

## A STUDY OF MICROBIAL CONTAMINATION IN PUNARNAVA MANDURA

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### ABSTRACT

**Introduction:** Herbal medicines are being used extensively in many countries to treat many diseases, as seen in the COVID-19 pandemic, so the global market for herbal medicines has grown suddenly. In India, under the *Ayurveda* system of medicine, apart from plants, animal and mineral products have also been used as medicine since long ago. Both herbal and animal products contain primarily organic components; therefore, like food ingredients (biological ingredients), herbal medicine and animal products are at risk of microbial growth and contamination. *Punarnava Mandura* is a popular *Ayurvedic* medicine described in various classical and authoritative texts of *Ayurveda*. It is a perfect example of a medicine made from substances derived from plants, minerals, and animals. It contains *Mandura Bhasma* as a mineral component apart from other herbal ingredients, while cow urine (*Gomutra*) is of animal origin. It is widely used in infectious and non-infectious fever, cold, indigestion, and other diseases of the Gastrointestinal tract. Microbial contamination of medicines is defined as the unintended habitation of pathogenic microbes that cause their degradation. In pharmaceuticals, various factors may be a source of microbes, including the ingredients of drugs, manpower, machines, and the environment of the pharmacy. Microbial contamination is a pertinent issue and essential in quality control steps in pharmaceutical manufacturing and food processing. Microbial contamination of medicines and food products may also affect human health. **Objective:** The primary aim and purpose of the present study was to determine the microbial contamination of an *Ayurvedic* Herbo-mineral drug sample of *Punarnava Mandura*. **Material and methods:** The drug sample (Self-made) was made in the institute pharmacy. Another drug sample (market sample) of *Punarnava Mandura* of a pharmacy was also kept as a control to compare the results. Quantitative and qualitative assessment of microbial loads in *Punarnava Mandura* was assessed. The

following strains of microbes: E. Coli, Salmonella spp., Staphylococcus aureus, Fungus, and Total viable aerobic bacterial count were evaluated. **Results:** Both the samples of *Punarnava Mandura* have shown an insignificant load of selected microbes.

**Key words:** *Ayurveda, Gomutra (Cow urine), Punarnava Mandura, Microbial contamination*

## INTRODUCTION

Herbal medicines have been used for the treatment of many diseases in many countries for a long time and it has increased significantly after COVID-19, hence the global market for the use of herbal medicines has also increased suddenly.<sup>[1]</sup> In India, under the *Ayurveda* system of medicine, apart from plants, animal and mineral products have also been used as medicine since long ago. *Ayurveda* system of medicine, a branch of the alternative medicine system, provides its services to the health sector, and its contribution is continuously increasing. This traditional medical system is entirely based on long-standing experience-based practices and knowledge specifically designed for the prevention and management of diseases. The approach of this medical system is holistic and customised according to the patient's characteristics. Its popularity is increasing tremendously all over the world.<sup>[2, 3]</sup> All these system features are the cornerstone of the emerging concept of integrating AYUSH medicines into the primary health care system to enhance the strength of the health care system. Herbal drugs are becoming popular as a non-toxic and non-harm form of healthcare therapy and people are getting various therapeutic benefits from the use of herbs: but this alone does not ensure the safety and effectiveness of herbal medicines. This fact often misleads people into believing that herbal medicine never causes any harm. Contamination (microbial), adulteration, and toxicity are essential issues related to herbal medicine that must be comprehensively addressed.<sup>[4]</sup> To counter and balance this perception, traditional testing methodology has generated efficacy and safety evidence over time. Pharmaceutical faculties are also taking the initiative to create quality standards of herbal preparation with the help of established conventional methodologies. Deterioration of pharmaceutical products and drugs is defined as the change in physical and chemical

