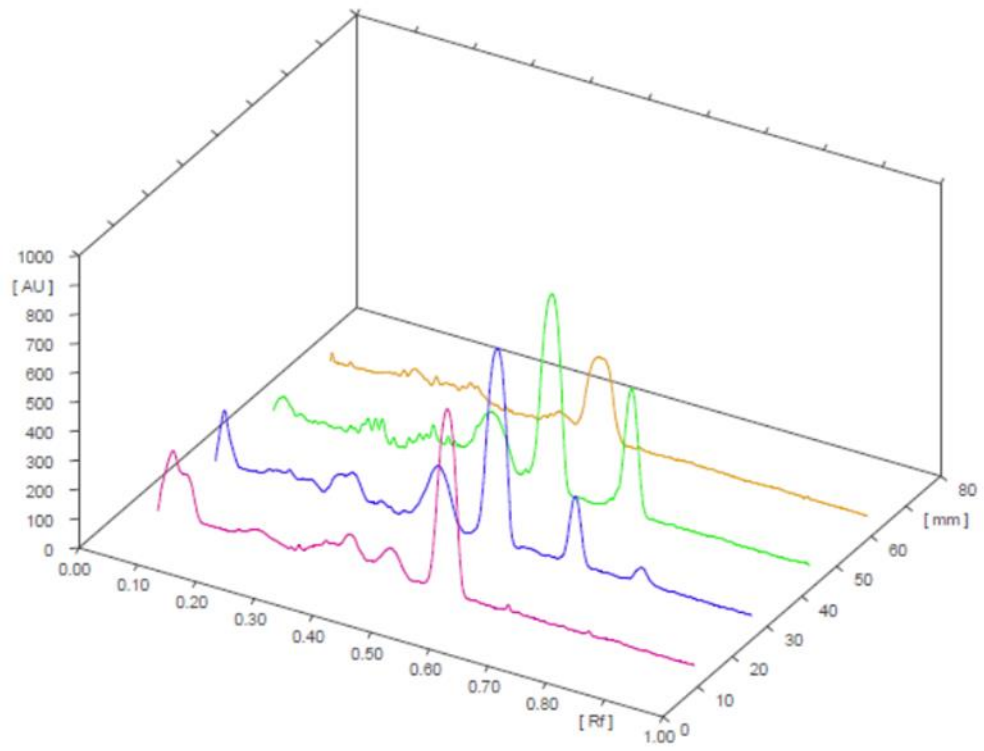


Fig.5.2. Chromatogram for HPTLC analysis of *Haritaki*, *Bhibhitaki* and *Amalaki*

5.8. Collection of *Lauha:*

Iron scrap was purchased from the local market

5.9. Authentication of *Lauha*:

Lauha authentication was done from Multani Pharmaceutical Pvt. Ltd. The authentication report number is AYR20220317101

5.10. Elemental Analysis of *Lauha*:

Elemental analysis for *Lauha* was performed in *Varsha* Bullion Elemental Analab, Mumbai.

Table 5.15. Percentage of component present in the Raw *Lauha*

S.No.	Component	Result (mass%)
1	Fe	98.4
2	Mn	0.611
3	Si	0.513
4	S	0.152
5	P	0.117
6	Cu	0.082
7	Ni	0.064
8	Cr	0.055
9	Mo	0.010
10	Mg	<0.0001

The elemental analysis of raw *Lauha* indicates that the presence of Iron (Fe) in the sample is maximum i.e., 98.4% and very trace amount of other compounds are present. Due to larger particle size the analysis was done through FP method. The Fundamental Parameter (FP) method is a technique used in X-ray fluorescence (XRF) analysis to determine the concentration of chemical elements in a sample.

Fig. 5.3. Iron Scrape

5.11. *Lauha Shodhana*:

Steps involved in *Shodhana* process are as follows: - *Shodhana* of *Lauha* involved two methods i.e., first *Samanya Shodhana* then *Vishesh Shodhana*.

5.11.1. Process of *Samanya Shodhana*:

Samanya Shodhana was done with *Ashuddha Lauha*, *Lauha* was taken and quenched in *Taila, Takra, Kulattha Kwatha, Go-Mutra* and *Kanji* one by one for seven times in each liquid media, every time fresh liquid media was used, the process was repeated in 6 batches.

5.11.1.1. Ingredients: *Aashuddha Lauha, Taila, Takra, Go-Mutra, Kulattha Kwatha and Kanji.*

- *Taila*: - Marketed *Taila* was used for the process.
- *Takra: - Takra* was prepared by adding equal quantity of water to curd (*Dadhi*) and diluting the same by churning. The pH of *Takra* prepared was 4.9.
- *Kanji*: - *Kanji* was prepared by placing rice along with small quantity of white radish cutted into pieces were placed in an earthen pot and 4 times of water was added. The mixture was kept for fermentation for 3 weeks. The prepared sour mixture was used for *Shodhana*.

Table 5.16. Preparation of *Kanji*

Sr.No.	Parameters	Batch					
		I	II	III	IV	V	VI
1.	Anna (kg)	18	18	18	18	18	18
2.	Water (l)	54	54	54	54	54	54
3.	pH	6.5	6.5	6.5	6.5	6.5	6.5
4.	Atmospheric temperature(°C)	30	29	29	30	29	29
5.	Total duration (days)	14	15	15	15	16	15

Final yield of Kanji prepared was 69.43 ± 0.87, In 14-15 days the Kanji was prepared completely, initially the pH was observed 6.5 which was reduced after the completion of the process.

Table 5.17. Observation during *Kanji* Preparation

Sr. no.	Observation		Batch					
			I	II	III	IV	V	VI
1.	Effervescence (days)	Prsnt	2nd	3rd	2nd	2nd	3rd	2nd
2.	Burning match test (days)	Absnt	3rd	4th	3rd	4th	4th	3rd
		Prsnt	15th	15th	15th	15th	15th	15th
3.	Fermentation completion		Successful	Successful	Successful	Successful	Successful	Successful
4.	pH		3.5	3.5	3.5	3.5	3.5	3.5

Prsnt- Preasent, Absnt- Absent

Effervescence was present from day 2nd, burning match test was initially absent but on 15th day it was present, showing that the completion of the process, pH was 3.5 after the completion of the process.

Table 5.18. Observation during Kanji Preparation

Observation	Batch						STD
	I	II	III	IV	V	VI	
Final yield (ml)	69.9	70.2	68.7	68.4	70.7	68.7	69.43 ± 0.87

- ***Kulattha Kwatha: -*** *Kulatha Kwatha* was prepared by adding 16 times of water then reducing to 1/4th. pH of *Kulattha Kwatha* was 5.6.

Table 5.19. Preparation of *Kulattha Kwatha*

Sr. no	Ingredients	Batch						Total
		I	II	III	IV	V	VI	
1.	***Kulattha* (*Dolichos***	3.5	3.5	3.5	3.5	3.5	3.5	21

	***biflorus*) (kg)**							
2.	**Water (RO) (L)**	56	56	56	56	56	56	336
3.	**Total time taken for the preparation of *Kwatha* (hrs.)**	8	8	8	8	8	8	8
4.	**Final quantity of *Kwatha* obtained (L)**	14.450	14.385	14.250	14.470	14.300	14.474	14.38±0.08

Total *Kwatha* prepared was 14.38±0.08 (L), in around 8 hrs *Kwatha* was prepared.

• ***Go-Mutra*: -** Fresh *Go-Mutra* was collected at the time of *Shodhana.* The pH of Go-mutra was in the range of 7.6-8.0.

Equipment: *Lauha Kadai,* spatula, gas stove, Lighter

Results: In each batch 1000gm of *Aashuddha Lauha was* taken for *Samnya Shodhana* process. The results are mentioned in the tables below.

Table 5.20. Weight of *Lauha* after *Samanya Shodhana*

Media	**Wt. of *Lauha* after *Shodhana* (gm)**						**Std. Dev.**
	Batch						
	I	**II**	**III**	**IV**	**V**	**VI**	
Taila	992	995	995.4	994.5	997.3	994	994.7±1.58
Takra	982	989	982	985.3	984.7	982	984.1±2.54

Go-mutra	984	987.8	979	981	974	978	980.6±4.40
Kulattha Kwatha	973	969.4	972.2	979.3	965.6	966.8	971.05±4.54
Kanji	968	962.7	965.2	970.5	957.5	958.3	963.7±4.75

During *Samanya Shodhana* process, total weight loss was found to be around 40gm, the repeated process of heating and quenching leads to destruction in the particles of the *Lauha*, leads to increased brittleness, reduction in hardness which further leads to reduction in particle size. After *Samanya Shodhana* colour, shape, size of particles was changed. This process made the metal brittle and removed various impurities making it suitable for further process.

Fig 5.4. Picture showing the different steps of *Vishesh Shodhana*. (A) *Ashuddha Lauha*, (B) Quenching in *Taila* (C) Quenching in *Kulatha Kwatha* (D) Quenching in *Takra* (E) Queching in *Go-mutra* (F) Quenching in *Kanji*

5.11.2. Process of *Vishesh Shodhana*:

5.11.2.1. Ingredients: *Samanya Shodhit Lauha, Triphala Kwatha*

5.11.2.2. Preparation of *Yavkut*: Individual *Yavkut* of each fruit was prepared. Details are mentioned below.

Table 5.21. Table showing the amount of *Yavkut* prepared in each batch

S.No.	Ingredients	Batch (Kg)						Std. Dev.
		I	II	III	IV	V	VI	
1	***Amlaki***	2.76	2.75	2.85	2.88	2.72	2.88	2.80±0.06
2	***Haritaki***	2.85	2.97	2.85	2.87	2.92	2.82	2.88±0.05
3	***Bhibhitaki***	2.78	2.74	2.86	2.82	2.70	2.88	2.78±0.06

3kg of each fruit was taken in 6 batches and individually converted into coarse powder. Individual pounding was done to ensure proper size reduction of each fruit, since *Amalaki* takes more time as compared to *Haritaki* and *Bhibhitaki*. During the process around 0.6% ±0.06 of *Amalaki*, 0.5%±0.0r of *Haritaki* and 0.6%±0.06 of *Bhibhitaki* loss was observed, which was due to sticking of the material on the surface of the *Khalwa Yanta*.

5.11.2.3. Preparation of *Triphala Yavkut*: *Triphala Yavkut* was prepared by homogenously mixing *Yavkut* of individual 3 drugs i.e, *Amalaki, Haritaki, Bhibhitaki* in equal proportion.

Table 5.22. Table showing the amount of *Triphala Yavkut* prepared in each batch

S.No.	Ingredient	Batch (Kg)					
		I	II	III	IV	V	VI
1	***Haritaki***	2.5	2.5	2.5	2.5	2.5	2.5
2	***Bhibhitaki***	2.5	2.5	2.5	2.5	2.5	2.5
3	***Amalaki***	2.5	2.5	2.5	2.5	2.5	2.5
4	**Total**	7.5	7.5	7.5	7.5	7.5	7.5

Homogenous mixing was done by taking all the three ingredients in a vessel and mixing thoroughly by hands. This step ensures that the *Triphala Yavkut* prepared has equal proportion of each fruit in each batch. 6 batches were prepared.

5.11.2.4. Preparation of *Triphala Kwatha*: *Triphala Kwatha* was prepared as per the method mentioned in 4.2.1. The details are shown below in the table. And the process of making *Triphala Kwath* is shown in pictures below. *Kwatha* was prepared in 6 batches.

Ingredients: *Triphala Kwatha,* water

Equipment's used: Gas stove, vessels, weighing balance, measuring cylinder, lighter, spatula, starrier.

Result: In each batch 15 lit. of the *Kwatha* was prepared and used for the process of *Shodhana.*

Table 5.23. *Triphala Kwatha* prepared in each batch

Ingredients	Batch						Std. Dev.
	I	II	III	IV	V	VI	
Amount of *Triphala* taken (kg)	7.5	7.5	7.5	7.5	7.5	7.5	-
Amount of water added (lit)	60	60	60	60	60	60	-
Reduced to	1/4th	1/4th	1/4th	1/4th	1/4th	1/4th	-
Final *Kwath* obtained (lit)	15.5	15.3	15.3	15.2	15.3	15.1	15.28±0.12

The final *Kwatha* obtained from each batch was around 15.28±0.12. in each batch 7.5 kgs of *Triphala* was taken and 60 lit of water was added and reduced to 1/4th. The obtained *Kwatha* was used for further process.

Evaluation Parameters of *Triphala Kwatha*: The prepared *Kwatha* was first analyzed based on Organoleptic Characteristics, Physico-chemical analysis Qualitative anslysis, Heavy metal analysis and microbial overload.

Table 5.24. Organoleptic Characteristics of *Triphala Kwatha*

S.No.	Organoleptic Characters	Observation

1	**Colour**	Dark Brown
2	**Odour**	Characteristic
3	**Appearance**	Dark
4	**Taste**	Bitter

The Organoleptic Characteristics of *Triphala Kwatha* shows that the Colour of obtained *Kwatha* was Dark brown, odour characteristic appearance dark and the taste was bitter.

Table 5.25. Physico-chemical analysis of *Triphala Kwatha*

Parameters	**Batches**						**Std. Div.**
	I	**II**	**III**	**IV**	**V**	**VI**	
Total Solid content (%w/v)	2.68	2.93	2.62	2.87	2.86	2.69	2.7±0.11
pH	6.2	6.0	6.7	6.2	6.4	6.5	6.3±0.2
Specific Gravity	1.007	1.005	1.007	1.007	1.008	1.003	1.0±0.001
Viscosity	1.233	1.227	1.232	1.234	1.229	1.230	1.2±0.002
Refractive Index	1.347	1.329	1.332	1.345	1.348	1.336	1.3±0.007

Physico-chemical analysis of *Triphala Kwatha* indicates that the Total Solid content, pH, Specific Gravity, Viscosity, Refractive Index were 2.7±0.11, 6.3±0.2, 1.0±0.001, 1.2±0.002, 1.3±0.007 respectively, which are under prescribed standard limit.

