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STANDARDIZATION OF STERILIZATION METHOD FOR MEDICINAL PLANT POWDERS

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ABSTRACT

Background: Sterilization is a method to eliminate or kill the growth of microorganisms. Sterilization can be accomplished by several methods. Plant-based products have been greatly in use due to their pharmacological activities and it is necessary to ensure the plant products are sterile and safe to consume. **Objective:** The objective of this study was to standardize the best sterilization method for plant-based medicinal products. **Methods:** *Tinospora*, *Eclipta*, *Phyllanthus, a*nd *Rauwolfia* plant powders were sterilized by several methods such as microwave pasteurization, keeping in a hot air oven at different temperatures, exposure to ultraviolet rays at different time intervals and autoclaving the plant powders. The unsterilized and sterilized plant powders were subjected to microbial screening by streaking onto nutrient agar and sabouraud dextrose agar. **Results:** The microwave pasteurization method was successful as no bacterial or fungal growth was observed on nutrient agar and sabouraud dextrose agar for all four types of plant powders. **Conclusion:** The contaminants present in medicinal plant powders can be eliminated through sterilization by the pasteurization method.

Keywords: Pasteurization, Medicinal plant, Microbial screening, Contamination, Microorganisms

INTRODUCTION

Sterilization is a process that destroys all microorganisms including the most resistant bacteria and spores. It is a necessary process for many studies as contaminations can alter the result. Currently, the sterilization method of plant-based products is very uncertain. The most common form of sterilization for plant-based products is fast sterilization. However, if the temperature is too high, the components of the plants may be destroyed and if too low, microorganisms won't be killed.

Sterilization can be performed by combining chemicals, radiation, heat, filtration, and high pressure [1]. An effective sterilization method is essential to eliminate the presence of microorganisms. The different sterilization methods used to eradicate the growth of microorganisms are steam sterilization, dry heat sterilization, filtration, radiation sterilization, and sterilization by chemicals. In recent years, the consumption of plant-based products has been a major interest in our society. Plant-based products are used widely due to their pharmacological activities, higher nutritive values, lack of side effects, affordability, and more [2]. However, it is of utmost importance to ensure that plant-based products are sterile and safe to be consumed [3]. In order to identify the effective sterilization method, *Tinospora*, *Eclipta*, *Phyllanthus,* and *Rauwolfia* plant powders were chosen in this study.

Tinospora is a medical plant that has been used for generations by medical practitioners for its curative properties. It is commonly known as *"Guduchi"* and belongs to the *Menispermaceae* family [6]. It has been used in ayurvedic medicine for the treatment of skin problems, allergies, inflammation, rheumatism, urinary disorder, and more [7]. *Eclipta* plays important role in Siddha and Ayurvedic medicine. The plant possesses a strong hepatoprotective agent and it is certified in Indian Herbal Pharmacopoeia and The Ayurvedic Pharmacopoeia of India [4, 5]. Udayashankar et al. (2019) identified several other ethnopharmacological properties of *Eclipta* such as analgesic activity, antioxidant, anti-cancerous, antimicrobial, diuretic, anti-inflammatory, and anti-depressant [8].

Phyllanthus is a small herb that comprises several homoeopathic properties and is broadly used globally. *Phyllanthus* is known for its vital uses in managing stomach ailments, the genitourinary system, kidney, spleen, and liver issues. This plant also possesses antiseptic properties. The whole plant *Phyllanthus* is used in genital infections such as gonorrhoea, menorrhagia and others [9]. *Rauwolfia* is a medicinal plant used throughout India in the 1940s and throughout the world in the 1950s. The plant is well-known for its efficacy in treating hypertension. *Rauwolfia* was widely used to treat a variety of diseases such as malaria, abdominal pain, dysentery and also insect and snake bites [10]. Therefore, the present study aimed to standardize the best sterilization method that effectively eliminates the microorganisms present in the selected medicinal plant powders.