properties. The formulation or therapeutic agent has deteriorated and is no longer suitable. Contamination is the unwanted introduction of impurities of any nature (organic and inorganic) that cause spoilage. Microbial contamination of the drug is spoilage of pharmaceutical entities by contaminating microbes. Contamination of pharmaceutical preparations with microorganisms is found many times. This has been reported for several non-sterile drugs. Many factors such as ingredients, water content, pH, and temperature cause microbial contamination to spoil pharmaceutical products.<sup>[5]</sup> It has been observed that spoilage of medicines can also potentially impact therapeutic efficacy and sometimes threaten patients' health. The manufacturing of herbal medicines involves a series of processing steps using various equipment, including personnel, so there are many opportunities for contamination of raw materials, intermediates, or packaging materials during the manufacturing process.<sup>[6,7,8]</sup> *Punarnava Mandura* is a classical poly herbal formulation including *Mandura Bhasma*. Herbal ingredients *Boerhavia diffusa (Punarnava)*, *Operculina turpethum Linn. (Trivrita)*, *Zingiber officinale (Sunthi)*, *Piper longum (Pippali)*, *Piper nigrum (Marica)*, *Embelia ribes (Vidanga)*, *Cedrus deodara (Devadaru)*, *Plumbago zeylanica (Chitraka)*, *Saussurea lappa (Kushtha)*, *Curcuma longa Linn. (Haridra)*, *Berberis aristata (Daruharidra)*, *Phyllanthus emblica (Amalaki)*, *Terminalia bellirica (Vibhitaki)*, *Terminalia chebula Retz. (Haritaki)*, *Baliospermum montanum (Danti)*, *Piper retro fractum (Chavya)*, *Holarrhena antidysenterica (Indrayava)*, *Piper longum (Pippali)*, *Pippalimula* and *Cyperus Rotundus Linn. (Musta)*, along with cow urine, is used in the preparation of *Punarnava Mandura*.<sup>[9]</sup> Since several herbal drugs are used in its manufacturing and cow urine is also employed as a liquid substance, both of these can make it prone to microbial

contamination. Drug contamination with microbial agents can occur through various sources from any stage of drug development—from collecting raw materials from their sources to pharmaceutical procedures and packaging. In the present study, *Punarnava Mandura* was prepared in the pharmacy per the standard operative procedure mentioned in *Ayurveda's* reference books. Later, the microbial load (qualitative and quantitative determination of micro-organisms) was evaluated in the finished product of *Punarnava Mandura* as per guidelines of relevant pharmacopeia.

## MATERIAL & METHODS:

### Literary Review:

A review has been conducted to compile the available literature on microbes, microbial contamination, and spoilage in the *Ayurveda* texts. A review has also been undertaken to compile literature on *Punarnava Mandura* related to its preparation, ingredients and their proportions, and preparation method. Microbial contamination, microbial contamination of food and pharmaceutical products, various causes of microbial contamination, techniques used in the analysis of microbial contamination, and factors preventing microbial contamination all these issues were reviewed.

### Preparation of *Punarnava Mandura*:

Ingredients of *Punarnava Mandura* of acceptable variety and standards were obtained from the pharmacy. Fresh Cow urine was collected in a sterile container from *Gau-Shala*. *Punarnava Mandura* was prepared as per methods of the Ayurvedic Formulary of India<sup>[10]</sup>

### Microbial Study of *Punarnava Mandura*:

In the present study, the microbial load (qualitative and quantitative determination of micro-organisms) was evaluated in the finished product of *Punarnava Mandura*. Tests for specific pathogens were carried out. The following strains of microbes *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, Fungus, and Total viable aerobic bacterial count were evaluated. Microbial enumeration tests were conducted according to the Pharmacopeia technique. To validate the analysis results, a market sample of *Punarnava Mandura* (control) was also analysed and compared with the finished product. The microbial study of the sample of *Punarnava Mandura* was conducted in two study centers: Apex center and Oasis test lab.

## RESULTS AND DISCUSSION:

### Review:

*Punarnava Mandura* is a classical formulation consisting of *Mandura Bhasma* and several herbal drugs and cow urine. Herbo-minerals medicines including *Mandura Bhasma* became popular as *Mandura Kalp*.<sup>[11]</sup> It is especially used for anemia (*Pandu roga*) and swelling (*Sotha*).<sup>[9]</sup> *Mandura* is an ore of iron and chemically it is a mixture of ferrous and ferric oxides and other trace elements.<sup>[12]</sup> This may also include basic ferrous and ferrous carbonates. However, for practical purposes, it can be considered as ferric oxide  $Fe_2O_3$ . For the first time, this medicine has been mentioned by *Agnivesh* in his book *Charak Samhita* in the treatment of *Pandu* disease (anemia). It is also prescribed in Mal-absorption syndrome (*Grahani*), Splenic diseases (*Pleeha roga*), Intermittent fever (*Vishamjwara*), Hemorrhoids (*Arsha*), Skin diseases (*Twaka roga*), and Worm infestation (*Krimi roga*).<sup>[9]</sup>