Table 5.26. Phytochemical analysis of *Triphala Kwatha*

Compound	**Test Performed**	**Result (Batch)**					
		I	**II**	**III**	**IV**	**V**	**VI**
Alkaloids	Mayer's reagent	+	+	+	+	+	+
Saponins	Foam test	+	+	+	+	+	+
Carbohydrates	Molisch's reagent	+	+	+	+	+	+
Tannin	Neutral $FeCl_3$	+	+	+	+	+	+
Glycosides	Keller-Killani test	+	+	+	+	+	+
Phenols	$FeCl_3$ test	+	+	+	+	+	+

Protein	Millon's reagent	+	+	+	+	+	+
	Ninhydrin reagent	+	+	+	+	+	+

The result for phytochemical analysis shows that all the 6 batches show positive results for various phytochemicals like Alkaloids, Saponins, Carbohydrates, Tannins, Amino acids, Phenolic compounds proteins and Glycosides. * '+'- Positive.

Table 5.27. Heavy metal test of *Triphala Kwatha*

S.No.	Test Parameters	Results	Specifications
1	**Lead (Pb) ppm**	0.15	<10.0
2	**Arsenic (As) ppm**	<0.30	<3.0
3	**Cadmium (Cd) ppm**	0.08	<0.3
4	**Mercury (Hg) ppm**	<0.13	1.0

The above table shows that the *Kwatha* prepared has very less amount of Heavy metal i.e., 0.15 ppm of Lead, <0.30ppm As, 0.08ppm Cd, <0.13ppm Hg which are under prescribed limit and are present in very trace amount.

Table 5.28. Microbial limit test of *Triphala Kwatha*

S.No.	Test	Results	Specifications
1	**Total bacterial count (cfu/g)**	11032	10^5
2	**Yeat and mould count (cfu/g)**	285	10^3
3	**E. Coli**	Absent	Should be absent/g
4	**S. Aureus**	Absent	Should be absent/g
5	**P. Aeruginosa**	Absent	Should be absent/g
6	**Salmonella sp.**	Absent	Should be absent/g

The result of the total bacterial count, total yeast and mould count shows that 11032 and 285 while E. coli, S.Aureus, P.Aeruginosa, Salmonella sp. were absent, which are as pre the standard specifications.

Equipment's: *Lauha Kadai,* spatula, gas stove, Lighter

Result: In each batch 1 kg *Lauha Shodhana* was done. And the final *Lauha* obtained after *Shodhana* was stored in a container and used for further process as per the requirements. *Shodhana* was done in 6 batches, observation is mentioned in the table below.

Table 5.29. Weight of *Lauha* after *Vishesh Shodhana*

No. of process	Batch (gm)						Std. Dev
	I	II	III	IV	V	VI	
1	998	993	995	994	994	995	994.8±1.5
2	992	992	995	990	991	991	991.8±1.5
3	989	985	986	989	986	984	986.5±1.8
4	985	983	985	989	982	981	984.1±2.6
5	983	980	979	981	984	982	981.5±1.7
6	981	976	978	981	980	979	979.1±1.7
7	977	975	977	980	981	979	978.1±2.0

Fragments of *Lauha* became more brittle as compared to *Samanya Shodhana*. Particle were converted into more small particles, which is due to the presence of tannins present in *Triphala* which leads to the destruction of the particles. The colour of *Lauha* was changed to black, and around 20gm loss was observed during the process.

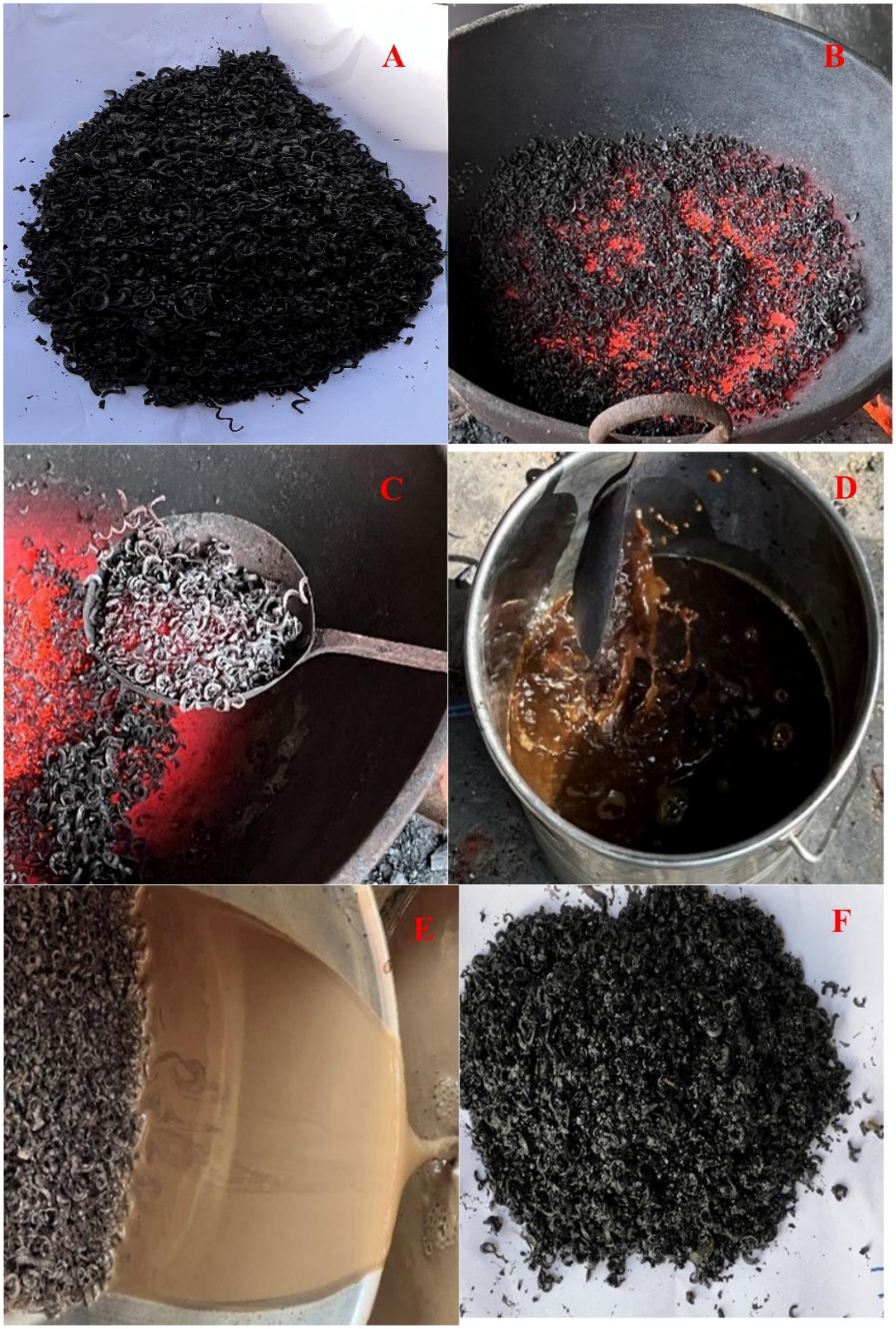


Fig. 5.5. Different stages of *Vishesh Shodhana* (A) *Samanya Shodhit Lauha (*B*) Lauha* subjected to red hot (C) Red hot *Lauha* (D) *Lauha* quenched in *Triphala Kwatha* (E) Filtration (F) *Lauha* after *Vishesh Shodhana*

5.12. Data for heating pattern of *Shodhana:*

Table 5.30. Table showing the heating pattern during the process of *Shodhana*

Time and temperature	***Taila***						
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
Time taken to become red hot (minutes)	68	59	52	48	46	40	42
Temperature at the time of red hot *Lauha* (°C)	700	723	728	732	735	741	739
	Takra						
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
Time taken to become red hot (minutes)	58	46	42	44	38	40	42
Temperature at the time of red hot *Lauha* (°C)	742	758	757	762	758	750	748
	Go-mutra						
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
Time taken to become red hot (minutes)	55	49	52	53	55	60	58
Temperature at the time of red hot *Lauha* (°C)	734	742	738	743	745	743	746
	Kulattha Kwatha						
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
Time taken to become red hot (minutes)	55	49	52	49	55	59	57
Temperature at the time of red hot *Lauha* (°C)	732	752	748	747	745	749	746
	Kanji						
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
Time taken to become red hot (minutes)	52	58	55	51	53	57	54

Temperature at the time of red hot *Lauha* (°C)	732	741	739	733	735	743	742
	Triphala Kwatha						
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
Time taken to become red hot (minutes)	55	58	57	51	55	56	52
Temperature at the time of red hot *Lauha* (°C)	731	740	734	737	736	747	750

Table showing the heating pattern during *Shodhana*, it was observed that around 1-1.5 hrs were taken each time to red hot the *Lauha* and temperature required was 700-750°C.

5.13. *Lauha Marana*:

***Marana* of *Lauha* was conducted in two different methods:**

5.13.1. Method 1: *Trividpaka (Bhanupaka, Sthalipaka, Putapaka)*

5.13.1.1. Process of *Bhanupaka:* *Shodhit Lauha* was taken 500 gm and was kept in a *Kharal* to it *Triphala Kwatha* was added and the *Kharal* was kept in high Sunlight, till the decoction (*Triphala Kwatha*) evaporated completely. Once the decoction (*Triphala Kwatha*) was evaporated completely then the *Lauha* was taken and weighed and again according to the weight of *Lauha* again *Triphala* was added to it. This process was done 7 times. Details of the process is given below in the Table 3.1. and relevant pictures of the process are added.

5.13.1.1.1. Method of making *Triphala Kwatha* for *Bhanupaka* Process: For this equal amount of *Triphala* was taken as of *Lauha* and to it 2 times water was added and reduced to 1/4th. This *Triphala Kwatha* was added to *Lauha* each time by increasing the quantity.

Table 5.31. Observation during *Bhanupaka* process

No. of B.P.	*Kwatha* added (ml)	Batch (wt. in kg)						Std. Dev.
		I	II	III	IV	V	VI	
1	250	0.63	0.69	0.63	0.66	0.62	0.68	0.65±0.02
2	315	0.76	0.8	0.81	0.78	0.76	0.75	0.77±0.02
3	380	0.89	0.88	0.9	0.91	0.85	0.87	0.88±0.01
4	445	1.2	1.23	1.1	1.32	1.21	1.31	1.22±0.07
5	600	1.31	1.31	1.31	1.34	1.32	1.33	1.32±0.01
6	655	1.43	1.41	1.41	1.44	1.41	1.4	1.41±0.01
7	715	1.54	1.52	1.53	1.55	1.52	1.56	1.53±0.01

During the *Bhanupaka* process, equal amount of *Triphala Kwatha* was added according to the weight of the *Lauha* (everytime the amount of *Triphala Kwatha* was increased as per the weight of the *Bhasma*) as mentioned in the above table, during the *Bhanupaka* process total 1 kg weight gain was observed i.e., from 500gm initial weight to final weight after 7th *Puta* it was around 1.53kg which indicates around 95% of the weight gain in the *Lauha* which was due to the addition of *Triphala Kwatha* in it. In this process

most of the *Lauha* became soft and brittle colour was jet black. Later the *Lauha* was converted into powder by pounding.

Fig 5.6. Picture showing *Bhanupaka* process of *Lauha*

5.13.1.2. Process of *Sthalipaka*: After the completion of *Bhanupaka* process, the *Lauha* was taken and processed for *Sthalipaka*. In this process, in a steel vessel equal amount of *Triphala Kwatha* and *Bhanupakawa Lauha* was added and subjected to heat till all the liquid media evaporated completely. After complete evaporation, liquid media (*Triphala Kwatha*) was added again according to the weight of completely dried *Lauha.* The process was done for 3 times. The detail of the process is explained in the table below and picture of the process is attached below.

Table 5.32. Weight of *Lauha* after *Sthalipaka* process

No. of Process	Batch						Std. Dev.
	I	II	III	IV	V	VI	
1st	1.65	1.68	1.57	1.62	1.59	1.62	1.62±0.03
2nd	1.78	1.75	1.78	1.74	1.69	1.72	1.74±0.03
3rd	1.92	1.98	1.95	1.92	1.9	1.89	1.92±0.03

During the *Sthalipaka* process after 1st *Puta* the weight was not increased much, colour of *Lauha* was dark brown particles were hard, after 2nd and 3rd *Puta* increase in weight was observed which was due to the presence of solid content in *Triphala Kwatha.* Also, after 2nd *Puta* the colour of the *Lauha* was slightly darker in comparison with the 1st *Puta*, particle size of the *Lauha* was decreased slightly and after 3rd *Puta* colour of *Lauha* was black and the particle were brittle and became soft, easily breakable. The *Paka* during *Sthalipaka* process was *Madhyam Paka*.

Fig 5.7. Picture showing different stages of *Sthalipaka*

5.13.1.3. Process of *Putapaka*: After the completion of *Sthalipaka* process the *Lauha* was taken and further processed for *Putapaka*. In this process firstly *Lauha* was subjected to *Mardana* after the completion of *Mardana, Chakrikas* were prepared and dried under sunlight. Then after that dried *Chakrika* were subjected to *Gajaputa* (at 800℃ for 3 hrs.). Detail of the weight during *Putapaka* method is given below in the table and pictures of the process is attached below.