METHODOLOGY

Plant powders

Tinospora stem powder and coarse powders of whole plants of *Eclipta*, *Phyllanthus* and *Rauwolfia* were procured from an authorized supplier. The plant powders were further grounded using a commercial blender and sieved using a 200 cc sieve.

Plant powders sterilization

The plant powders were sterilized using various techniques to identify and standardize the best sterilization technique that eliminates the contaminants present in the plant powders. All the sterilization methods were standardized in the QIU laboratory.

Microwave pasteurization

All the plant powders were subjected to the microwave pasteurization method. About 5 g of each plant powder were microwaved for different time periods (3, 4 and 9 minutes) at 78 °C and rapidly cooled at 4 °C for 4 consecutive cycles. After that, the plant powders were kept under ultraviolet (UV) light for 30 minutes.

Hot air oven

All the plant powders were subjected to a hot air oven sterilization technique. The plant powders were sterilized by incubating the plant powders in a hot air oven. About 5 g of each plant powder was kept in a hot air oven at different temperatures, 70 °C and 80 °C, respectively.

Ultraviolet ray

The plant powders were also sterilized by keeping the plant powders under UV rays. About 5 g of each plant powder was kept under UV rays at different time periods, 30 minutes, 1 hour and 2 hours, respectively.

Microbial contamination testing

The procedure of microbial screening was standardized in the QIU laboratory. The unsterilized and sterilized plant powders were tested for bacterial and fungal contaminations. The suspensions of unsterilized and sterilized plant powders for microbial screening were prepared as follows. About 50 mg of each unsterilized and sterilized plant powder were dissolved in 1 mL of double-distilled water (ddH_2O), respectively. The plant suspensions were evenly streaked on the nutrient agar (NA) and sabouraud dextrose agar (SDA) plates. The agar plates were incubated for 24 hours at 37 °C. After 24 hours the plates were examined for microbial growth.

RESULTS

Before sterilization

Table 1 represents the result of microbial screening for unsterilized plant powders. Based on Table 1, there were bacterial and fungal growth observed on NA and SDA plates for all four types of unsterilized plant powders. Figure 1(a) shows bacterial colonies grown on the NA plate for *Tinospora* plant powder whereas figure 2 (a) shows the growth of fungal colonies on the SDA plate for *Tinospora* plant powder. Figures 3 (a) and (h) show the growth of bacterial and fungal colonies on NA and SDA plates, respectively for *Eclipta* plant powder. Figures 4 (a) and (h) show bacterial and fungal growth on NA and SDA plates, respectively for *Phyllanthus* plant powder. Figures 5 (a) and (h) show the growth of bacterial and fungal colonies on NA and SDA plates,

respectively for *Rauwolfia* plant powder. This observation shows that before sterilization, there were microorganisms present in all the plant powders.

Table 1

Before sterilization

Agar plates	Observation			
	Tinospora	***Eclipta***	***Phyllanthus***	***Rauwolfia***
NA	+	+	+	+
SDA	+	+	+	+

Note. Symbol "+" indicates the presence of microorganisms; Symbol "-" indicates the absence of microorganisms.

After sterilization

Microwave pasteurization

Table 2 represents the effectiveness of microwave pasteurization method in sterilizing plant powders. According to Table 2, there was microbial growth observed on NA and SDA plates when all the plant powders were subjected to microwave pasteurization for 3 minutes. Based on figures 1 (b) and 2 (b), immense microbial growth was observed on NA and SDA plates, respectively when *Tinospora* plant powder was sterilized by microwave pasteurization for 3 minutes. Figures 3 (a) and (h), figures 4 (a) and (h), and figures 5 (a) and (h) shows the growth of microorganisms on NA and SDA plates, respectively when *Eclipta*, *Phyllanthus* and *Rauwolfia* plant powders were sterilized by microwave pasteurization for 3 minutes. This indicates that microwave pasteurization for 3 minutes is insufficient to eliminate and kill the microorganisms present in all the plant powders.

