Research Article

A Comparative Study of Bioburden Reduction on Ayurvedic Powders Using Physical Techniques Individually and in Combination

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ABSTRACT

People consume Ayurvedic Powders in different ways (topically, oral etc.). These powders are obtained from natural products and hence contain large amount of micro-organisms. To make such products suitable for consumption, there is a need to reduce the bacterial number to an approbatory limit. The objective of this research was to compare the efficiency of different bioburden reduction techniques. Ashwagandha (*Withania somnifera*) and Manjishtha (*Rubia cordifolia*) powders were used throughout the process. Microbial limit test was carried out and both the powders were found to be contaminated with *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa (P.aeuroginosa*) and *Salmonella Typhi (S. typhi)*. By the spread plate method, the average microbial count of the untreated powders was found out to be very high. The count was 5.86×10^{12} CFU/mL for Ashwagandha and 40×10^{6} CFU/mL for Manjishtha. These powders were subjected to 3 physical methods and 2 combinations of the physical methods viz. Dry heat, Ultraviolet Germicidal Irradiation (UVGI), Ultrasonication, Dry heat & Ultrasonication and Ultrasonication & UVGI. After treatments, the count was found out to be within the permissible limits stated by WHO guidelines. The count being reduced to 233 CFU/mL for Ashwagandha and 7 CFU/mL for Manjishtha with the combination UVGI and Ultrasonication. Thus, the combination of techniques was proved to be much more effective in reducing the microbial count than the technique individually.

Keywords: Bioburden, dry heat, microbial limit test, ultrasonication, ultraviolet germicidal irradiation.

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INTRODUCTION

Bio burden is defined as the number of bacteria living on the surface [1].

It is measured in CFU (colony forming units) per unit of product. Bioburden reduction is necessary for preventing the degradation and spoilage of the product. The Powders obtained from natural sources are generally contaminated with microbes[2]. Therefore, reducing the number of microorganisms is mandatory in order to bring the microbial load into the permissible limits.

The Powders were found out to be contaminated with *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa (P.aeuroginosa*) and *Salmonella Typhi (S. typhi)*, determined by Microbial Limit test [3]. *E.coli* is a gram negative bacterium. This facultative anaerobe, culture on MacConkey agar and is known to cause diseases like urinary tract infection, neonatal meningitis and other gastrointestinal diseases. *Salmonella typhi* is also a Gram negative intestinal rod known to cause intestinal and extraintestinal diseases. *Pseudomonas aeruginosa* causes illness to individual with impaired immune defenses. They cause systemic and localized diseases [4].

A collaborative study of bio burden reduction and techniques were carried out on the powders of Ashwagandha (*Withania somnifera* family: Solanaceae) and Manjishtha (*Rubia cordifolia* family: Rubiaceae). The techniques used were Dry heat, Ultraviolet Germicidal Irradiation (UVGI) and combination of Ultrasonication and Dry heat and Ultrasonication and UVGI.

In Dry heat, the lethality of the micro-organisms is due to the destructive oxidation of essential cell constituents. The organisms are killed in an exponential fashion. It also causes depyrogenation.

Higher temperatures and long exposure times are required. As per British Pharmacopoeia 1988, typical time cycle as follows: 1) 160ºC for 120minutes 2) 170ºC for 60 minutes 3) 180ºC for 30minutes. Positive pressure should be present in the chamber to prevent the entry of non-sterile air. HEPA filters may be incorporated into the chambers. Although, the heat is not spread evenly and rapidly, it can used for powders that are not penetrated by steam. Dry heat sterilization cannot be employed to thermo labile powders [5].

Moist heat sterilization is more effective in killing micro-organism than dry heat sterilization but it cannot be employed for bio burden reduction of powders because it makes the powder moist.

Ultrasonication produces cavitation phenomena when acoustic power inputs are sufficiently high to allow the multiple productions of micro bubbles at nucleation sites in the fluids is called as Cold Boiling. The bubbles grow in size during the rarefying phase of the sound wave, and then are collapsed during the compression phase. On collapse, a violent shock wave passes through the medium. The whole process of gas bubble nucleation, growth and collapse due to the action of intense sound waves is called cavitation. The collapse of the bubbles converts sonic energy into mechanical energy in the form of shock waves equivalent to several thousand atmospheres (300 MPa) pressure. This energy imparts motions to parts of cells which disintegrate when their kinetic energy content exceeds the wall strength. An additional factor which increases cell breakage is the micro streaming which occur near radially vibrating bubbles of gas caused by the ultrasound [6].

Ultraviolet germicidal irradiation (UVGI) is a physical method that uses ultraviolet (UV) light at sufficiently short wavelength to kill microorganisms. UV has been a known mutagen at the cellular level for more than one-hundred years. UVGI utilizes short-wavelength ultraviolet radiation (UV-C) that is harmful to microorganisms. It is effective in destroying the nucleic acids in these organisms so that their DNA is disrupted by the UV radiation, leaving them unable to perform vital cellular functions [7].

OBJECTIVE

The objective of the performed study was to compare the bioburden reduction efficiency of different physical methods used either alone or in combination.

Spread plate method was used to determine the original microbial count of each powder [8], [9].

A serial dilution scheme was prepared for the same [10].