Table 5.33. Weight of *Bhasma* during *Putapaka* Process

No. of *Puta*	Batch						Std. Dev.
	I	II	III	IV	V	VI	
1st	392	432	412	389	423	404	408.6±15.5
2nd	495	498	502	497	499	501	498.6±2.3
3rd	504	504	502	506	505	501	503.6±1.6
4th	512	515	517	515	519	512	515±2.5
5th	520	521	517	526	528	522	522.3±3.6
6th	528	531	529	532	535	530	530.8±2.2
7th	532	538	538	537	531	537	535.5±2.8
8th	538	537	532	542	539	543	538.5±3.5
9th	542	540	546	541	543	548	543.3±2.8
10th	547	546	551	538	540	550	545.3±4.8
11th	550	552	557	548	546	552	550.8±3.4
12th	552	554	557	550	559	551	553.8±3.2

During the *Putapaka* Process, initially 0.5kg *Lauha* was taken, *Bhawana* was given, *Chakrikas* were prepared, dried and then subjected for *Puta*, during the *Puta* process maximum temperature given was 800°C after attaining the maximum temperature the temperature was maintained for 30 mins and then automatically the temperature was reduced. After the first *Puta*, gradual decrease in the weight was observed which was due to the burning of *Triphala Kwatha,* added during the *Bhanupaka* and *Sthalipaka*. After that in rest all *Puta*, gradual increase in the weight was observed which was due the presence of total solid content of *Triphala* in it.[81]

5.13.2. Observation: The following observation were observed during the process of *Bhasma*, the method prepared by this process, started passing *Varitaratwa* and *Rekhapurnatwa* test but did not attain the desired color and other tests like *Nischandratwa* etc, the observation has been listed below in the table. Pictures of the tests are also attached below.

Table 5.34. Observation during the (*Trividpaka) Putapaka* process

No. of *Puta*	***Varitaratwa* test**	***Rekhapurnatwa* test**
1	Not Present	Not Present
2	Not Present	Not Present
3	Not Present	Not Present
4	Very slightly observed	Very slightly observed
5	Increased floating particles	Some particles were fine
6	Increased floating particles	*Bhasma* started filled in the furrows
7	Floating for some time then settled down	*Bhasma* filled in the lines
8	Most of the *Bhasma* was floating	*Bhasma* filled the lines, but some particles were bigger in size
9	Most of the *Bhasma* was floating	*Bhasma* filled in the lines
10	Particles were lighter and floating properly	*Bhasma* was finer, slight irritation was observed during rubbing.
11	*Bhasma* was floating properly; no shrinking was observed.	*Bhasma* filled in the lines properly
12	*Bhasma* was floating completely	*Bhasma* filled in the lines completely

The prepared *Bhasma* was checked after each *Puta*, for the quality and preparation of *Bhasma*, it was observed that after 5th *Puta* the *Bhasma* started showing positive results for *Rekhapurnatva* and *Varitaratwa.* At 12th *Puta* the *Bhasma* was completely prepared and was black in colour with no shinny particles in it.

Fig. 5.8. Picture showing different stages of *Bhawana* and *Chakrikakaran* in *Trividpaka* process.

5.13.3. Method 2: *Putapaka*:

In this *Putapaka* method *Shuddha Lauha* was taken and subjected for *Mardana* along with *Bhaat* and *Triphala Kwatha* for 6 hours then *Chakrikas* were made and dried under sun light. After the *Chakrikas* were dried completely it was subjected to *Saravasamputa* and *Gajaputa* (at 800℃ for 3 hrs.) was given. In this process total 15 *Puta* were given, then the *Bhasma* of desired quality was obtained.

Table 5.35. Weight of *Bhasma* during *Putapaka* process

No. of *Puta*	Batches (gm)						Std. dev.
	I	II	III	IV	V	VI	
1st	448	450	452	449	448	451	449.6±1.49
2nd	503	500	502	505	501	503	502.3±1.59
3rd	507	501	505	508	509	505	505.8 ±2.60
4th	511	514	514	517	513	518	514.5 ±2.36
5th	520	521	525	523	521	518	521.3 ±2.21
6th	526	524	529	531	526	522	526.3 ±2.98
7th	526	527.7	531	527	527	525	527.2 ±1.87
8th	531.8	530	535	528	530	529	530.6 ±2.26
9th	538	533	542	539	537	534	537.1 ±3.02
10th	549	545	547	543	547	549	546.6 ±2.13
11th	552	550	551	550	553	552	551.3 ±1.10
12th	557	553	556	556	559	560	556.8 ±2.26
13th	564	563	559	553	552	551	573.6 ±5.25
14th	566	565	563	559	558	559	561.6 ±3.14
15th	567	569	570	565	564	571	567.6 ±2.56

During the *Putapaka* Process, initially 0.5kg *Lauha* was taken, *Bhawana* was given, *Chakrikas* were prepared, dried and then subjected for *Puta*, during the *Puta* process maximum temperature given was 800°C after attaining the maximum temperature the temperature was maintained for 30 mins and then automatically the temperature was reduced. After the first *Puta*, gradual decrease in the temperature was observed which was comparatively less from the *Trividpaka* Process, because in this method no

excessive *Triphala* was present. After that in all *Puta*, gradual increase in the weight was observed which was due the presence of *Triphala* in ash form.

5.13.3.1. Observation: The following observation was observed during the process of *Bhasma*, it has been listed below in the table.

Table 5.36. Observation of *Bhasma* during *Putapaka* method

S.no	*Puta*	Observation
1.	1st	Color black, no test passed, particle size was large
2.	2nd	Color black, material become slightly soft, no test passed
3.	3rd	Color dusty black, particle size reduced
4.	5th	Color slightly turned brown initially but on trituration turned black again, no test passed
5.	7th	Pellets were hard, color blackish red
6.	9th	Brownish red color, particle size reduced
7.	11th	Brownish color, pellets slightly fragile, slight *Varitaratwa* observed
8.	12th	Brownish color, hardness of pellets reduced slightly, slight *Varitaratwa* observed.
9.	14th	75% *Rekhapurnatva* and 70% *Varitaratva* test positive, metallic taste absent.
10.	15th	Metallic taste present, *Pakwa Jambu* varna color, *Bhasma* passed all the *Pariksha*.

The prepared *Bhasma* was checked after each *Puta*, for the quality and preparation of *Bhasma*, in this process it was observed that after 9th *Puta* the *Bhasma* started showing positive results for *Rekhapurnatva* and *Varitaratwa*. At 15th *Puta* the *Bhasma* was completely prepared and was black in colour with no shinny particles in it.

5.14. Data for time, temperature and duration of *Puta* during *Marana*:

The table below represents the desired temperature required, average time required to reach maximum temperature during *Puta* process, for how the desired temperature is maintained and the maximum duration of heat.

Table 5.37. Time, temperature and duration of *Puta* during *Marana*

Parameters	Batch						Std. Dev.
	I	II	III	IV	V	VI	
Max. desired temperature (°C)	800						-
Time taken to reach the max temperature (mins)	150	156	150	162	157	155	155±4.16
Temperature maintained (min)	28	32	27	29	30	32	29.6±1.88
Total duration of heat (mins)	178	188	177	191	187	187	184.6±5.24

Fig 5.9. Picture showing different stages of *Bhawana* and *Chakrikakaran* in *Putapaka*

5.15. Evaluation Parameters:

Evaluation was done based on two parameters that is based on classical parameters and on the basic of modern parameters.

5.15.1. Evaluation of *Bhasma* through Classical parameters:

Rekhapurnatava, Nischandratva, Varitaratva, Unnaman and *Nirdhoom* these parameters were analyzed after each *Puta* observations are mentioned below in the Table 5.38

Table 5.38. Result of *Trividpaka Bhasma* Classical evaluation

Parameters	Batch					
	I	II	III	IV	V	VI
Rekhapurnatava	+	+	+	+	+	+
Nischandratva	+	+	+	+	+	+
Varitaratva	++	++	++	++	++	++
Unnaman	++	++	++	++	++	++
Nirdhoom	+	+	+	+	+	++

'+'- Passed, '++'- more clear result

Table 5.39. Result of *Putapaka Bhasma* Classical evaluation

Parameters	Batch					
	I	II	III	IV	V	VI
Rekhapurnatava	+	+	+	+	+	+
Nischandratva	+	+	+	+	+	+
Varitaratva	+	+	+	+	+	+
Unnaman	+	+	+	+	+	+
Nirdhoom	+	+	+	+	+	+

'+'- Passed

The prepared *Bhasma* shows positive results for all the classical parameters like *Rekhapurnatava, Nischandratva, Varitaratva, Unnaman, Nirdhoom.* Both the *Bhasma* passed all the parameters, but *Trividpaka Bhasma* shows more prominent results then *Putapaka Bhasma*.

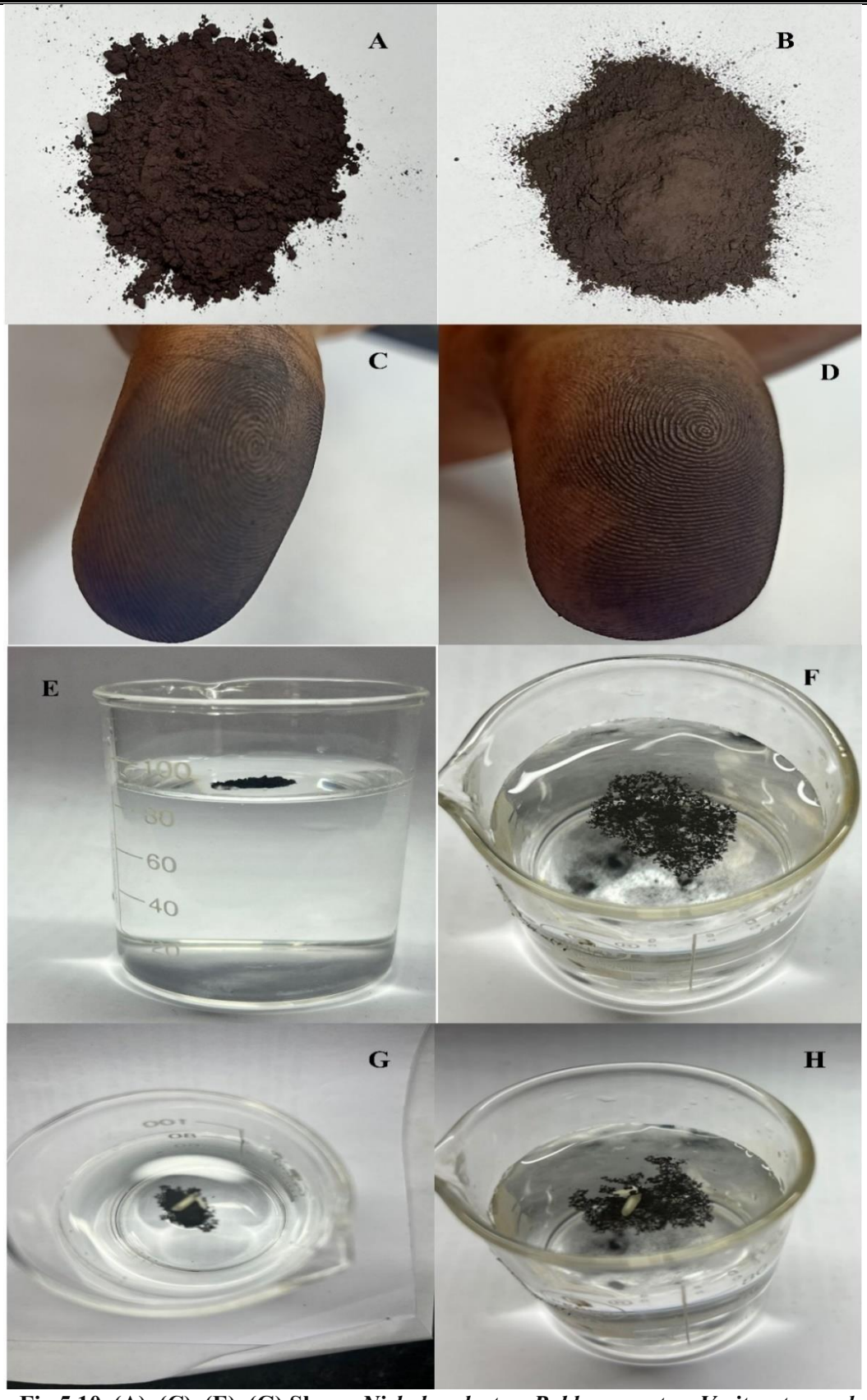


Fig.5.10. (A), (C), (E), (G) Shows *Nishchandratva, Rekhapurnatva, Varitaratva and Unman* in *Trividpaka* (B), (D), (F), (H) Shows *Nishchandratva, Rekhapurnatva, Varitaratva and Unman* in *Putapaka.*

5.15.2. Evaluation of *Bhasma* through Modern parameters:

5.15.2.1. Physico-Chemical analysis: Various parameters like Organoleptic characteristics, loss on drying, ash value, Acid insoluble ash and water-soluble ash were evaluated. Each test was repeated 7 times, the average result is mentioned below in the Table 5.40.

Table 5.40. Result of Organoleptic analysis of *Bhasma*

Parameters	***Trividhpaka Bhasma***	***Putapaka Bhasma***
Organoleptic Characteristics		
Color	*Pakwa Jambu Varna*	Dull black to *Jambu Varna*
Taste	Tasteless	Tasteless
Texture	Amorphous	Amorphous

The organoleptic Characteristic shows that the taste and texture of both the *Bhasma* was same i.e., Tasteless and Amorphous while the color of the *Bhasma* was different i.e., *Pakwas Jambu Varna* in *Trividpaka Bhasma* and Dull black to *Jambu Varna* in *Putapaka Bhasma.*

Table 5.41. Result of Physico-chemical analysis of *Bhasma*

Parameters	***Trividpaka Bhasma* (Batch)**						**Std. Dev.**
	I	**II**	**III**	**IV**	**V**	**VI**	
Loss on drying (w/w%)	0.3	0.36	0.28	0.32	0.31	0.30	0.3±0.02
Ash Value (w/w%)	99.24	98.26	98.22	98.45	99.13	98.2	98.5±0.43
Acid-insoluble ash (w/w%)	27.46	27.42	27.59	28.12	27.34	27.33	27.5±0.27
Water-soluble ash (w/w%)	4.38	4.47	4.44	4.39	4.38	4.56	4.43±0.06
Parameters	***Putapaka Bhasma* (Batch)**						**Std. Dev.**
	I	**II**	**III**	**IV**	**V**	**VI**	
Loss on drying (w/w%)	0.3	0.26	0.29	0.32	0.31	0.35	0.3±0.02
Ash Value (w/w%)	98.24	100.36	98.28	99.45	99.42	98.2	98.9±0.81

Acid-insoluble ash (w/w%)	27.43	28.47	27.59	29.12	27.34	28.73	28.1±0.69
Water-soluble ash (w/w%)	4.33	4.52	4.39	4.46	4.37	4.56	4.4±0.08

Physico-chemical test for the *Bhasma* were repeated 6 time so that standardized results were obtained. Loss on drying Ash Value Acid-insoluble ash Water-soluble ash for *Trividpaka* was 0.3±0.02, 98.5±0.43, 27.5±0.27, 4.43±0.06 while for *Putapaka Bhasma* was 0.3±0.02, 98.9±0.81, 28.1±0.69, 4.4±0.08 respectively. Both the *Bhasma* had similar results indicating that both the *Bhasma* have similar physico-chemical properties.