Table 2 also represents the efficacy of 4 minutes of microwave pasteurization on *Tinospora*, *Eclipta*, *Phyllanthus* and *Rauwolfia* plant powders. When *Tinospora* plant powder was sterilized for 4 minutes by microwave pasteurization, massive growth of microorganisms was observed on NA and SDA plates, as shown in figures 1 (c) and 2 (c), respectively. Thus, 4 minutes of microwave pasteurization is insufficient to eliminate the microbes present in *Tinospora*. However, it can be seen that there were no microbial growth observed on NA and SDA plates of *Eclipta*, *Phyllanthus* and *Rauwolfia* when the plant powders were microwaved and pasteurized for 4 minutes as represented in Table 2 and figures 3 (b) and (i), figures 4 (b) and (i), and figures 5 (b) and (i), respectively. This study results indicate that the duration of 4 minutes of microwave pasteurization is effective in eliminating the microorganisms present in *Eclipta*, *Phyllanthus* and *Rauwolfia* plant powders. Therefore, 4 minutes of microwave pasteurization is sufficient to eliminate the microorganisms present *Eclipta*, *Phyllanthus* and *Rauwolfia* plant powders.

Based on Table 2, it can be observed that there were no microbial contaminations observed on NA and SDA plates of *Tinospora* when the plant powder was subjected for 9 minutes of microwave pasteurization. Figures 1 (d) and 2 (d) shows the NA and SDA plates with the absence of microbial growth when streaked with sterilized *Tinospora* plant powder. This indicates that 9 minutes of microwave pasteurization is the effective duration to completely eradicate the microorganisms present in *Tinospora* plant powder.

Table 2

Microwave pasteurization

Sterilization duration	Agar plates	Observation			
		Tinospora	***Eclipta***	***Phyllanthus***	***Rauwolfia***
3 minutes	NA	+	+	+	+
	SDA	+	+	+	+
4 minutes	NA	+	-	-	-
	SDA	+	-	-	-
9 minutes	NA	-	x	x	x
	SDA	-	x	x	x

Note. Symbols "+" indicates the presence of microorganisms; Symbol "-" indicates the absence of microorganisms; Symbol "x" indicates that the test was not performed.

Hot air oven

All the plant powders were incubated in a hot air oven at two different temperatures. The plant powders were sterilized at 70 °C and 80 °C for 24 hours. According to Table 3, there were bacterial and fungal colonies observed on NA and SDA plates of all the plant powders that were sterilized at 70 °C and 80 °C. Figures 1 (e) and 2 (e), 3 (c) and (j), figures 4 (c) and (j), and figures 5 (c) and (j) shows the growth of bacterial and fungal colonies on NA and SDA plates respectively, that were streaked with plant powders sterilized at 70 °C. Figures 1 (f) and 2 (f), 3 (d) and (k), figures 4 (d) and (k), and figures 5 (d) and (k) show the growth of bacterial and fungal colonies on NA and SDA plates respectively, that were streaked with plant powders sterilized at 80 °C. As the temperature of hot air oven increased, the growth of bacterial and fungal colonies were slightly reduced. However, the temperatures of 70 °C and 80 °C to sterilize the plant powders were insufficient in eliminating the microorganisms present in the plant powders.

Table 3

Hot air oven

Temperature	Agar plates	Observation			
		Tinospora	***Eclipta***	***Phyllanthus***	***Rauwolfia***

70 °C	NA	+	+	+	+
	SDA	+	+	+	+
80 °C	NA	+	+	+	+
	SDA	+	+	+	+

Note. Symbol "+" indicates the presence of microorganisms; Symbol "-" indicates the absence of microorganisms.

