



Research Article

## A STUDY OF MICROBIAL CONTAMINATION IN SANJIVANI VATI, A COW URINE PREPARED DRUG

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### ABSTRACT

*Gomutra* (Cow urine) has been used in various diseases as a medicine since long. It is recommended alone or in combination with other herbal drugs. *Sanjivani vati* is a popular *Ayurvedic* drug described in various classical and official text of *Ayurveda* and it is a very good example of that combination. It has equal parts of *Vidanga, Sunthi, Pippali, Haritaki, Amalaki, Vibhitaki, Vaca, Guduchi, Bhallataka* and *Vatsanabha*. *Gomutra* (Cow urine) is an ingredient that is used to levigate the powder of all herbal drugs and helps in making pills.

Microbial contamination is defined as deterioration of pharmaceutical products by the microbes. In the pharmaceuticals, various factors may be as a source of microbes. Preparation of drug is a long process and occurring in open environment. A big number of machine and man power are involved in this production. All these factors including ingredients of drug may contribute the deterioration of the drugs due to contamination of microbes. Even though fresh Cow urine is sterile, it may be prone to contamination and it may produce microbial load during processing of various formulations. Microbial growth may develop in the *Sanjivani Vati* that encompasses cow urine as an ingredient.

The primary objective of this study was to determine microbial load. *Sanjivani Vati* was made in the institute pharmacy and evaluated for microbial load through outsource lab. Another market sample of *Sanjivani Vati* of a pharmacy was also evaluated to compare the results.

Quantitative and qualitative assessment of microbial loads in *Sanjivani Vati* was assessed. The following strains of microbes *E. Coli, Salmonella spp., Staphylococcus aureus, Fungus,* and Total viable aerobic bacterial count were evaluated. Both the sample of *Sanjivani Vati* has shown insignificant load of selected microbes.

### INTRODUCTION

*Ayurveda* System of Medicine, a branch of Alternative Medicine System, has considerable space in health segment and it is increasing continuously. Its popularity is increasing in both developing as well developed countries very much.

Various surveys conducted by different agencies including WHO shows that a large proportion of the world in developing countries accept the herbal medicines for health cares.

*Ayurveda* system of Medicine is a great proponent of the use of natural drugs prepared by plants, animal's products & minerals. As this system accepted that every substance in the universe has potential to become drug so it has been advocated their uses in diseases by this system since long. History of using these natural drugs is very old; perhaps it is since origin of life on the earth. It is very long period to substantiate its credentials.

As these drugs are natural in origin and made up of organic materials, so during administration, the safety issues such as quality of ingredients and method of preparation becomes very important. Both these issues may produce various impurities in the drugs. It is ensured that the medicine must be free from all type of impurities (Physical, Chemical and Biological) before using in the patients.

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The microbiological contamination (Biological impurities) of the pharmaceutical preparations is a one of the major issues and very common problem that has potential impacts on drugs and it can be very harmful to the patients as it may produce various changes in the medicines.

*Sanjivani Vati* is a combination of herbal drugs and Cow urine that helps to mitigate the diseases and strengthen the body by improving the immune power. Since many decades *Sanjivani Vati* have been recommended in the treatment of diseases of diverse origin as it comprises such herbal drugs that acts *Rasayana*; rejuvenator of all body tissues. In the *Ayurvedic* text, it is recommended in *Ajirna*, *Gulma*, *Visuchika*, *Sarpadansta* and *Sannipata*. It is widely used in high grade fever like typhoid & malaria, helpful in allergic rhinitis, diarrhea due to GI infections & worm infestation. Basically, it improves the *Vata*, *Kapha* and *Vata-Kapha* dominant conditions.

Cow urine has been used as medicine since ancient times and it is considered to be the best of medicines. As per *Ayurvedic* text, the cow urine is most preferable compare to the urine of other animals for medicinal and other purposes. [1] It is used in *Ayurvedic* formulation either alone or in combination. It must be fresh for drinking use or for formulation.