METHODOLOGY

1. Dry Heat: The 5gm each powders was subjected to 80°C for 120minutes.
2. Ultra-Violet Germicidal Irradiation: The powders were placed in UVGI chamber for 1 hour.
3. Ultrasonication: Each powder was subjected to the Ultrasonication machine, with Frequency range of 25 KHz, for 1 hour.
4. Dry Heat and US: The powders were put in the oven for 1hour at 80°C and then subjected to Ultrasonication for 1 hr.
5. UVGI and US: Keeping the Ultrasonication technique common, here, the powders were first subjected to UVGI for 1 hour and Ultrasonication for 30minutes.

A serial dilution was prepared of each of the treated powders and the microbial count was determined by Spread Plate Method. The sterility of the procedures was confirmed by incubating uninnoculated controlled plates.

RESULT AND DISCUSSION

As shown in **Table 1**, the original microbial count of the Ashwagandha powder was 5.86×10^{12} CFU/mL. By application of dry heat, Ultrasonication and UVGI; the microbial count was reduced to 516 CFU/mL respectively. Moreover, by applying the techniques in combination, the microbial count was reduced to greater extent. By using the in combination, the microbial count was reduced to 700 CFU/mL. Furthermore, with UVGI and Ultrasonication the count was reduced to 233.3 CFU/mL.

Table 1: It shows the calculated microbial count on untreated and treated Ashwagandha powders

Method	Dilution Factor			Average Count (CFU/mL)
	10^{12}	10^{11}	10^{10}	
Untreated	15	18	79	5.86×10^{12}
	10^{3}	10^{2}	10^{1}	
Dry Heat	1	10	45	516.6
Ultrasonication	0	6	30	300
UVGI	1	35	60	1700
UVGI+ Ultrasonication	0	5	20	233.3
Dry Heat + Ultrasonication	1	8	30	700

Table 2: It shows the calculated microbial count on untreated and treated Manjishtha powders

Method	Dilution Factor			Average Count
	10^{9}	10^{8}	10^{7}	
Untreated	0	1	2	40×10^{6}
	10^{3}	10^{2}	10^{1}	
Dry Heat	0	0	25	83
Ultrasonication	0	2	3	76.66
UGVI	0	0	35	116.6
Dry Heat + Ultrasonication	0	0	3	10
UVGI + US	0	0	2	7

Alike Ashwagandha, Manjishtha powder was subjected to similar treatment. The original microbial count was found to be 40×10^{6} CFU/mL. The dry heat method was found to reduce the microbial load to 83 CFU/mL. The Ultrasonication technique and UVGI reduced the load to 76.66 CFU/mL and 116.6 CFU/mL respectively. Moreover, the combination of techniques was found out to be more effective than individual techniques. The combination of dry heat and Ultrasonication repressed the microbial load to 10 CFU/mL. Furthermore, the combination of Ultrasonication and UVGI decreased the count to 7 CFU/mL.

Inactivation Factor: It is the degree to which the viable population of organism is reduced by the treatment applied, and is obtained by dividing the initial viable count by the final viable count [11].

Table 3: Inactivation Factors for Treated Ashwagandha Powders

TREATMENT	INACTIVATION FACTOR
1. Dry Heat	1.134×10^{10}
2. Ultrasonication	1.955×10^{10}
3. Ultraviolet Germicidal Irradiation	3.44×10^{9}
4. UVGI+ Ultrasonication	2.512×10^{10}
5. Dry heat + Ultrasonication	8.371×10^{9}

Table 4: Inactivation Factors for Treated Manjishtha Powders

TREATMENT	INACTIVATION FACTOR
1. Dry Heat	4.81×10^{5}
2. Ultrasonication	5.22×10^{5}
3. Ultraviolet Germicidal Irradiation	3.43×10^{5}
4. UVGI+ Ultrasonication	4×10^{6}
5. Dry heat + Ultrasonication	5.71×10^{6}

As shown in **Table 3** and **Table 4**; the inactivation factors have decreased considerably from individual techniques to combination of techniques. The inactivation factor for Ashwagandha and Manjishtha are 1.134×10^{10} and 4.81×10^{5} respectively for dry heat; 1.955×10^{10} and 5.22×10^{5} respectively for Ultrasonication; and 3.44×10^{9} and 3.43×10^{5} respectively for UVGI. For Combination technique; inactivation factor for Ultrasonication and UVGI was found out to be 2.512×10^{10} for Ashwagandha for 4×10^{6} for Manjishtha. In addition, the combination of Ultrasonication and Dry heat gave an inactivation factor of 8.371×10^{9} for Ashwagandha and 5.71×10^{6} for Manjishtha.

All the 5 methods can be employed to bring down the microbial count within the permissible limit, as stated by WHO i.e. 10^{5} CFU/mL, though the most effective being the combination of UVGI and Ultrasonication.

CONCLUSION

The Research has been carried out in the laboratory to reduce the microbial contamination in the Ayurvedic powders of *Withania somnifera* (Ashwagandha) belonging to family Solanaceae and *Rubia cordifolia* (Manjishtha) belonging to family Rubiaceae. Both the Powders were found to be contaminated with *E.coli*, *Salmonella* sp. and *P. aeuroginosa*. The initial count was found out to be 5.86×10^{12} for Ashwagandha and 40×10^{6} for Manjishtha. After employing the physical methods with the objective of reducing the microbial load, the microbial load was found to be reduced for both the powders. Though all the techniques namely Dry heat, Ultrasonication, Ultraviolet Germicidal Irradiation and the combination of Ultraviolet Germicidal Irradiation and Dry Heat and Ultraviolet Germicidal Irradiation and Ultrasonication were effective in bringing down the Bio-burden reduction, within the limits specified by WHO i.e. 10^{5} CFU/mL, the most efficient technique was found to be the combination of UVGI and Ultrasonication.

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