UV rays

Table 4 shows the effectiveness of UV rays in sterilizing all the plant powders. The plant powders were exposed to UV rays at three different time intervals. The plant powders were sterilized under UV rays for 30 minutes, 1 hour and 2 hours. According to Table 4, microbial growth was seen on NA and SDA plates for all the sterilized plant powders at all three different time intervals. Figures 1 (g) and 2 (g) show *Tinospora* plant powder sterilized under UV rays for 30 minutes on NA and SDA plates, respectively. Figures 1 (h) and 2 (h) show microbial growth on NA and SDA plates for *Tinospora* plant powder sterilized under UV rays for 1 hour. Figures 1 (i) and 2 (i) represent growth of microorganisms on NA and SDA plates for *Tinospora* plant powder sterilized under UV rays for 2 hours. It is clearly visible that NA plates had immense bacterial growth compared to the fungal growth on the SDA plates.

Minimal bacterial and fungal growth was observed when *Eclipta*, *Phyllanthus* and *Rauwolfia* plant powders were subjected to UV rays sterilization at different time intervals. Based on figures 3 (e) and (l), growth of microorganisms was observed on NA and SDA plates streaked with *Eclipta* plant powder sterilized for 30 minutes. Figures 3 (f) and (m) show the growth of microorganisms on NA and SDA plates streaked with *Eclipta* plant powder sterilized for 1 hour. Furthermore, figures 3 (g) and (n) show the growth of microorganisms on NA and SDA plates that were streaked with *Eclipta* plant powder sterilized for 2 hours.

When *Phyllanthus* plant powder was sterilized under UV rays for 30 minutes, bacterial and fungal colonies were present on NA and SDA plates as shown in figures 4 (e) and (l). As the UV rays sterilization period for *Phyllanthus* increased to 1 hour, the growth of microorganisms was observed on NA and SDA plates as shown in figures 4 (f) and (m). Figures 4 (g) and (n) show the NA and SDA plates streaked with *Phyllanthus* plant powder sterilized for 2 hours. Based on figures 4 (g) and (n), the growth of microorganisms on NA and SDA plates indicate that contaminants are still present in the plant powder. There was a minimal presence of bacterial and fungal colonies observed on NA and SDA plates as shown in figures 5 (e) and (l).

Figures 5 (f) and (m) show the growth of microorganisms on NA and SDA plates streaked with *Rauwolfia* plant powder sterilized for 1 hour. Similarly, figures 5 (g) and (n) show the growth

of microorganisms on NA and SDA plates for *Rauwolfia* plant powder sterilized for 2 hours. The growth of microorganisms on NA and SDA plates after 30 minutes, 1 hour and 2 hours of UV rays sterilization indicate that UV rays sterilization of *Rauwolfia* plant powder is ineffective to eliminate the microorganisms present in the plant powder.