Cow urine has many beneficial properties in the areas of therapeutics. In India, drinking of Cow urine has been practiced for thousands of years. It has various biological activities such as Anti-oxidant activity, Anti-diabetic activity, Anti-bacterial activity, Immuno-modulatory effect, Anti-fungal activity, Anti-clastogenic activity and Wound healing property, etc. [2]

It has various soluble solutes that possess various other properties which protect the cow urine from microbes. It consist sodium chloride, sodium nitrate, sodium benzoate, ascorbic acid, propionic acid, lactic acid that prevent or delay the growth of pathogenic bacteria and have been used as preservatives. [3] In various studies it has been proved that fresh cow urine at low pH has better antibacterial activity and it can be used in the control of bacteria. [4]

Microbial contamination is defined as deterioration of pharmaceutical products by the microbes. In the pharmaceuticals various factors may be as a source of microbes. Preparation of drug is a long process and occurring in open environment. A big number of machine and man power are involved in this production. All these factors including ingredients of drug may contribute the deterioration of the drugs due to contamination of microbes. Even though fresh Cow urine is sterile, it may be prone to contamination and it

may produce microbial load during processing of various formulations.

## MATERIAL & METHODS

### Literary Review

A comprehensive review has been made to compile the available literature on microbes and related issues in the texts of *Ayurveda* for showing their presence in diverse aspect. A review has been also made to compile the various literatures on *Sanjivani Vati* to present its formulary i.e. ingredients & their proportion, the possible impact of ingredients for producing microbial contamination in the drugs.

### Procurement of Ingredients & Preparation of *Sanjivani Vati*

Herbal ingredients of *Sanjivani Vati* of acceptable variety & standards were obtained from N.I.A. pharmacy. Fresh Cow urine was collected in a sterile container from nearby *Gau-Shala*, at Jaipur, Rajasthan. *Sanjivani Vati* was prepared as per methods of Ayurvedic Formulary of India (AFI-1, Section 12/35, P.No.154) [5] and *Sarangdhar Samhita* under *Vatikalpana*(Sha. S. M. K. 7th Ch.18- 21). [6]

### Microbial Study

The microbial composition during contamination varies and depends upon the ingredients that are used during preparation of drugs and the atmospheric condition of the area. In the present study, the evaluation of the microbial load (quantification and determination of presence of micro-organisms) of finished product of *Sanjivani Vati* was done. 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 evaluate the result of finished product, a market sample of *Sanjivani Vati* was also analyzed and compared. The microbial study was conducted of both the sample of *Sanjivani Vati* in two study center: Apex center and Oasis test lab.

## RESULTS AND DISCUSSION

### Review:

In 13<sup>th</sup> century, *Sarangdhar* wrote this formulation in his book *Sarangdhar Samhita* under *Vati-kalpana*. This formulation encompasses *Vidang* (*Embelia ribes*), *Nagara* (*Zingiber officinale*), *Pippali* (*Piper longum*), *Haritaki* (*Terminalia chebula*), *Amalaki* (*Phyllanthus emblica*), *Vibhitaka* (*Terminalia bellirica*), *Vaca* (*Acorus calamus*), *Gudduci* (*Tinospora cordifolia*), *Bhallataka* (*Semecarpus anacardium*), and *Vatsanabha* (*Aconitum ferox*) in equal quantity. The *Gomutra*(Cow urine) is a levigating agent for triturating the powders and helps in making pills. It has been indicated in *Ajirna*, *Gulma*, *Visuchika*,

*Sarpadansta* and *Sannipata*. *Sarangdhar* mentioned 'Adrakaswarasa' as *anupana* in all aforesaid disorders. This formulation is mentioned by others upcoming authors in their books also. Most of the authors followed same formulary in their text as in *Sarangdhar*

*Samhita* but some authors includes *Chitraka* (*Plumbago zeylanica*) in place of *Amalaki*. Table No. 1 shows two group of authors, one of the groups follow *Sarangdhar* formulation and authors of another group follow other formulary.