5.15.2.2. Semi-Quantitative analysis: Both *Bhasma* samples were evaluated for semi-qualitative analysis by FP analysis method. Results for the various parameters are mentioned in the table below.

Table 5.42. Table showing the compound present in the *Bhasma*

No.	**Component**	**Result (mass %)**	
		Trividhpaka Bhasma	***Putapaka Bhasma***
1	**Fe2O3**	92.3	86.6
2	**SiO2**	3.96	6.85
3	**Al2O3**	1.12	1.13
4	**K2O**	0.645	1.05
5	**CaO**	0.527	0.955
6	**SO3**	0.428	0.914
7	**P2O5**	0.360	0.906
8	**Cl**	-	0.562
9	**CuO**	0.0725	0.422
10	**MnO**	0.0653	0.257
11	**Cr2O3**	0.416	0.159
12	**ZnO**	0.0233	0.0492
13	**NiO**	0.1	0.0316
14	**SnO2**	0.0151	0.0292

15	**V2O5**	-	0.0292
16	**HgO**	0.0038	0.0141
17	**PbO**	0.0041	0.0141
18	**As2O3**	0.0070	0.0103
19	**CdO**	<0.0001	-

The table above shows that the *Trividpaka Bhasma* has 92.3% of Iron oxide in it while *Putapaka Bhasma* have 86.6% of Iron oxide in it. Indicating that *Trividpaka Bhasma* prepared have more amount of Iron oxide also indicating more purity of *Bhasma*.

5.15.2.3. FTIR Analysis

FTIR spectra with wavelength range from 400-4000 is shown in the (**Fig. 5.10**) From both the spectra it is observed that both the samples do not show much vital peaks which indicated that the sample is free from any major groups and impurities. The weak peaks observed at 1044 and 1050 cm-1 indicated the presence of C-H bond (weak to medium bond) while the strong peaks at 528, 432 and 549 cm-1 indicate the presence of oxides group nanoparticles.

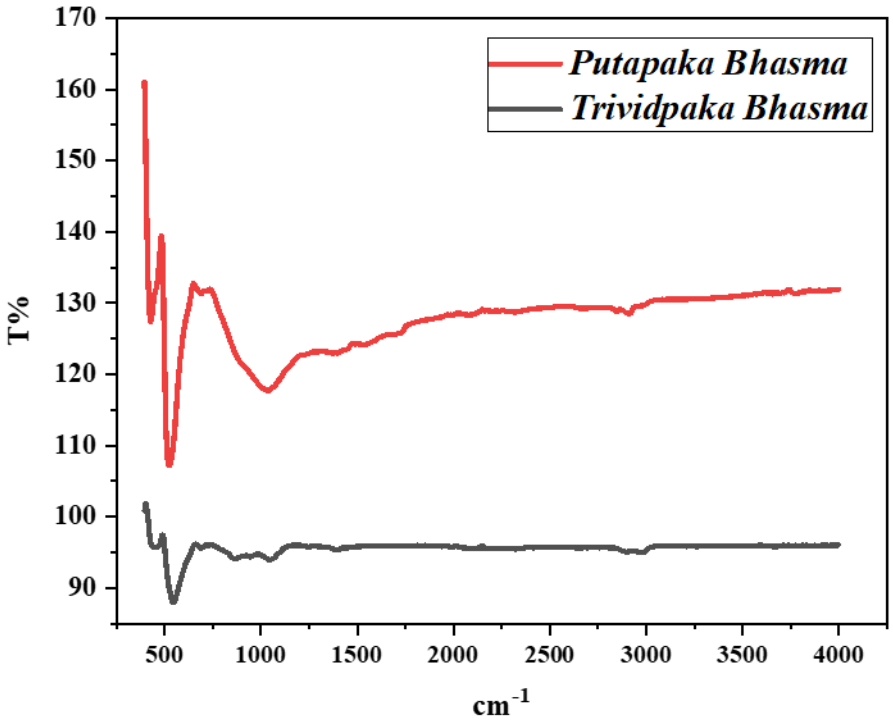


Fig. 5.11. Graph Showing result of FTIR.

5.15.2.4. FESEM-EDAX Analysis

5.15.2.4.1. FESEM Analysis:

The morphology and size of *Bhasma* prepared with *Putapaka* and *Trivipaka* was determined by FESEM analysis as shown in (**Fig.5.11**). SEM result shows particle size range from 20.6nm to 25.3nm in *Putapaka Bhasma* and in *Trividpaka Bhasma* 22.5 nm to 28.3 nm. The average particle size of the *Putapaka Bhasma* is 28nm and the average particle size of *Trividpaka Bhasma* is 22nm, the particle size is smaller, but *Trividpaka Bhasma* is finer as compared to the *Putapaka* method. This analysis indicates the reduction of particle size after proper incineration in less amount of *Puta*.

5.15.2.4.2. EDAX Analysis:

Elemental composition of the *Bhasma* using EDAX Mapping are shown in (**Fig. 5.12**). The concentration of the elements present in *Bhasma* is shown in (Fig 4). Also, the abundancy of the elements in depicted in **(Fig 5)**. EDAX analysis indicates that the *Bhasma* contain Fe as a first abundant element and O as a second abundant element. In *Putapaka*, percentage of Oxygen (40.40%) was lesser as compared to *Trividpaka* (47.19%). Some other elements like phosphorous, potassium and zinc were also reported to be present in both the *Bhasma* samples but in very trace amount.

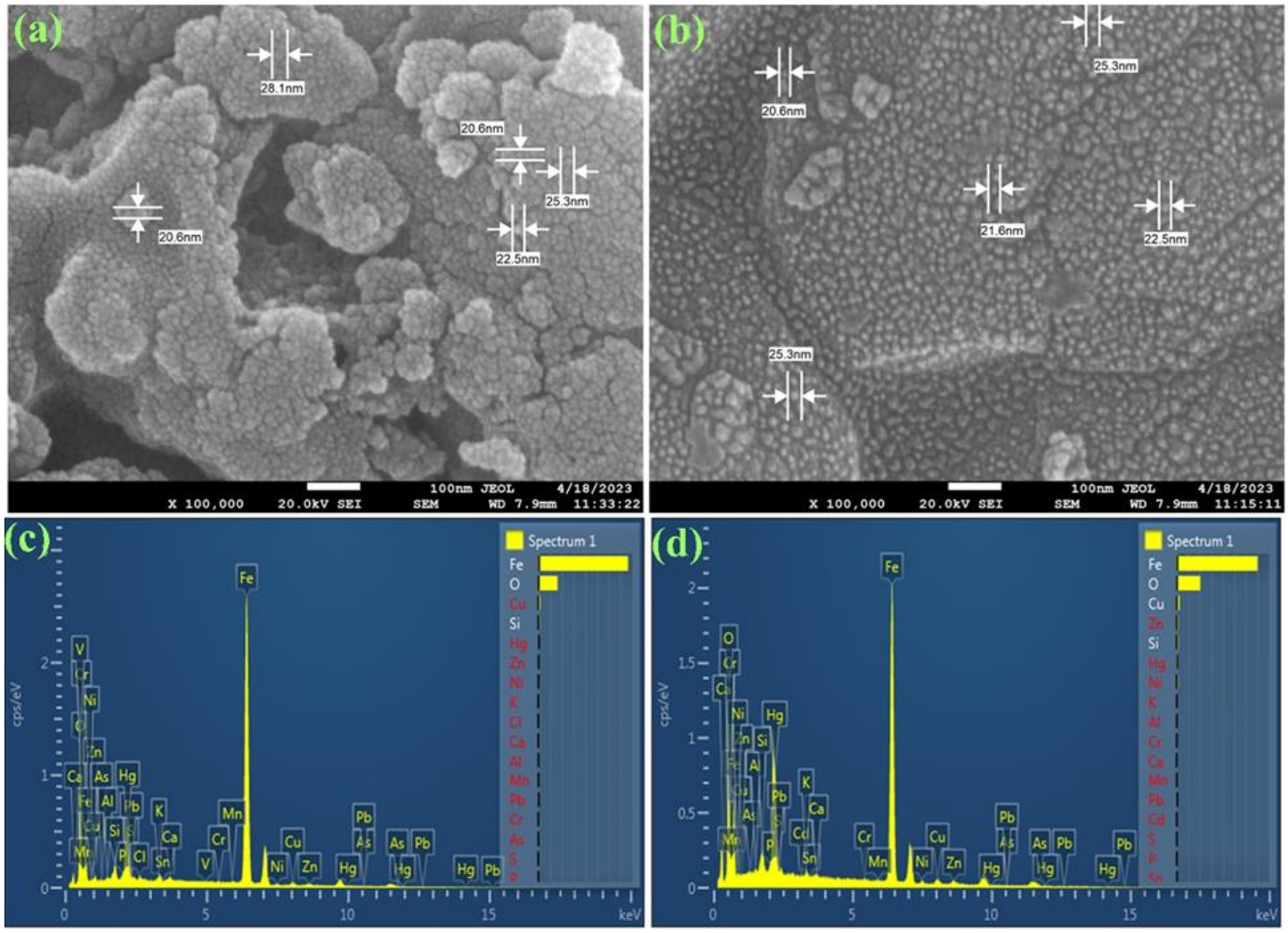


Fig.5.12. SEM microgram of *Bhasma* (a) Particle size of *Putapaka* (b) Particle size *Trividpaka* (c) Elemental Composition of *Putapaka* (d) elemental composition of *Trividpaka*

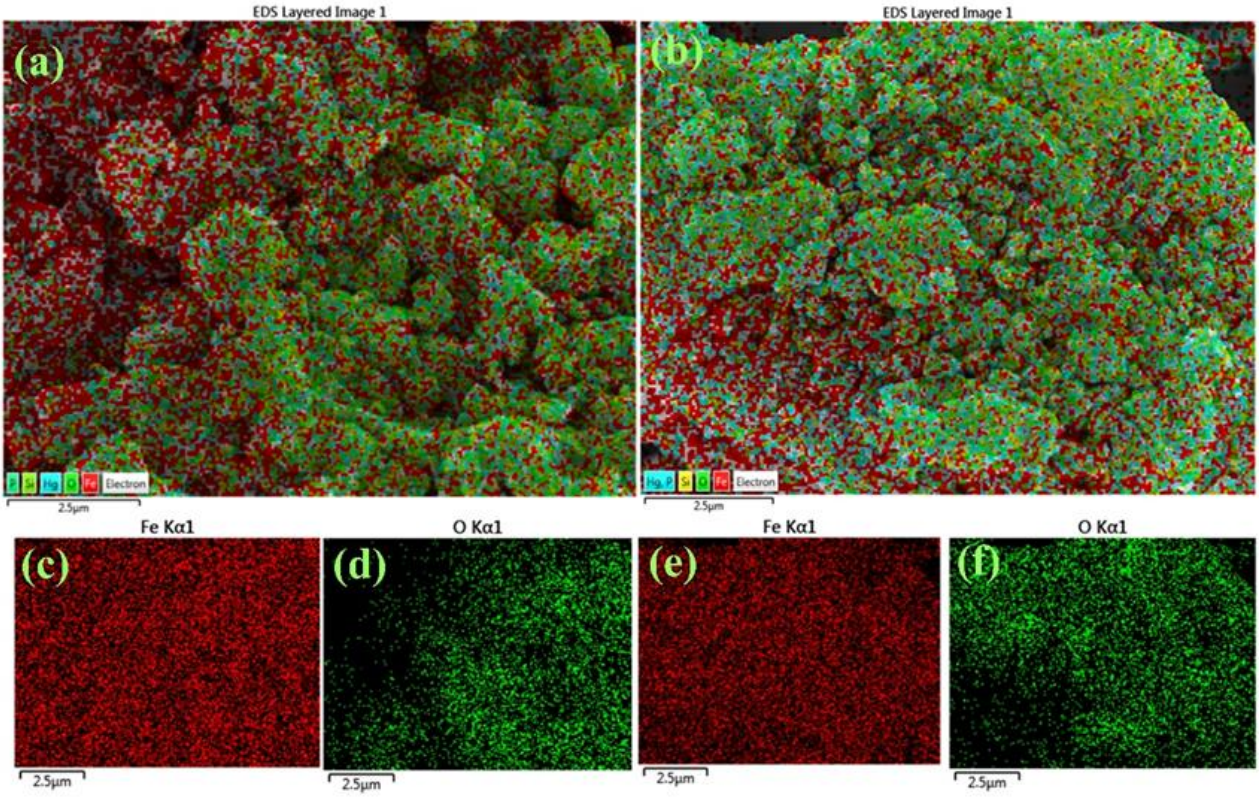


Fig.5.13. Picture showing the abundancy of the elements (a,c,d) ***Trividpaka Bhasma*** **(b,e,f)** ***Putapaka Bhasma***

5.15.2.5. XRD Analysis

Lauha Bhasma prepared from the two different methods through ayurvedic procedure was examined by X-ray Diffraction, Bruker, D8 Advanced. XRD graphs of *Trividpaka Bhasma* and *Putapaka Bhasma* are shown in **(Fig. 3)** and Table 2 gives the detail of XRD result of both the *Bhasma* sample shows the presence of crystallite Iron oxide metal, located at 2θ 18°-65°.