Table 4

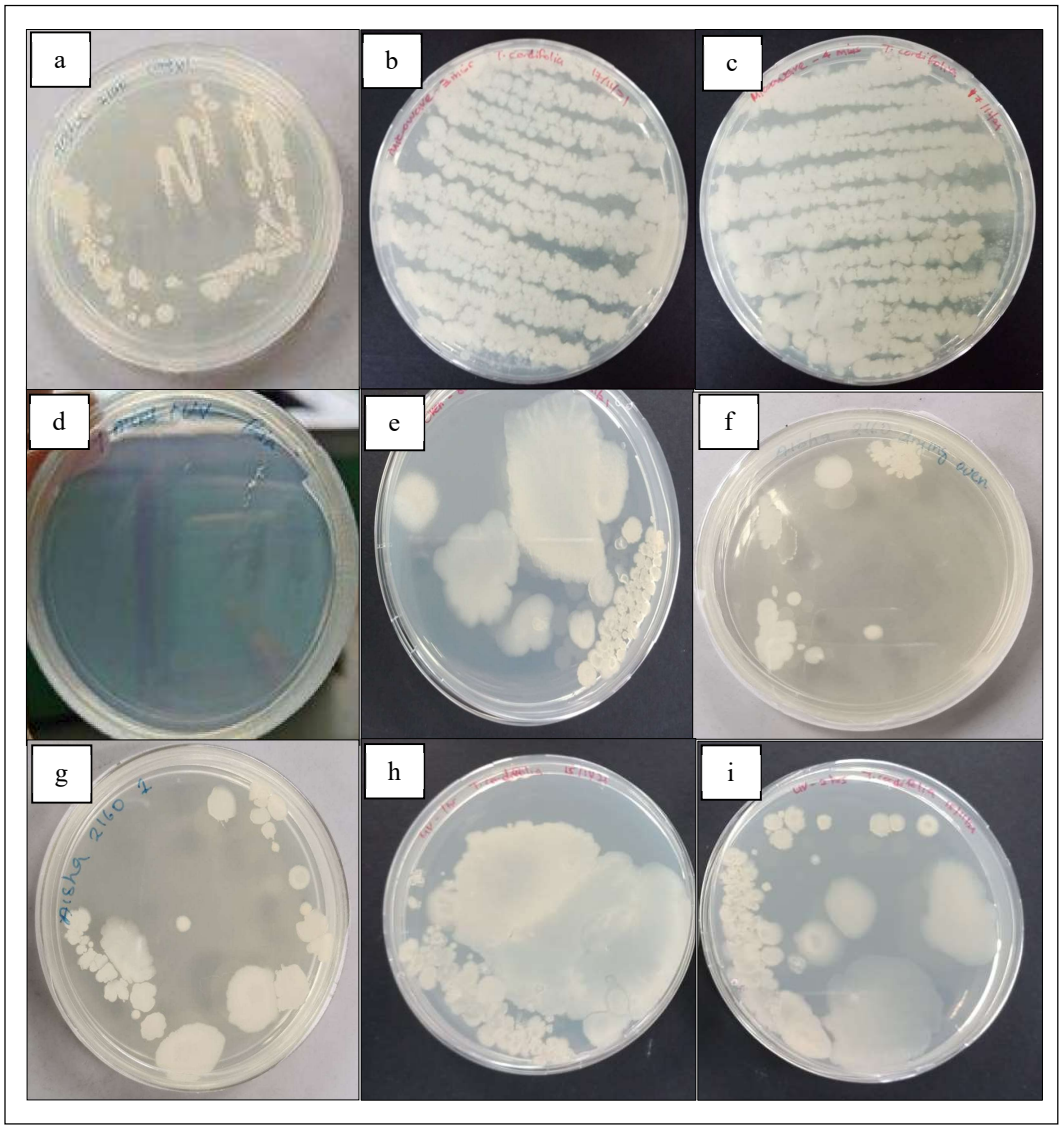
UV rays

Exposure period	Agar plates	Observation			
		Tinospora	***Eclipta***	***Phyllanthus***	***Rauwolfia***
30 minutes	NA	+	+	+	+
	SDA	+	+	+	+
1 hour	NA	+	+	+	+
	SDA	+	+	+	+
2 hours	NA	+	+	+	+
	SDA	+	+	+	+

Note. Symbol "+" indicates the presence of microorganisms; Symbol "-" indicates the absence of microorganisms.