**Table 1: Ingredients of Sanjivani Vati as per different texts [7]**

Group	Name of textbooks	Ingredients
1.	<i>Sarangdhar Samhita, Nighantu Ratanakar, Yoga Chintamani, Yoga Ratanakar, Vaidya Rahasya, Sidha Yoga Sangraha, Ayurveda Sara Sangraha, Rasa Tantra Sara, Ayurvedic Formulary of India and Pharmacoepial Standard of Ayurvedic Formulation.</i>	<i>Vidanga, Nagara, Pippali, Haritaki, Amalaki, Vibhitaki, Vaca, Guduchi, Bhallataka Vatsanabha and Gomutra</i>
2.	<i>Vaidya Chintamani, Vrihat Yoga Tarangini, Vrihat Nighantu Ratanakar and Basavragiyam</i>	Replaced <i>Amalaki</i> with <i>Chitraka</i>

### Review on Microbial Contamination and its Impact on Pharmaceutical Product

*Charaka, Sushruta* and *Vagabhata* have accepted that food material whether fresh or rotten, is good hosts for microbes or worms (*Krimi*) growth that provide them nutrition for their survival. *Puti ahar, Klinnaahar*, [8] *Sandustaahar* and *Ashuchiahar*, [9] these are synonym words, used for rotten and contaminated food in *Ayurvedic* literature. Such food material is a good vehicle for microbes or worms (*Krimi*) to get enter in the host.

In *Charaka (Kalpa sthana)* [10], *Sushruta (Sutra sthana)* [11] and *Astangahridaya (Kalpa sthana)* [12], relevant rules are mentioned regarding drug selection and collection. They told that physician should use fresh and un-rotten drug whether it is used for single drug therapy or to make formulation. Rotten and contaminated drugs (herbal, animal origin) are not good for use. They not only get down efficiency of treatment but also defame to the physician. *Maharishi Sushruta, Charaka* and *Vagbhatta* used term *Krimi-anupahata, Janturbhi-anupahata* and *Jantava-ajagdham* for uncontaminated drug respectively.

In ancient *Ayurvedic grantha*, the *Rakshoghna* is described as a group of drugs possessing *krimighna* properties (Anti-microbes & Vermicidal activities). These drugs keep the environment free from *krimi* when used through different activities such as *Dhoopan* (Fumigation). In the ancient time *Rakshoghna dhoopan* have been used for the sterilization of rooms, kitchen, *Rasayanshala, Shastragar* etc. [13]

Microbial contamination is defined as deterioration of pharmaceutical products by the contaminant microbes. There are many factors such as ingredients, water content, pH and temperature affecting microbial spoilage of pharmaceutical products. Contamination of pharmaceutical preparations with microorganism is not a rare situation; it has been reported for several non-sterile

drugs. [14] It was noticed to cause spoilage of numerous products and induce potential health hazard to the patients several times. Contamination of Pharmaceuticals products with micro-organisms can bring about changes in their characteristics such as odors, color etc. [15] It may produce potential impact on therapeutic efficacy of the drug also. According to the WHO technical guidelines, determination of microbiological contaminants and limit tests for total viable aerobic bacteria and fungi, indicate the quality of herbal preparations. [16] According to the British Pharmacopoeia (2004) standards, *Salmonella* and *Shigella* species must not be present in herbal medicines intended for internal use, at any stage. [17] Other microorganisms should be tested and should comply with limits set in regional, national or international pharmacopoeias.

### Preparation of Sanjivani Vati

Fresh and dried part (usable part) of every ingredient was powdered and sieved. Total 150 gm (15gm from each ingredient) powder was taken into a big steel utensil and then mixed properly. Then whole mixture was kept into mortar. 500 ml of fresh cow urine was poured into the mortar very slowly. It was blended properly to make homogenous form. When mixer became dry, it kept in shade. Next day, 500 ml of fresh cow urine was poured again and the whole process was repeated. This trituration was continued for third day also. At the end of process pills of appropriate shape and measurement was made and kept in shade for one or two days to dry.

### Result of Microbial Study of Sanjivani Vati

*Sanjivani Vati* (self made and pharmacy sample) was evaluated as per the recommended guidelines & methods and compared the pathogenic load with the microbiological standards for herbal preparations given by the Indian Pharmacopoeia (IP). Both the laboratory used same methods during the test.

**Analytical assessment of microbial contamination of self-made sample of *Sanjivani Vati*:** In the Table No. 2 and 3, results of self made sample of *Sanjivani Vati* are stated.