The average crystallite size was calculated by using the prominent peaks from the result obtained using the Debye-Scherrer equation ($D = 0.89\lambda/\beta cos\,\theta$), where D is the crystallite diameter, λ is the x-ray wavelength (0.154060), β is the full width at half maximum intensity (FWHM) of the diffraction peak and θ is the diffraction angle of the peak pattern of the *Bhasma* sample. The average crystallite size of *Trividpaka Bhasma* was found as 39.08, while the average crystallite size of *Putapaka Bhasma* was found as 28.80

More characteristic peaks of Iron oxide were observed in *Trividpaka Bhasma* as compared to *Putapaka Bhasma*, indicating high purity of obtained Fe_2O_3 in *Lauha Bhasma*. While comparing the data of *Trividpaka Bhasma* with standard JCPDS data (card no. 39-1346) contains hkl planes at (220), (311), (511), (440) showing the highest peak at 35° indicating Fe_2O_3 compound. Similarly, comparing the data of *Putapaka Bhasma* with standard JCPDS data (card no. 33-0664) contains hkl planes at (012), (104), (110), (113), (024), (116), (214) showing the highest peak at 33° indicating Fe_2O_3 compound. XRD data of both *Lauha Bhasma* are shown in the table 2 below:

Table 5.43. Result of XRD analysis

Angle (2θ)	d value (Å)	hkl	FWHM	Crystallite size
Putapaka Bhasma				
18.297	4.84700	(1,1,1)	0.08849	94.974
30.085	2.96800	(2,2,0)	0.19495	44.0717
35.510	0.26525	(3,1,1)	0.26525	32.845
53.811	1.71300	(4,2,2)	0.82269	11.30911
57.063	1.61500	(5,1,1)	0.35538	26.57533
Trividpaka Bhasma				
24.251	3.67242	(0,1,2)	0.08689	97.671864

33.116	2.69288	(1,0,4)	0.56954	15.198812
35.700	2.51030	(1,1,0)	0.41339	21.086588
54.287	1.69043	(1,1,6)	0.4006	23.2757
57.729	1.59534	(0,1,8)	9.42939	1.0047

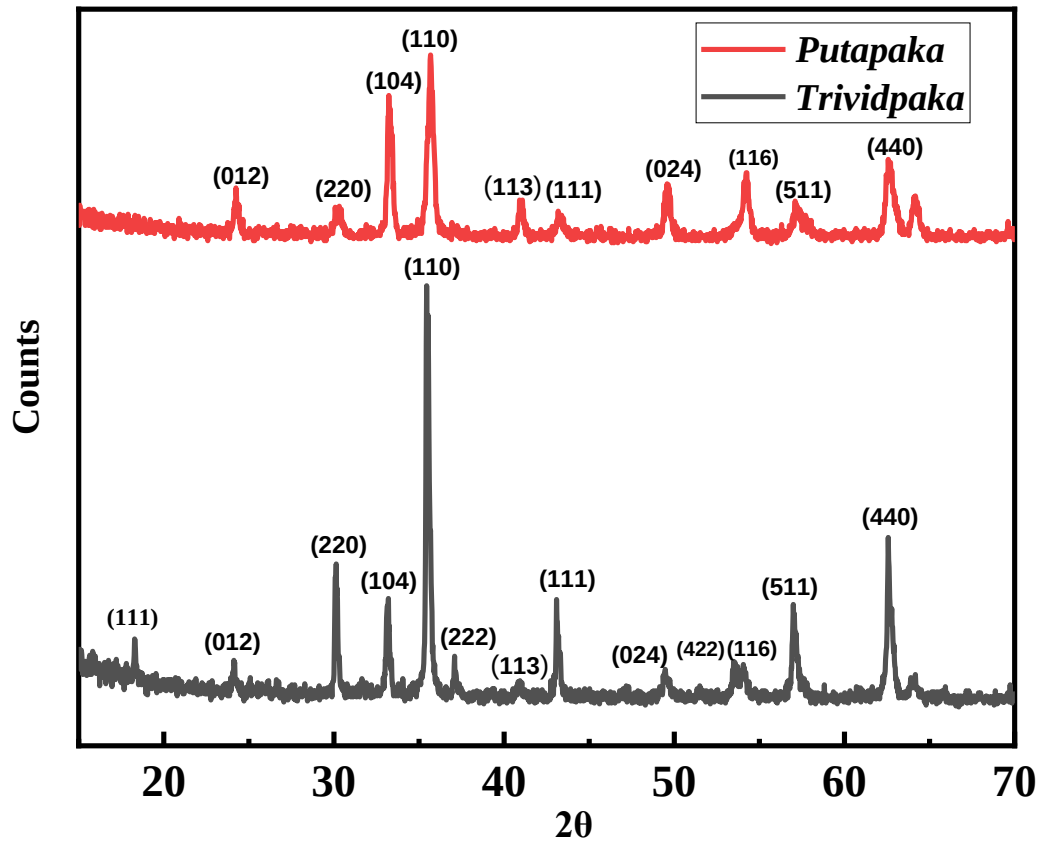


Fig. 5.14. XRD spectra for *Lauha Bhasma*

5.15.2.6. Thermogravimetric analysis

TGA curve of *Lauha Bhasma Putapaka* as shown in the **(Fig 5.15)**. The temperature range is 30°C to 800°C. The analysis shows that in *Putapaka Bhasma* the total weight loss percentage of the sample is 5.5%, the initial weight loss observed below 250°C, it corresponds to the liberation of adsorbed moisture on the surface of the sample and later the weight loss was observed at 300°C, it corresponds to the elimination of the carbon compounds group, and in the final stage, at 300-500°C the weight loss was seen which corresponds to the phase transformation. Although, TGA curve of *Trividpaka Bhasma* as shown in **(Fig 5.15)** shows total 0.8% of weight loss the initial weight loss observed below 250°C, it corresponds to the liberation of moisture on the surface and later very minute amount of loss was observed at 300°C-700°C which corresponds to the elimination of the carbon compound group. As per the analysis *Trividpaka Bhasma* is more stable as compared to *Putapaka Bhasma*.

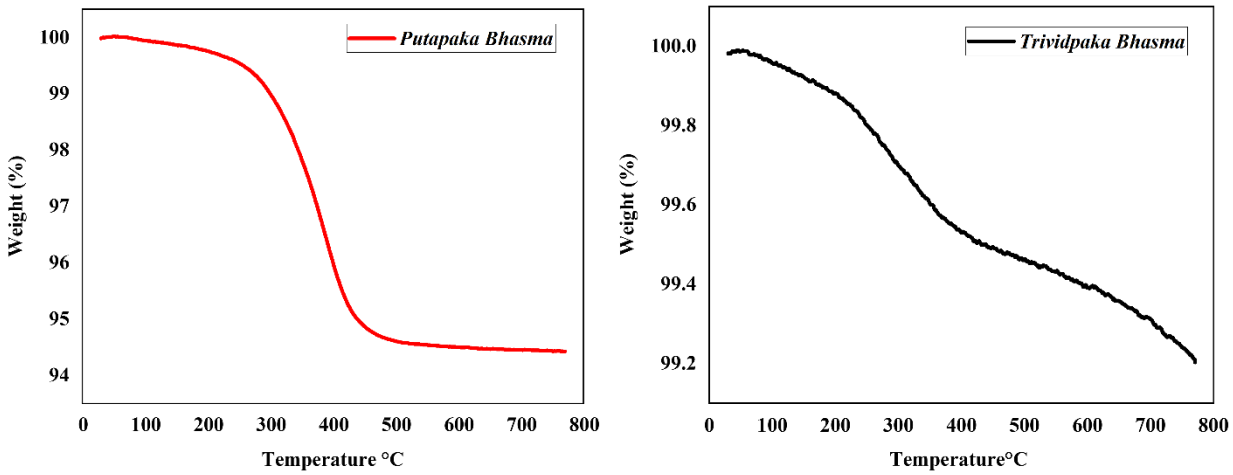


Fig 5.15. TGA graph of *Lauha Bhasma* Sample

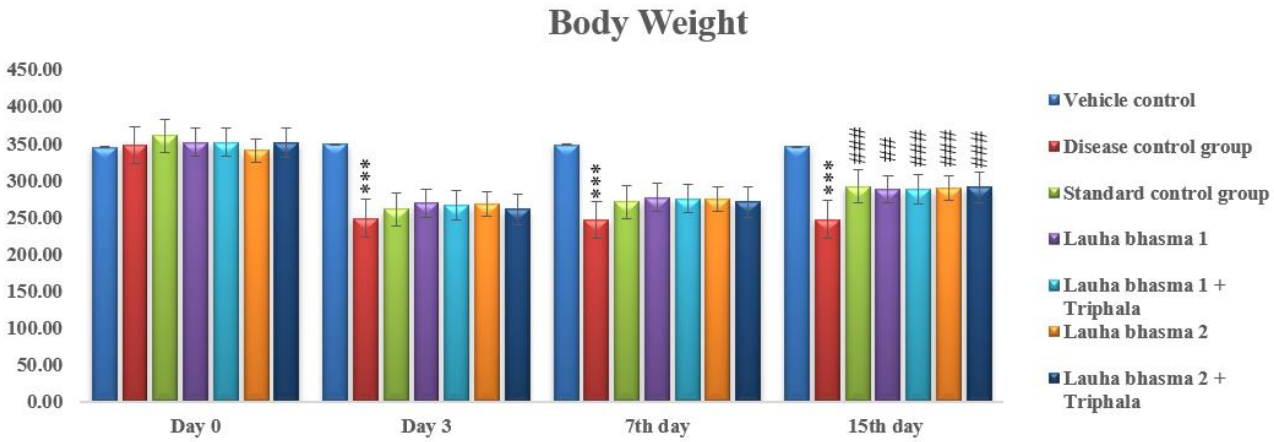
5.16. Animal Study: Study was conducted as per the approval by the IAEC committee. Protocol number for the study was LPU/IAEC/2023/38.

5.16.1. Blood Parameters:

The following results were obtained after the evaluation of blood

5.16.1.1. Body Weight:

Fig.5.16 Body weight of Animals in different groups: Data represented by means ±



SEM. Statistical significance level was considered as $p<0.05$(*), $p< 0.01$(**) and $p<0.001$ (***)

*** denotes $p<0.001$ statistically significant difference as compared to vehicle control group, ##, ### denotes $p<0.01$, $p<0.001$ statistically significant difference as compared to disease control group.

Body weight for vehicle control did not show any change throughout the study. In all the rest groups, significant decrease in weight was observed after disease induction. The disease control shows the statistically significant decrease in the weight of rat from day 3 to day 15. Standard control group showed slight increase in the body weigh after treatment. All other treatment groups also showed increase in the weight after treatment from day 3 to 15. On day 15th significant increase in the weight of rats were observed as compared to disease control group.

5.16.1.2. Hemoglobin (Hb):

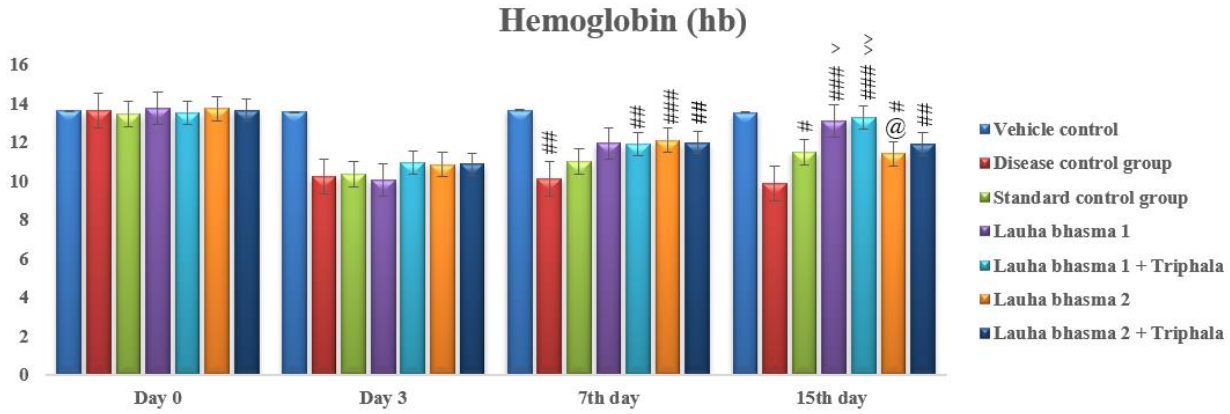


Fig. 5.17. Hemoglobin (Hb) in Animals in different groups: Data represented by means ± SEM. Statistical significance level was considered as p<0.05(*), p< 0.01(**) and p<0.001 (***)

*,**,*** denotes p<0.05, p<0.01, p<0.001 statistically significant difference as compared to vehicle control group, #,##,### denotes p<0.05, p<0.01, p<0.001 statistically significant difference as compared to disease control group, ^,^^,^^^ denotes p<0.05, p<0.01, p<0.001 statistically significant difference as compared to standard control group, @ denotes p<0.05 statistically significant difference when *Lauha Bhasma* 1 compared with *Lauha Bhasma* 2.

On 0th day normal hemoglobin was observed in the rats. On day 3 significant decrease in the Hb was observed in the animals since disease was induced. After 3rd day treatment was started then significant increase in the Hb was observed. On day 7th *Lauha Bhasma* 1+*Triphala* group, *Lauha Bhasma* 2 group, *Lauha Bhasma* 2 + *Triphala* group shows slight increase in the Hb of rats in comparison disease control group. On day 15th *Lauha Bhasma* 1, *Lauha Bhasma* 1 +*Triphala* group, *Lauha Bhasma* 2 group, *Lauha Bhasma* 2 + *Triphala* shows significantly increase in the Hb of rats, also in comparison with standard group, *Lauha Bhasma* 1 group and *Lauha Bhasma* 1 +*Triphala* shows slight increase in the Hb.