Figure 1

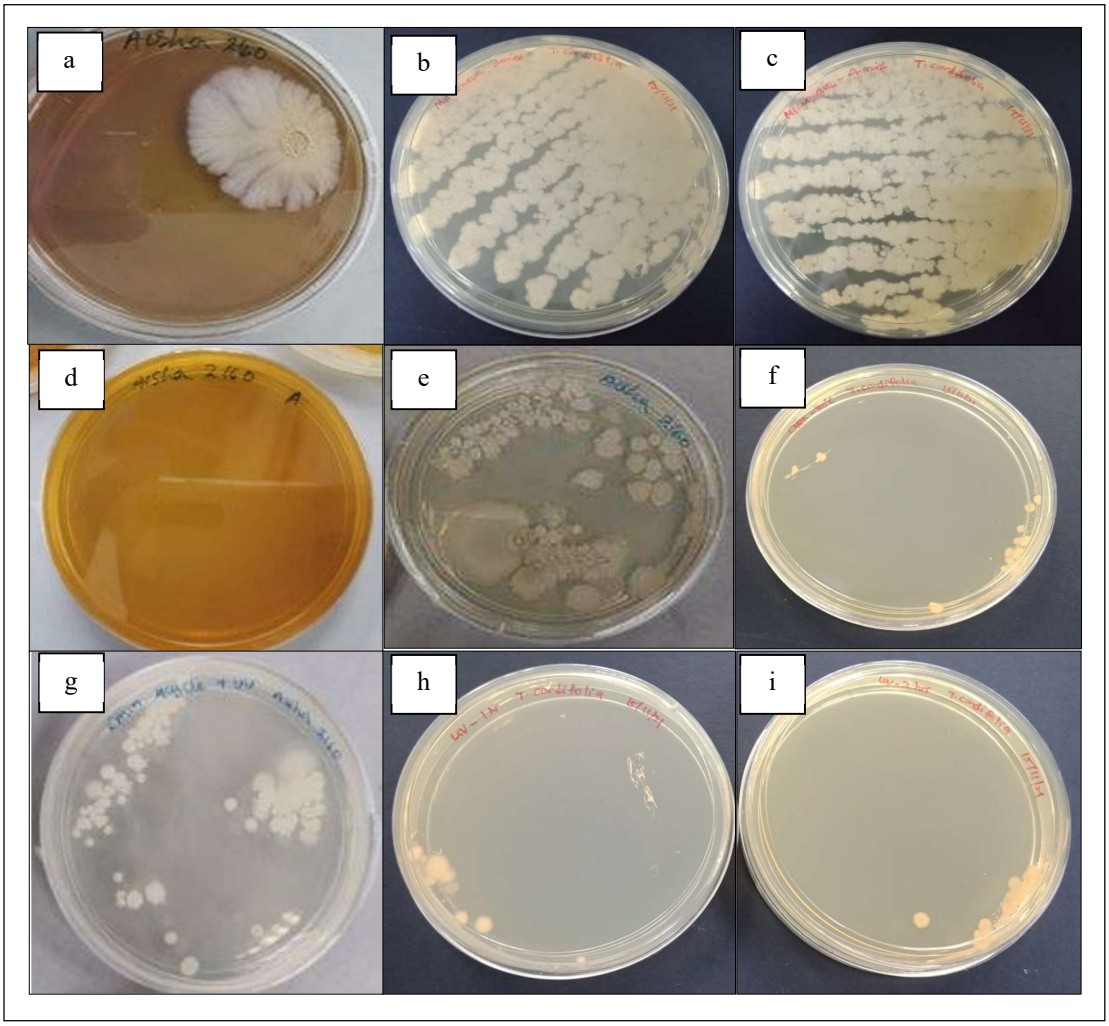
Sterility of Tinospora plant powder streaked on NA plates



Note. (a) *Tinospora* plant powder before sterilization; (b) *Tinospora* plant powder after 3 minutes of microwave pasteurization; (c) *Tinospora* plant powder after 4 minutes of microwave pasteurization; (d) *Tinospora* plant powder after 9 minutes of microwave pasteurization; (e) *Tinospora* plant powder after 70 °C of hot air oven sterilization; (f) *Tinospora* plant powder after 80 °C of hot air oven sterilization; (g) *Tinospora* plant powder after 30 minutes of UV ray sterilization; (h) *Tinospora* plant powder after 1 hour of UV ray sterilization; and (i) *Tinospora* plant powder after 2 hours of UV ray sterilization.