As per the text, in the preparation of *Punarnava Mandura*, 48 grams (1 pal) of the powder of each herbal medicine was used. *Mandura Bhasma* was twice the total weight of the powder and 6.4lt. (1 *Adhaka*) of Cow urine was used.<sup>[9]</sup> Many other authors *Vangasena*, *Cudamani Mishra*, *Vrinda*, *Yadavji Trikamji Acharya*, *Sodhal*, *Cakrapanidatta* etc. have also mentioned this formulation in their texts. They have made some changes in the formulation and indications of this medicine.<sup>[13]</sup>

### Review on Microbial Contamination and its Impact on Pharmaceutical Products:

#### The available literature on microbes and the concept of contamination in Ayurveda:

Both *Acharya Charak* and *Sushruta* have accepted the existence and role of external factors (micro-organisms) in causing disease. *Acharya Sushruta* named such diseases *Aupasargika roga* (Infectious diseases).<sup>[14]</sup> *Acharya Charak* described the term epidemic (*Janapadodhvansha*) in detail and also defined its causative factors comprehensively.<sup>[15]</sup> *Acharya Sushruta* used the term *Marak*, while *Acharya Bhela* named it *Janamara*.<sup>[16, 17]</sup> *Charaka* acknowledged that food items are good hosts for the growth of microbes or insects (*Krimi*), providing them with nutrition for survival. *Puti-kilinn-ahaar* is the word used for rotten and

contaminated food. Such food items are a suitable medium for germs or insects (worms) to enter the host.<sup>[18]</sup> Some guidelines and directives are mentioned in *Charaka (Kalpa Sthana)*, *Sushruta (Sutra Sthana)* and *Ashtangahridaya (Kalpa-Siddhi Sthana)* for the selection and collection of raw material before the preparation of medicine. *Acharya* advocated the use of fresh and unspoiled drugs. *Janturbhi-Anupahta*, *Krimi-Anupahta*, and *Jantav-Ajagadham* are some synonyms used by *Acharyas* for impure drugs, respectively.<sup>[19, 20]</sup><sup>21</sup> Rotten and contaminated medicines (herbal, animal origin) are unsuitable.

#### **The available literature on microbes and the concept of contamination in modern science:**

Microorganisms like bacteria, viruses, etc., are known pathogens (disease-causing agents) that contaminate food, drugs, and other consumable items and can endanger human health. In general, not all microbes are always harmful. Microbial contamination of organic compounds is considered to be the presence of some unwanted and pathogenic microbes. Microbial contamination is defined as the spoilage of pharmaceutical entities of any nature by harmful microbes and making them unfit for use due to the presence of pathogenic microorganisms. Contamination of pharmaceutical preparations with microorganisms has been reported many times. The microbiological quality of medicinal products became noteworthy in 1966 when over 200 cases of salmonellosis were reported from consuming contaminated thyroid tablets, demonstrating that microbial contamination of medications can result in clinical infection.<sup>[22]</sup> Fungal contamination has been noted to affect the chemical composition of the raw materials and thereby decrease the medicinal potency of herbal drugs.<sup>[23]</sup> Harmful microbes can enter the drug through the manufacturing process including ingredients and become part of the final product. There are many routes of drug microbial contamination, e.g. raw materials, air in the manufacturing area, chemicals and equipment employed, surfaces used in the process, or working personnel. Contamination of pharmaceutical products with microorganisms may lead to changes in their

characteristics such as odor, color, etc.<sup>[6]</sup> Plants, including herbs and their exudates, have high levels of different types of carbohydrates, minerals and vitamins; key factors that influence the growth of microbes.<sup>[24]</sup> According to WHO technical guidelines, determination of microbiological contaminants and limit tests for total viable aerobic bacteria and fungi indicate the quality of herbal preparations. According to British Pharmacopoeia standards, *Salmonella* and *Shigella* species should not be present at any level in herbal medicines intended for internal use. Other microorganisms should be tested for and follow limits set in regional, national or international pharmacopoeias.<sup>[25, 26]</sup>

#### **Preparation of Punarnava Mandura:**

The fresh and dried parts of the ingredients are powdered and sieved. The powder of all ingredients was taken in equal quantities, and the quantity of *Pippali* was twice for each ingredient. The powder of all ingredients was appropriately mixed. The required quantity of fresh cow urine was poured into an iron utensil and put on a flame. After some time, the purified and incinerated *Mandura Bhasma* was mixed in the hot cow urine. The quantity of *Mandura Bhasma* in this was twice the sum of all the ingredients combined. Keep stirring the entire substance continuously for proper mixing. Later the fine powder of ingredients was added and the utensil was taken down. The stirring process was continued so that it got mixed properly. Later the dry powder was collected and stored.

