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# BIOBURDEN REDUCTION METHODS FOR AYURVEDIC MEDICINAL POWDERS: A COMPARATIVE STUDY

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## ABSTRACT

Medicinal powders obtained directly from natural sources are generally contaminated with a variety of pathogens. A reduction in the number of such organisms is necessary in order to bring the microbial load of the powder within the permissible limits and to make the final product fit for use. The objective of the present study was to compare the bioburden reduction efficiency of different physical methods. Ashwagandha (*Withania somnifera*) powder, procured from a local vendor was used in the present study. Original microbial count of the non-sterile powder was determined using pour-plate method. Four physical methods viz. dry heat sterilization; ultraviolet germicidal irradiation (UVGI), ultrasonication and moist heat sterilization were used for bioburden reduction of the sample. Microbial counts of sterilized samples were again determined with pour-plate method using the same medium. The sterility of these procedures was confirmed by incubating uninoculated control plates. The microbial count of the original non-sterile powder was found to be $6.23*10^{10}$ CFU /ml. According to WHO suggestions, the total number of viable organisms in products ready for internal use should not exceed $10^5$ CFU /ml The count after sterilization with dry heat sterilization, UVGI, ultrasonication and moist heat sterilization was found to be 289 , 380 , 8 and 0 respectively. Though moist heat sterilization can reduce bioburden by 100 %, it can not be employed for powders. Dry heat sterilization, UVGI , ultrasonication are effective in bringing down bioburden within permissible limits with ultrasonication having higher efficiency than the other two.

## INTRODUCTION

Medicinal powders obtained directly from natural sources are generally contaminated with a variety of pathogens. A reduction in the number of such organisms is necessary in order to bring the microbial load of the powder within the permissible limits and to make the final product fit for use.

## OBJECTIVE

Methods for the control of microorganisms are broadly divided into physical methods and chemical methods. In comparison, physical methods are preferred for their efficiency, simplicity, and dependability. The objective of the present study was to compare the bioburden reduction efficiency of different physical methods.

## EXPERIMENTAL METHODS

Ashwagandha (*Withania somnifera*) powder was used in the present study. Sample was procured from a local vendor at Dadar, Mumbai.

Original microbial count of the non-sterile powder was determined using pour-plate method. A serial dilution scheme of the powder was prepared. Nutrient agar medium is used to determine the total number of viable microorganisms.

Four physical methods were used for bioburden reduction of the sample.

*1. Dry heat sterilization:*

Sample was subjected to $80^0$ C for 6 hours in a hot air oven. Dry heat coagulates the proteins in any organism, causes oxidative free radical damage, causes drying of cells and can even burn them to ashes, as in incineration.

*2. Ultraviolet germicidal irradiation (UVGI)*

UV has been a known mutagen at the cellular level. Ultraviolet germicidal irradiation (UVGI) is a sterilization method that uses ultraviolet (UV) light at sufficiently short wavelength to break down microorganisms.

*3. Ultrasonication:*

These are high frequency sound waves. If propagated in fluids, sound waves cause microscopic bubbles to form, and the water appears to boil (also called cold boiling). The bubbles rapidly collapse, giving tiny cavities and sending out shock waves. Microorganisms in the fluid are rapidly disintegrated by the external pressures. Frequency range of (23 – 25 KHz) was used.

*4. Moist heat sterilization:*

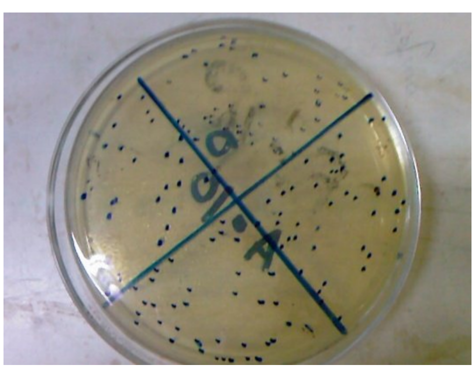
The procedure was performed using autoclave. A special valve increases the pressure to 15 lb/sq. inch above normal atmosphere pressure. The temperature rises to 121.5°C and super heated water molecules rapidly conduct heat into the microorganisms within about 15 minutes.

Microbial counts of sterilized samples were again determined with pour-plate method using the same medium.

The sterility of these procedures was confirmed by incubating uninoculated control plates.

## RESULTS AND DISCUSSION

The microbial counts obtained before and after sterilization are summarized in the table below:



Original count in Nutrient Agar

Microbial count after moist heat

| Method of sterilization | Original count | | | | Count after sterilization | | | |
|---|---|---|---|---|---|---|---|---|
| | Dilution factor | | | Average (CFU/ml) | Dilution factor | | | Average (CFU/ml) |
| | $10^8$ | $10^9$ | $10^{10}$ | | $10^0$ | $10^1$ | $10^2$ | |
| Dry heat | 159 | 41 | 13 | $6.23*10^{10}$ | 196 | 27 | 4 | 289 |
| UVGI | | | | | 261 | 38 | 5 | 380 |
| Ultrasonication | | | | | 8 | 0 | 0 | 8 |
| Moist heat | | | | | 0 | 0 | 0 | 0 |

Moist heat sterilization method was found to be the most effective method for bioburden reduction of medicinal powders according to the data above. However, moist heat sterilization is not generally preferred mainly because of greater possibility of active content loss and loss of powder characteristics.

Therefore, alternative methods like dry heat sterilization, UVGI, ultrasonication may be employed. Out of these methods, ultrasonication was found to be the most efficient method having been able to produce about 40 fold more decrease in the number of pathogens as compared to dry heat and UVGI.

According to WHO suggestions, the total number of viable organisms in products ready for internal use should not exceed $10^5$ CFU /ml. Thus, all three methods can be employed to bring bioburden of powders within permissible limits.

In future, the selective effectiveness of each of these methods against different kind of pathogens may be tested. This will serve as a useful guide for the selection of a method to sterilize a product mainly contaminated with a particular pathogen.

## CONCLUSION

Bioburden reduction becomes mandatory for Ayurvedic medicinal powders. Though moist heat sterilization can reduce bioburden by 100 %, it can not be employed for powders. Dry heat sterilization, UVGI, ultrasonication are effective in bringing down bioburden within permissible limits with ultrasonication having higher efficiency than the other two.

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