**Result of Oasis test lab**

**Table 2: Microbial contamination in *Sanjivani Vati***

S.No.	Microbes	Methods	Findings
1.	E. Coli	IP-96	Nil
2.	Salmonella spp.	IP-96	Nil
3.	Staphylococcus aureus	IP-96	Present (Insignificant)
4.	Fungal	IP-96	72cfu /gm
5.	Total viable aerobic bacterial count	IP-96	30957/gm

**Result of Apex center**

**Table 3: Microbial contamination in *Sanjivani Vati***

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

**Analytical assessment of microbial contamination of pharmacy sample of *sanjivani Vati*:** In the Table No. 4 and 5, results of pharmacy sample of *Sanjivani Vati* are stated.

**Result of Oasis test lab:**

**Table 4: Microbial contamination in *Sanjivani Vati***

S.No.	Microbes	Methods	Findings
1.	E. Coli	IP-96	Nil
2.	Salmonella spp.	IP-96	Nil
3.	Staphylococcus aureus	IP-96	Present (Insignificant)
4.	Fungal	IP-96	Nil
5.	Total viable aerobic bacterial count	IP-96	7732/gm

**Result of Apex center**

**Table 5: Microbial contamination in *Sanjivani Vati***

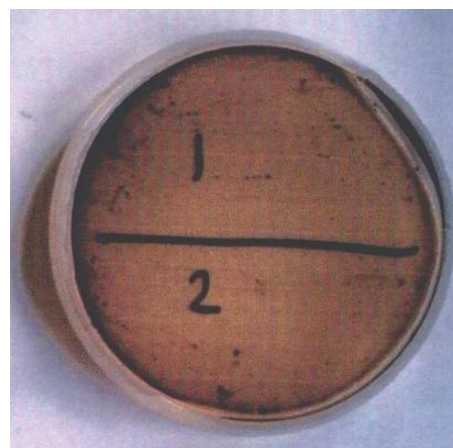
S.No.	Microbes	Methods	Findings
1.	E. Coli	IP-96	Nil
2.	Salmonella spp.	IP-96	Nil
3.	Staphylococcus aureus	IP-96	Nil
4.	Fungal	IP-96	Nil
5.	Total viable aerobic bacterial count	IP-96	Insignificant



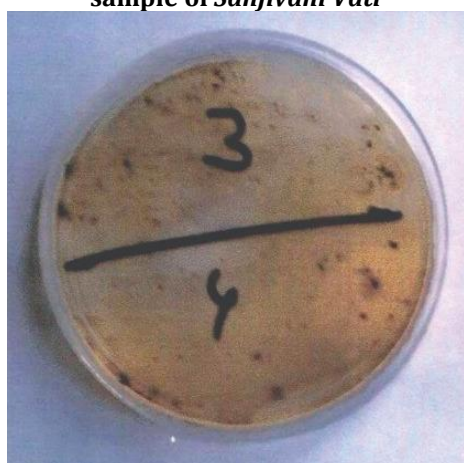
### In photos level of microbial load has been shown



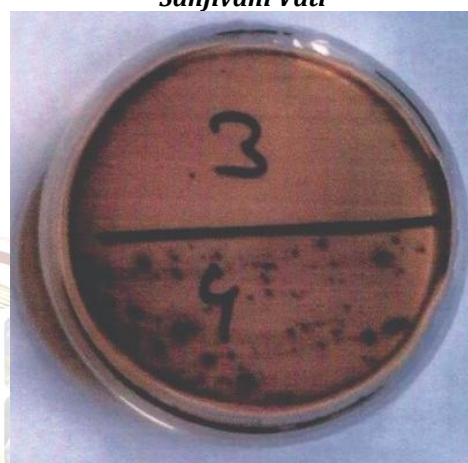
Portion of Petri dish below the line (2) shows Staphylococcus in the market sample of *Sanjivani Vati*



Portion of Petri dish below the line (2) shows E. Coli in the market sample of *Sanjivani Vati*



Portion of Petri dish above the line (3) shows Staphylococcus in the self-made sample of *Sanjivani Vati*



Portion of Petri dish above the line (3) shows E. Coli in the self-made sample of *Sanjivani Vati*

### CONCLUSION

This study demonstrated that the aerobic and other bacteria as well as fungi was found in both the sample of *Sanjivani Vati* in nil or insignificant amount. Cow urine cannot be the cause of growth of microbial load in a drug. It may be due to the other process activities from beginning to the end up to final products. It is assumed to occur during processing activities.

**Conflict of Interest: Not any**

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