#### **Result of Microbial Study of Punarnava Mandura:**

*Punarnava Mandura* (self-made and pharmacy sample) was evaluated as per the recommended guidelines and methods and compared the pathogenic load with the microbiological standards for herbal preparations given by the Indian Pharmacopoeia (IP). (Figure:1) Both the laboratories used the same methods during the test.

- **Analytical assessment of microbial contamination of self-made sample of Punarnava Mandura:** In Tables No. 1 and 2, the results of a self-made sample of *Punarnava Mandura* are stated.

➤ **Result of Oasis test lab:**

**Table No.-1:** Microbial contamination in *Punarnava Mandura*

| S.No. | Microbes                             | Methods | Findings |
|-------|--------------------------------------|---------|----------|
| 1.    | E. Coli                              | IP-96   | Absent   |
| 2.    | Salmonella spp.                      | IP-96   | Absent   |
| 3.    | Staphylococcus aureus                | IP-96   | Present  |
| 4.    | Fungal                               | IP-96   | 124/gm   |
| 5.    | Total viable aerobic bacterial count | IP-96   | 36549/gm |

➤ **Result of Apex Center:**

**Table No.2:** Microbial contamination in *Punarnava Mandura*

| S.No. | Microbes                             | Methods | Findings      |
|-------|--------------------------------------|---------|---------------|
| 1.    | E. Coli                              | IP-96   | < 500cfu/gm   |
| 2.    | Salmonella spp.                      | IP-96   | Nil           |
| 3.    | Staphylococcus aureus                | IP-96   | Nil           |
| 4.    | Fungal                               | IP-96   | < 500cfu/gm   |
| 5.    | Total viable aerobic bacterial count | IP-96   | Insignificant |

2. **Analytical assessment of microbial contamination of pharmacy sample of *Punarnava Mandura*:** In Tables 3 and 4, the results of the pharmacy sample of *Punarnava Mandura* are stated.

➤ **Result of Oasis test lab:**

**Table No.3:** Microbial contamination in *Punarnava Mandura*

| S.No. | Microbes                             | Methods | Findings |
|-------|--------------------------------------|---------|----------|
| 1.    | E. Coli                              | IP-96   | Absent   |
| 2.    | Salmonella spp.                      | IP-96   | Absent   |
| 3.    | Staphylococcus aureus                | IP-96   | Present  |
| 4.    | Fungal                               | IP-96   | Nil      |
| 5.    | Total viable aerobic bacterial count | IP-96   | 9478/gm  |






➤ **Result of Apex Center:**

**Table No.4:** Microbial contamination in *Punarnava Mandura*

| S.No. | Microbes                             | Methods | Findings      |
|-------|--------------------------------------|---------|---------------|
| 1.    | E. Coli                              | IP-96   | Nil           |
| 2.    | Salmonella spp.                      | IP-96   | Nil           |
| 3.    | Staphylococcus aureus                | IP-96   | Nil           |
| 4.    | Fungal                               | IP-96   | Nil           |
| 5.    | Total viable aerobic bacterial count | IP-96   | Insignificant |



**Figure: 1** Level of microbial load in Self-made & Market Sample of *Punarnava Mandura*

|  |   |
|--|---|
|   |   |
| <p>Portion of Petri dish above the line (1) shows <i>E. Coli</i> in the market sample of <i>Punarnava Mandura</i></p>        | <p>Portion of Petri dish above the line (5) shows <i>E. Coli</i> in the self-made sample of <i>Punarnava Mandura</i></p>        |
|    |    |
| <p>Portion of Petri dish below the line (1) shows <i>Staphylococcus</i> in the market sample of <i>Punarnava Mandura</i></p> | <p>Portion of Petri dish below the line (5) shows <i>Staphylococcus</i> in the self-made sample of <i>Punarnava Mandura</i></p> |
|   |   |
| <p>Portion of Petri dish shows <i>Staphylococcus</i> in the self-made sample of <i>Punarnava Mandura</i></p>                 |   |

## CONCLUSION

The aerobic and other bacteria as well as fungi were found in nil or insignificant amounts (within the permissible limit as per pharmacopeia) in both the samples of *Punarnava Mandura*. However, the results showed that the potential risk in both the samples of *Ayurvedic* preparations is relatively very low, it is very necessary and highly recommended that quality assurance in the production of *Ayurvedic* medicines preparations should be monitored from collection of the drugs to the production and during packing.

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