5.16.1.3. Red Blood Cells (RBC):

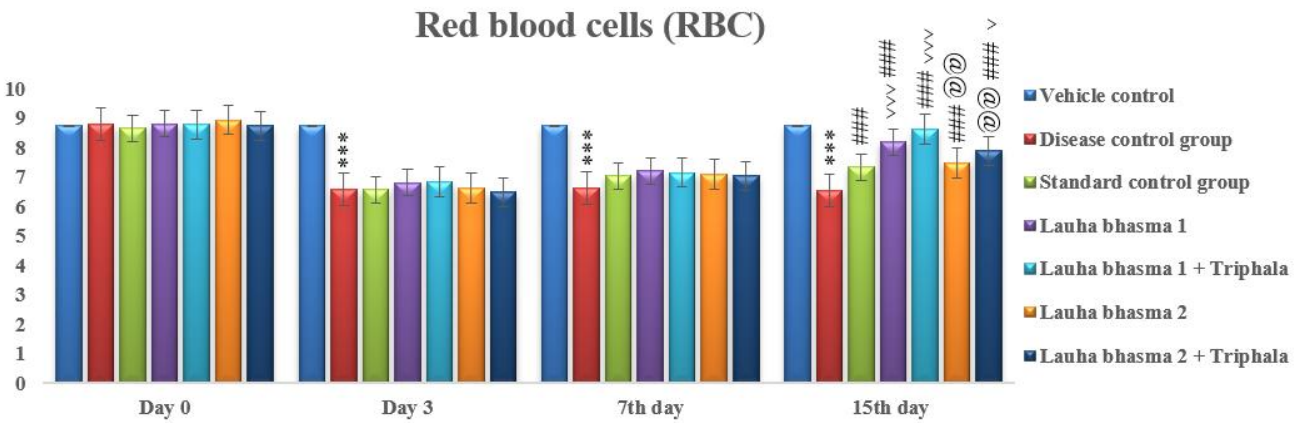


Fig.5.18. Red blood cells (RBC) in Animals in different groups: Data represented by means ± SEM. Statistical significance level was considered as $p<0.05$(*), $p<0.01$(**) and $p<0.001$ (***)

*Comparison between control to per se, # comparison between control to standard and treatment groups, ^ comparison between standard to treatment groups, @ comparison between treatment groups (*Lauha Bhasma* 1 + *Lauha Bhasma* 2*, Lauha Bhasma* 1 *(Triphala*) + *Lauha Bhasma* 2 (*Triphala*).

After disease induction sudden decrease in the RBC was observed on day 3. After that on 7th day slight increase in the red blood count was observed in the rats after treatment. On day 15th a statistically significant increase in all treatment group (*Lauha Bhasma* 1 + *Lauha Bhasma 2, Lauha Bhasma* 1 (*Triphala*) + *Lauha Bhasma 2* (*Triphala*) and standard group was observed. Also, *Lauha Bhasma* 1 + *Lauha Bhasma* 1 *Triphala* shows significantly increase in RBC in comparison with the standard group. In comparison with *Lauha Bhasma* 1 group with *Lauha Bhasma* 2 group, *Lauha Bhasma 2* group shows a slight increase in the RBC. Overall result of *Lauha Bhasma* 1 with and with *Triphala* shows better results as compared to standard group other treatment groups.

5.16.1.4. Packed Cell Volume (PCV):

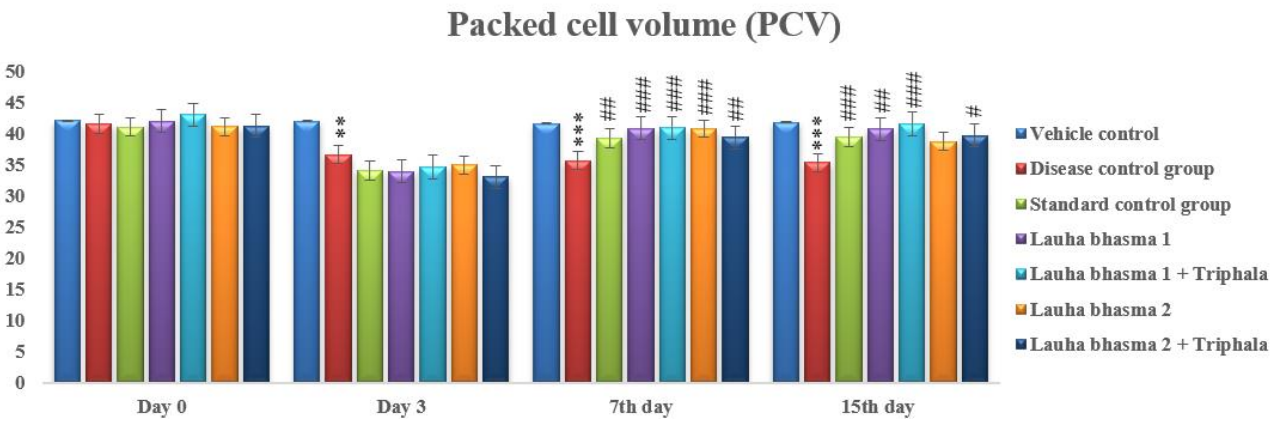


Fig.5.19. Packed Cell Volume (PCV) in Animals in different groups: Data represented by means ± SEM. Statistical significance level was considered as $p<0.05$(*), $p< 0.01$(**) and $p<0.001$ (***)

*Comparison between control to per se, # comparison between control to standard and treatment groups, ^ comparison between standard to treatment groups, @ comparison between treatment groups (*Lauha Bhasma* 1 + *Lauha Bhasma* 2, *Lauha Bhasma* 1(*Triphala*) + *Lauha Bhasma* 2 (*Triphala*).

Packed cell volume shows slight decrease on day 3 after the induction of disease. After the treatment on day 7th significant difference was observed in standard group and all the treatment groups (*Lauha Bhasma* 1 + *Lauha Bhasma* 2, *Lauha Bhasma* 1 *(Triphala)* + *Lauha Bhasma* 2 *(Triphala*). On day 15th day also, similar results were observed. Anemia does not show much major effect on PCV.

5.16.1.5. Mean cell Volume (MCV):

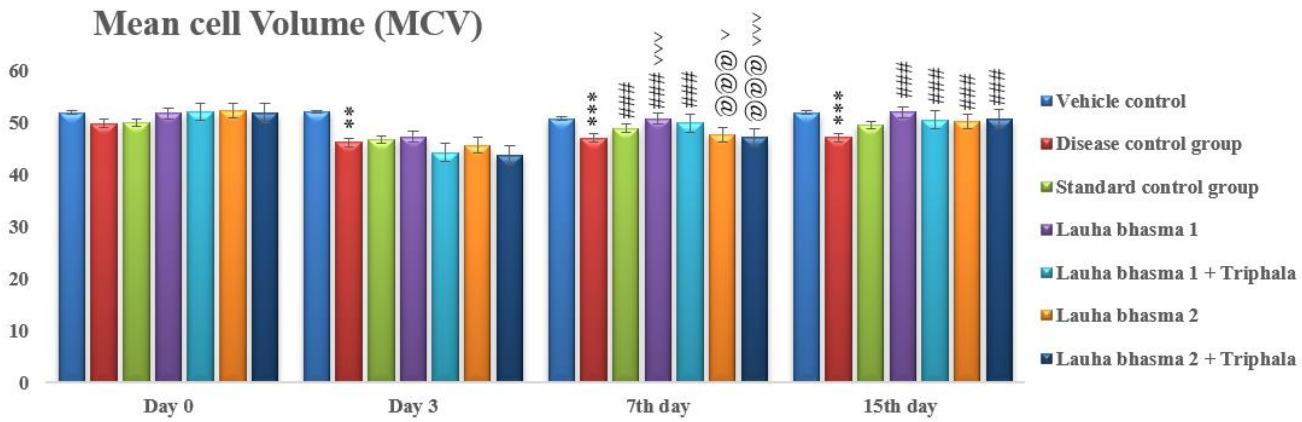


Fig.5.20. Mean Cell Volume (MCV) in Animals in different groups: Data represented by means ± SEM. Statistical significance level was considered as $p<0.05$(*), $p< 0.01$(**) and $p<0.001$ (***)

*Comparison between control to per se, # comparison between control to standard and treatment groups, ^ comparison between standard to treatment groups, @ comparison between treatment groups (*Lauha Bhasma Trividpaka* + *Lauha Bhasma Putapaka, Lauha Bhasma Trividpaka* (*Triphala*) + *Lauha Bhasma Putapaka* (*Triphala*).

Mean cell volume shows slight decrease on day 3 after the induction of disease. After the treatment on day 7th significant difference was observed in standard group and all the treatment groups (*Lauha Bhasma* 1 + *Lauha Bhasma* 2*, Lauha Bhasma* 1 (*Triphala*) + *Lauha Bhasma* 2 (*Triphala*). On day 7th *Lauha Bhasma* 1 shows statistically significant increase in the MCV in comparison with standard group. Also, *Lauha Bhasma* 2 and *Lauha Bhasma* 2 with *Triphala* shows significant increase in the MCV. On day 15th day, all the treatment drugs show significant increase in the MCV as compared to disease control group.

5.16.1.6. Mean cell Hemoglobin (MCH):

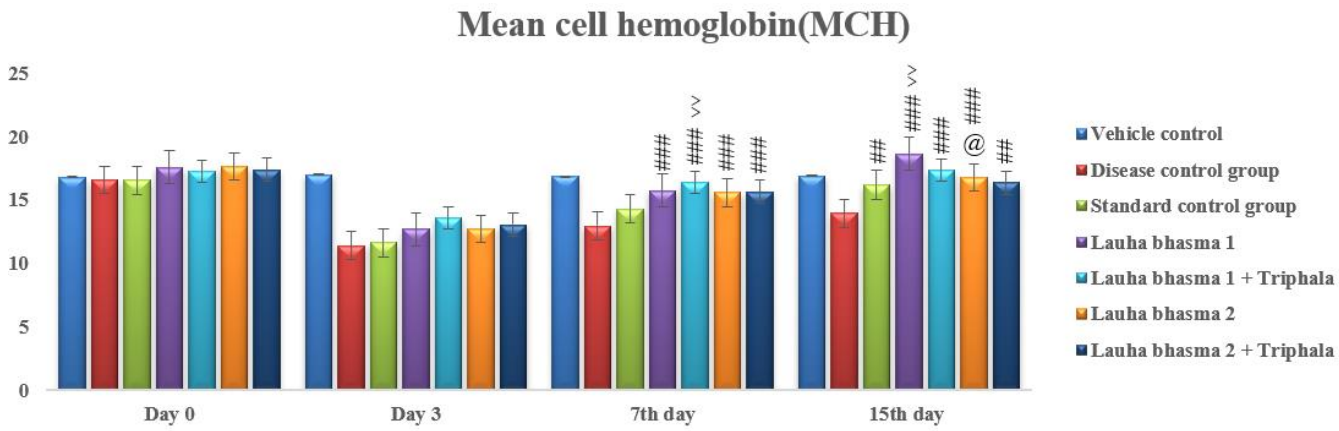


Fig.5.21. Mean Cell Hemoglobin (MCH) in Animals: Data represented by means ± SEM. Statistical significance level was considered as $p<0.05$(*), $p< 0.01$(**) and $p<0.001$ (***)

*Comparison between control to per se, # comparison between control to standard and treatment groups, ^ comparison between standard to treatment groups, @ comparison between treatment groups *(Lauha Bhasma Trividpaka + Lauha Bhasma Putapaka, Lauha Bhasma Trividpaka* (*Triphala*) + *Lauha Bhasma Putapaka* (*Triphala*).

MCH in the blood was decreased after the induction of disease, after treatment it was observed that the MCH in the blood got significantly increased on day 7 as compared to disease control group. On day 7 in comparison with standard group, *Lauha Bhasma* 1 shows moderate increase in the MCH. On day 15 significant increase in standard group as well as all treatment group was observed and *Lauha Bhasma* 1 group shows better result as compared to standard group, *Lauha Bhasma* 2 shows slight better result than *Lauha Bhasma* 1.

5.16.1.7. White blood cells (WBC):

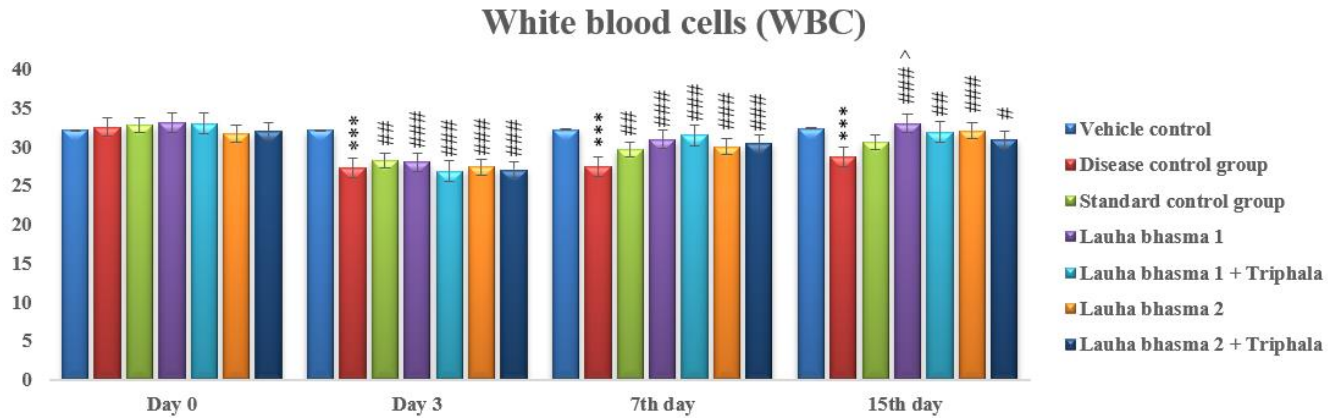


Fig.5.22. White Blood Cell (WBC) in Animals: Data represented by means ± SEM. Statistical significance level was considered as $p<0.05$(*), $p< 0.01$(**) and $p<0.001$ (***)

*Comparison between control to per se, # comparison between control to standard and treatment groups, ^ comparison between standard to treatment groups, @ comparison between treatment groups (*Lauha Bhasma Trividpaka* + *Lauha Bhasma Putapaka, Lauha Bhasma Trividpaka* (*Triphala*) + *Lauha Bhasma Putapaka* (*Triphala*).

In White blood cells, from day 3 onwards the statistical analysis shows that the standard group and all treatment groups have shown significant increase in the WBC in rats. Similarly, on day 7th same comparison was observed, on day 15th *Lauha Bhasma* 1 group shows better result in comparison with standard group, rest all the groups shows significantly better results as compared with the disease control group.

5.16.2. Histopathological Evaluation:

On 15th day Animals were Sacrificed and their spleen was isolated and kept in formaldehyde solution. The isolated spleen was sent to the Lab for evaluation of wall. The Histopathology reports are shown below in Fig. 5.22.

Normal control showing the presence of normal red, white pulp and adequate lymphoid cellular cells. This indicates a healthy spleen with normal structure and function. The red pulp, which is involved in filtering blood and removing old or damaged red blood cells, is normal. The white pulp, which plays a role in immune responses, also appears healthy with an adequate number of lymphoid cells.

Anemic control indicating focal depletion of cellular splenic parenchyma tissues. There is focal depletion of cellular splenic parenchyma tissues. This suggests that anemia has led to a decrease in the cellular components of the spleen's parenchyma, which might affect the spleen's ability to perform its functions effectively.

Standard Control group showing redevelopment of ruptured cells. Showing redevelopment of ruptured cells. This indicates a recovery process where previously damaged or ruptured cells in the spleen are regenerating. This suggests a response to previous injury or stress.

Section Show increased cellularity of white pulp. Red pulp shows normal cellularity along with areas of lipidosis and dilated, congested blood vessels. An increase in cellularity of the white pulp usually signifies an immune response or reactive process, indicating that the spleen is actively responding to some form of antigenic stimulation or infection. While the red pulp appears normal, the presence of lipidosis (fat accumulation) and dilated, congested blood vessels suggest some degree of metabolic disturbance or circulatory impairment.

Section shows almost normal looking white pulp and red pulp Negative for fibrosis and lipidosis. This indicates a spleen that is largely healthy, with no significant signs of fibrosis (scarring) or lipidosis. Any abnormalities present are minor and do not indicate serious pathology.

Section shows decrease cellularity of white pulp along with areas of fibrosis and dilated, congested blood vessels. A decrease in cellularity of the white pulp suggests a reduction in the immune function of the spleen. The presence of fibrosis indicates chronic damage or scarring, and dilated, congested blood vessels suggest circulatory issues.

Section shows normal red pulp and negative for fibrosis and lipidosis. This indicates a healthy spleen with no significant pathology affecting the red pulp. The absence of fibrosis and lipidosis further supports a normal histological appearance.

Each of these descriptions provides insights into the condition of the spleen's tissue and its potential implications for overall health and immune function.

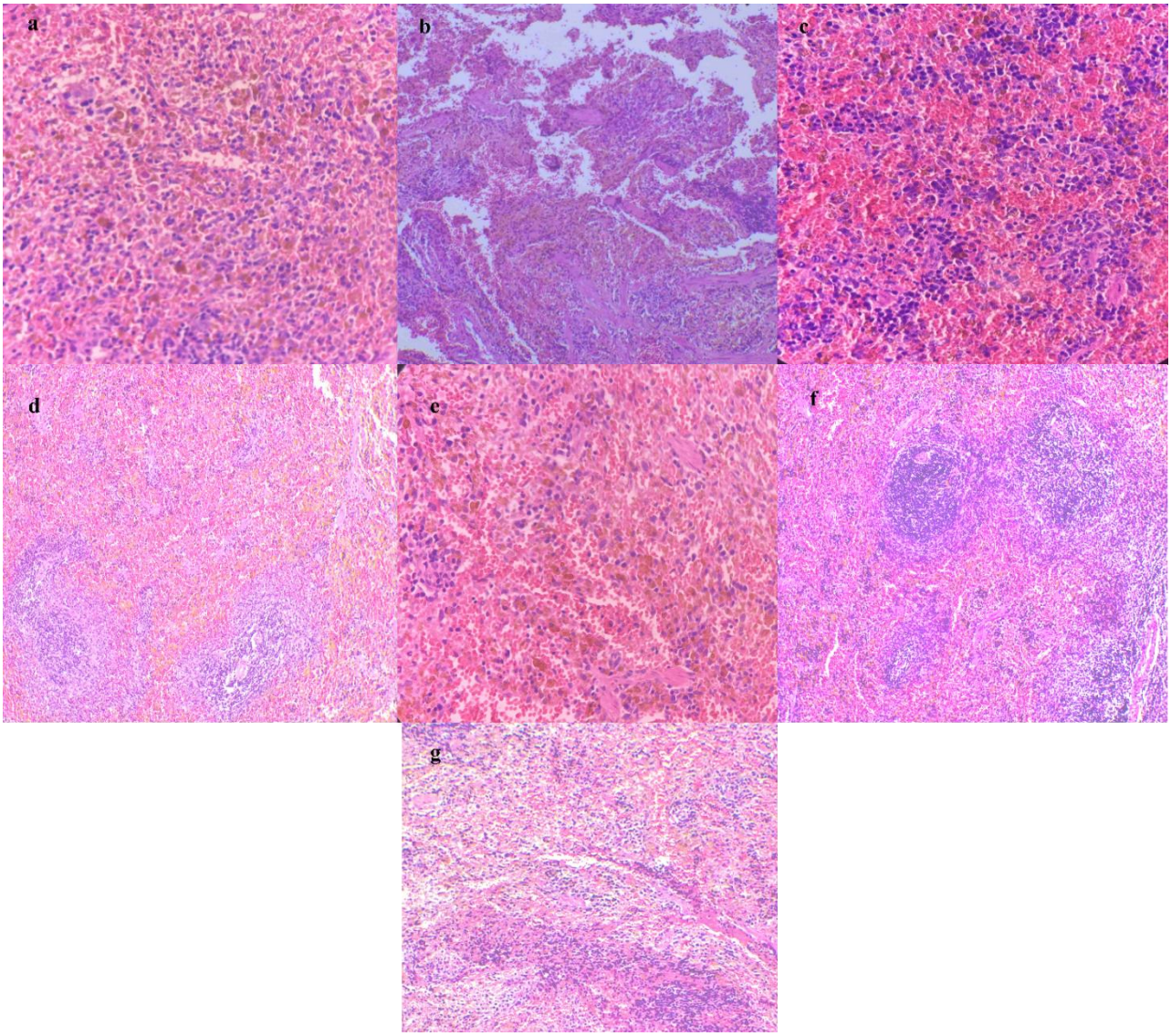


Fig. 5.23. (a) Control group (b) Disease control group (c) Standard control group (d) *Lauha Bhasma Trividpaka* (e) *Lauha Bhasma Trividpaka* with *Triphala* (f) *Lauha Bhasma Putapaka* (g) *Lauha Bhasma Putapaka* with *Triphala*

Summary and Conclusion

The comparative study of *Trividpaka Bhasma* and *Putapaka Bhasma* has revealed significant differences in their efficacy, particle characteristics, and physico-chemical properties, with *Trividpaka Bhasma* demonstrating superior outcomes.

The organoleptic characteristics of *Trividpaka Bhasma* and *Putapaka Bhasma* shows that there was slight difference in the color of both the *Bhasma* i.e., *Trividpaka Bhasma* have *Pakwa Jambu Varna* and *Putapaka Bhasma* shows dull black to *Jamun Varna* while taste and texture was same for both i.e., tasteless and amorphous. Physio-chemical analysis also shows similar results for both the *Bhasma*, *Trividpaka Bhasma* shows Loss on drying 0.3±0.013, Ash value 98.20±0.04, Acid-insoluble ash 27.42±0.27, water-soluble ash 4.36±0.32 and *Putapaka Bhasma* shows Loss on drying 0.3±0.012, Ash value 98.60±0.855, Acid-insoluble ash 28.93±0.161, water-soluble ash 3.83±0.43 both the *Bhasmas* have physico-chemical result under the standard limit.

The particle size analysis indicated that *Trividpaka Bhasma* has significantly smaller particles i.e., particle size ranging from 20.6nm to 25.3nm as compared to *Putapaka Bhasma* ranging particle size from 22.4nm to 28.3nm. This finer particle size contributes to the increased surface area, enhancing the bioavailability and therapeutic efficacy of *Trividpaka Bhasma*. The physico-chemical analysis further supported these findings, showing that *Trividpaka Bhasma* possesses better-defined structures and a higher degree of purity due to better crystallinity. These properties are crucial for the bio-efficacy and stability of the *Bhasma*.

EDAX Analysis indicates that *Trividpaka* 47.19% of iron while in *Putapaka* it was around 40.40%.

X-ray Diffraction (XRD) Evaluation revealed that (While comparing the data of *Trividpaka Bhasma* with standard JCPDS data (card no. 39-1346)) *Trividpaka Bhasma* has more distinct and sharper diffraction peaks compared to *Putapaka Bhasma*, indicating a higher degree of crystallinity. This higher crystallinity suggests better physicochemical stability and potentially more predictable pharmacokinetics, making *Trividpaka Bhasma* a more reliable and effective medicinal preparation.

Fourier Transform Infrared (FTIR) Spectroscopy analysis demonstrated that *Trividpaka Bhasma* has more clearly defined functional groups compared to *Putapaka Bhasma*. The FTIR spectra showed characteristic peaks indicating the presence of various

organic and inorganic compounds, suggesting a more homogenous and well-defined chemical composition in *Trividpaka Bhasma*. This implies better consistency and potentially greater therapeutic action. The weak peaks observed at 1044 and 1050cm-1 indicated the presence of C-H bond (weak to medium bond) while the strong peaks at 528, 432 and 549 cm^{-1} indicate the presence of oxides group nanoparticles.

Thermogravimetric Analysis (TGA) analysis showed that *Trividpaka Bhasma* exhibits greater thermal stability compared to *Putapaka Bhasma*. The weight loss profile of *Trividpaka Bhasma* shows 0.8% of weight loss which indicated a more stable composition under thermal stress, which is indicative of its superior quality and stability while the *Putapaka Bhasma* shows 5.5% of weight loss which indicates that the *Bhasma* is less stable. This property is crucial for maintaining the integrity and efficacy of the *Bhasma* during storage and application.

In the animal studies, *Trividpaka Bhasma* exhibited more pronounced therapeutic benefits, indicating its superior potency. Specifically, the *Trividpaka Bhasma* group showed better hematological parameters, improved cellular regeneration, and reduced pathological changes in splenic tissues compared to the *Putapaka Bhasma* group. This suggests that the finer particle size and superior physico-chemical properties of *Trividpaka Bhasma* facilitate better absorption and utilization by the body, leading to more effective treatment outcomes.

Conclusion

The comparative study reveals that *Trividpaka Bhasma* is superior to *Putapaka Bhasma* in terms of efficacy, particle size, and physico-chemical properties. *Trividpaka Bhasma* has finer particles (20.6nm to 25.3nm), a higher iron content (47.19%), better crystallinity, and greater thermal stability (0.8% weight loss), all contributing to its enhanced quality. Animal studies further confirm its superior therapeutic benefits, showing better hematological parameters, improved cellular regeneration, and reduced pathological changes in splenic tissues.

The advantages of *Trividpaka Bhasma* can be attributed to its unique preparation method, which involves the initial steps of *Bhasnupaka* and *Sthalipaka*, indicating that the role of liquid media and heat in different ways can affect a lot on reduction of particle size. These steps effectively reduce the particle size, resulting in a finer *Bhasma* that requires fewer *Puta* (heating cycles) compared to the *Putapaka* method. This more efficient preparation process enhances the physico-chemical properties of *Trividpaka Bhasma*, making it more bioavailable and therapeutically potent.

The finer particle size and superior physico-chemical properties of *Trividpaka Bhasma* facilitate better absorption and utilization by the body, leading to more effective treatment outcomes. The higher degree of crystallinity and thermal stability further ensures its integrity and efficacy during storage and application, making *Trividpaka Bhasma* a more reliable and effective medicinal preparation.

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Appendix-1

GSTIN : 09AAWFS0680G1ZO

SHRI AYURVED SEVA SADAN™

Seva Sadan
AYURVED THE WAY OF LIFE®

Ref. No. :SASS/231/21-22

Date : 04-02-2022

PLANT AUTHENTIFICATION CERTIFICATE

This is to certify that the following plant samples are well authenticated and verified. These studies have been accomplished in consultation with literature in the Ayurvedic Pharmacopoeia of India (Part-1, Volume-1).

Sr. No.	Common Name	Botanical Name
1-	HARITAKI	*Terminalia chebula*
2-	BHIBHITAKI	*Terminalia bellirica*
3-	AMALAKI	*Emblica officinalis*

We, here by confirm the authenticity of the above mentioned plant.

This certificate issued to Miss. Isha Agrawal, Phd scholar, Department of Ayurvedic Pharmaceutical Sciences, LPU, Phagwara (Punjab) Jalandhar. Registration No. : 12020548

For- **Shri Ayurved Seva Sadan**

Authorised Signatory

1, JAIN NAGAR, AGRA GATE, NH-2, FIROZABAD- 283 203 (U.P.)
9927093097, Fax: 05612-232503 e-mail: ayurvedsevasadan@yahoo.com, web: www.sevasadan.in

Appendix-2

E-mail : varshabullion@hotmail.com Phones : 2341 3626 / 6237 2880

VARSHA BULLION & ELEMENTAL ANALAB

M.H. DHARAMKANTA BLDG., 2nd FLOOR, 223, MUMBADEVI ROAD, OPP. MUMBADEVI TEMPLE, MUMBAI - 400 002.

Analyzed result

Sample Information

Sample name	1
File name	SHRI AYURVED SEVA SADAN
Application	FP_Metal
Date	15-03-2022 15:34
Analyzed by	MUKESH
Counts	1
Comment	All Metal

Analyzed result(FP method)

No.	Component	Result	Unit
1	Fe	98.4	mass%
2	Mn	0.611	mass%
3	Si	0.513	mass%
4	S	0.152	mass%
5	P	0.117	mass%
6	Cu	0.0822	mass%
7	Ni	0.0647	mass%
8	Cr	0.0550	mass%
9	Mo	0.0108	mass%
10	Mg	(<0.0001)	mass%

NOTE : THE ABOVE MENTIONED REPORT IS IN OXIDE FORM PERCENTAGE COMPOSITION. RIGHT HAND SIDE MASS % IS IN OXIDE FORM & LEFT HAND SIDE MASS % IS ITS CORRESPONDING METAL / ELEMENT CONTENT. THIS IS JUST A MATHEMATICAL CALCULATION OF MOLECULAR WEIGHTS RATIO.

FOR CHEMICALS THIS REPORT IS ON ANHYDROUS & DRY WEIGHT BASIS i.e. WATER CRYSTALLISATION AND MOISTURE CONTENT IS NOT CONSIDERED IN CALCULATION.

ANY VOLATILE MATERIAL LIKE CARBON, ORGANIC CONTENT OR GASEOUS PART LIKE CO_2 IN CARBONATES ETC., WHICH FORMS LOSS ON IGNITION AT 1000°C SHALL NOT BE CONSIDERED IN ELEMENT PERCENTAGE COMPOSITION. THIS MEANS ACTUAL METALLIC / ELEMENT CONTENT MAY BE MUCH LESS THAN SHOWN IN ABOVE REPORT.

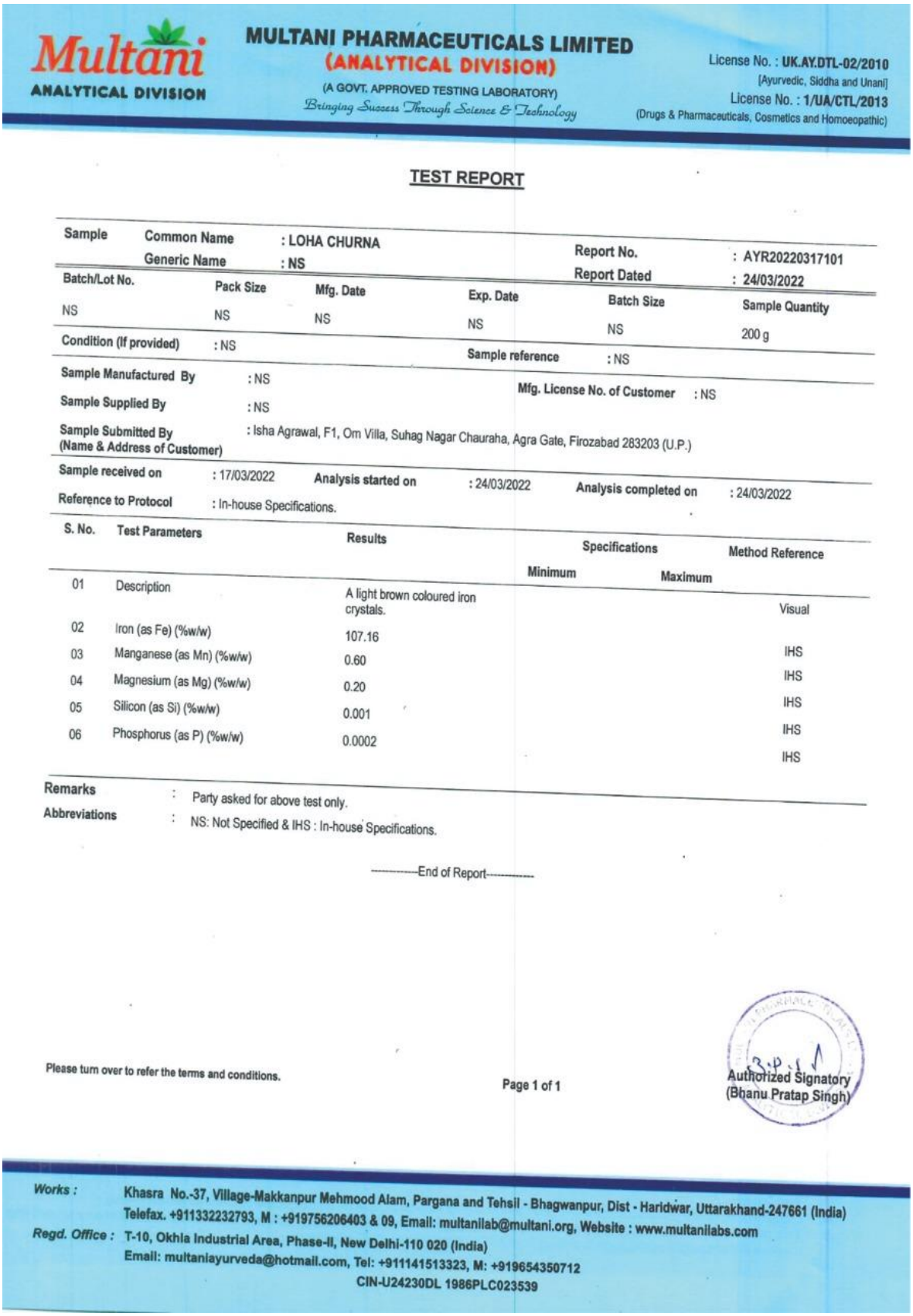
IF THE IDENTITY OF CHEMICAL OR ITS STRUCTURE OR ANION FORM IS KNOWN, THEN ACTUAL PERCENTAGE OF CHEMICAL (*within instrumental accuracy limits*) ALONGWITH ITS ELEMENT CONTENT AND IN ADDITION IMPURITIES PROFILE (above 100 ppm) IN GENERAL CAN BE KNOWN BY THIS REPORT.

For all practical purposes, this powder, mineral / ore or chemical report should be treated as SEMI-QUANTITATIVE ANALYSIS only OR Elemental Ratio between SODIUM (Atomic NO. 11) to URANIUM (Atomic NO. 92).

* **IF THE METAL / ELEMENTS CONTENT IN THIS POWDER ARE IN METALLIC STATE, SEE THE REPORT BACKSIDE.**

For **Varsha Bullion & Elemental Analab**

Partner / Authorised Signatory



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TEST REPORT

Sample	**Common Name**	: LOHA CHURNA		**Report No.**	: AYR20220317101
	Generic Name	: NS		**Report Dated**	: 24/03/2022

Batch/Lot No.	Pack Size	Mfg. Date	Exp. Date	Batch Size	Sample Quantity
NS	NS	NS	NS	NS	200 g

Condition (If provided) : NS **Sample reference** : NS

Sample Manufactured By : NS **Mfg. License No. of Customer** : NS

Sample Supplied By : NS

Sample Submitted By (Name & Address of Customer) : Isha Agrawal, F1, Om Villa, Suhag Nagar Chauraha, Agra Gate, Firozabad 283203 (U.P.)

Sample received on : 17/03/2022 **Analysis started on** : 24/03/2022 **Analysis completed on** : 24/03/2022

Reference to Protocol : In-house Specifications.

S. No.	Test Parameters	Results	Specifications		Method Reference
			Minimum	Maximum	
01	Description	A light brown coloured iron crystals.			Visual
02	Iron (as Fe) (%w/w)	107.16			IHS
03	Manganese (as Mn) (%w/w)	0.60			IHS
04	Magnesium (as Mg) (%w/w)	0.20			IHS
05	Silicon (as Si) (%w/w)	0.001			IHS
06	Phosphorus (as P) (%w/w)	0.0002			IHS

Remarks : Party asked for above test only.

Abbreviations : NS: Not Specified & IHS : In-house Specifications.

----------End of Report----------

Please turn over to refer the terms and conditions.

Page 1 of 1

Authorized Signatory
(Bhanu Pratap Singh)

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List of Conferences attended and Publications

List of Publications

1. Evaluation of Triphala Churna by different Physicochemical parameters.

List of Conferences

1. Oral presentation in the 3rd international conference of pharmacy (ICP-2022)
2. Oral presentation in the international conference on Feminine Hygiene Management- Beyond Taboo (ICHFM-2022)

Workshop attended

1. Short term course on tools and techniques of scientific writing and publishing.

Serial No. 31PC20221592

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Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. Isha Agrawal has successfully participated as Delegate & ~~Presented Poster~~/ Oral Presentation on Standardization of Tripholo Kwatha Churna with its ingredients through Phy.Chem, Microscopy & HPTLC in the 3rd International Conference of Pharmacy (ICP-2022) on the Theme of "Practice, Promotion & Publication of Innovation : A Way of Transforming Health" held on 09th & 10th November 2022 organized by School of Pharmaceutical Sciences in a collaboration with Indian Pharmaceutical Association (IPA) at Lovely Professional University, Punjab.

Mr. Suresh Khanna
National Hon. Gen. Secretary, IPA

Dr. Bimlesh Kumar
Organizing Secretary

Dr. T.V. Narayana
National President
IPA

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Dr. Monica Gulati
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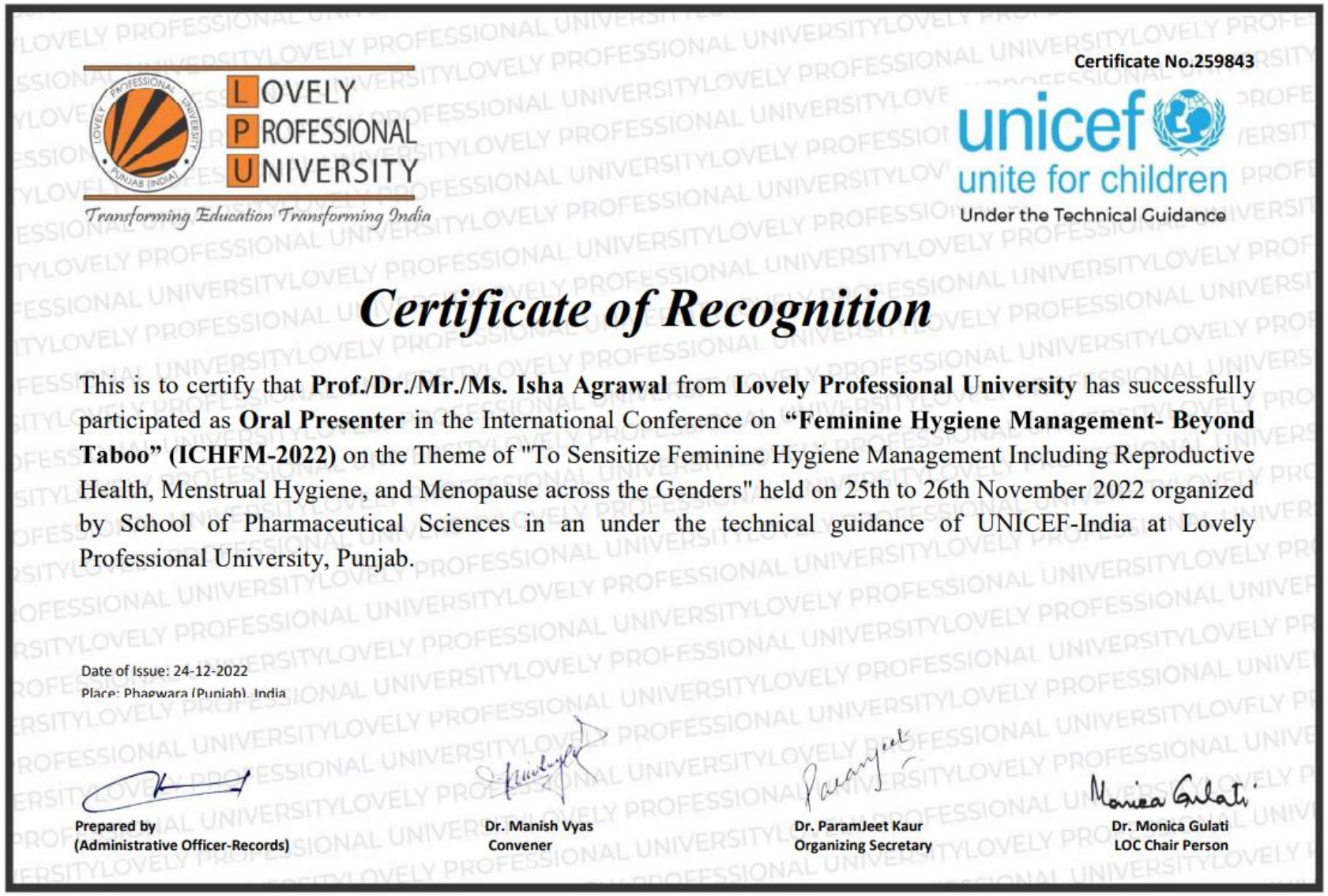

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Date of Issue: 24-12-2022
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Date of Issue : 16-07-2022
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References

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Evaluation of *Triphala Churna* by different physicochemical parameters

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ABSTRACT

Triphala, a revered herbal blend within Ayurvedic tradition, has attracted widespread interest for its multifaceted roles in promoting digestive health, detoxification, and overall well-being, alongside its prevalent inclusion in diverse Ayurvedic formulations. This study undertakes a detailed examination of individual *Triphala* fruit and *Triphala Churna*, employing a range of pharmaceutical parameters to evaluate its quality, safety, and potential health benefits comprehensively. Through scrutiny of key parameters such as phytochemical composition, physical and chemical properties, and microbial contamination, a comprehensive understanding of *Triphala's* therapeutic potential is elucidated. The analysis not only sheds light on its inherent pharmaceutical attributes but also reinforces its relevance and efficacy within Ayurvedic practice. By delving into *Triphala's* intricate molecular profile, structural integrity, safety profile, and antioxidant capacity, this study provides valuable insights into therapeutic mechanisms, by comparing individual *Triphala* fruits and *Triphala Churna.*

Keywords: *Triphala, Triphala Churna*, Ayurveda, Physico-chemical.