Figure 2

Sterility of Tinospora plant powder streaked on SDA plates

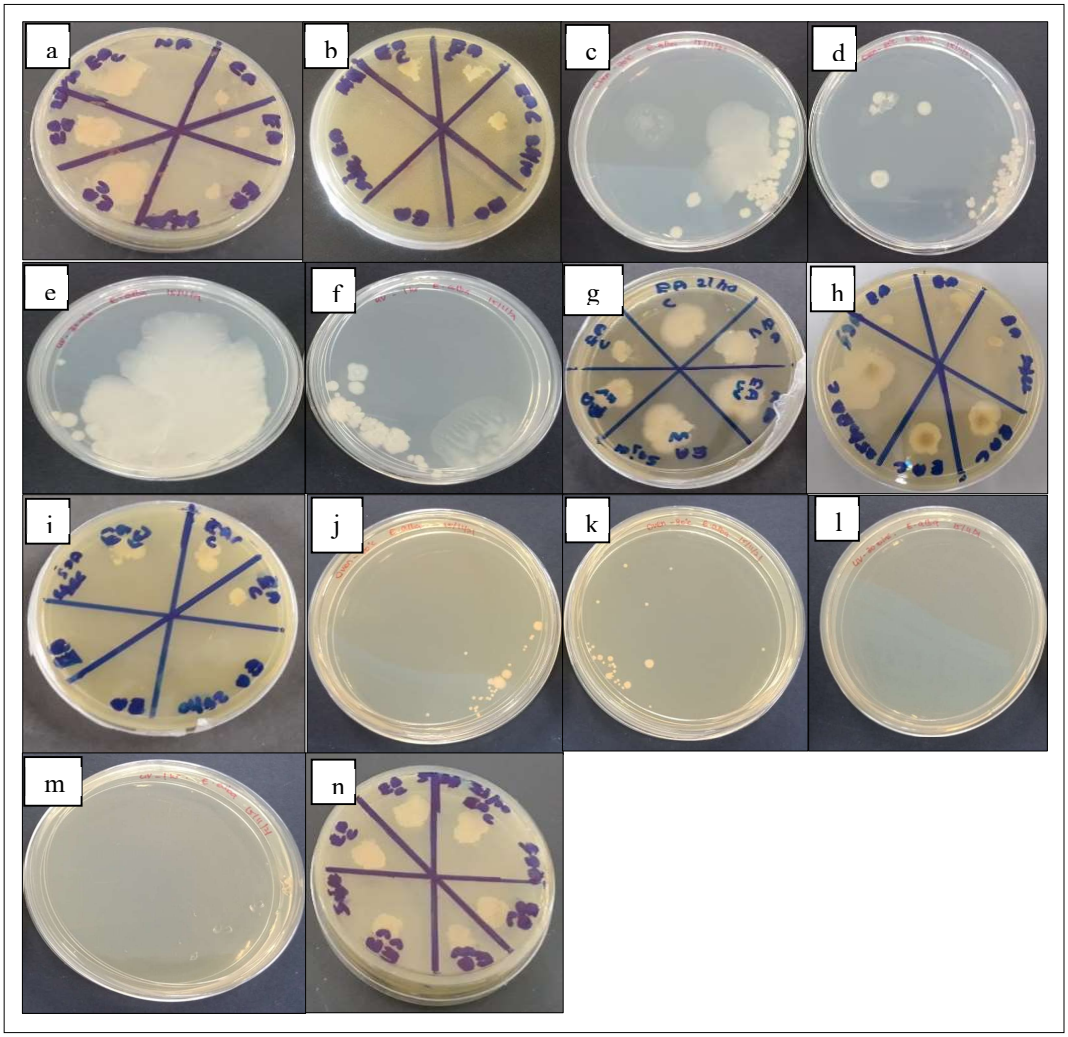


Note. (a) *Tinospora* plant powder before sterilization; (b) *Tinospora* plant powder after 3 minutes of microwave pasteurization; (c) *Tinospora* plant powder after 4 minutes of microwave pasteurization; (d) *Tinospora* plant powder after 9 minutes of microwave pasteurization; (e) *Tinospora* plant powder after 70 °C of hot air oven sterilization; (f) *Tinospora* plant powder after 80 °C of hot air oven sterilization; (g) *Tinospora* plant powder after 30 minutes of UV ray sterilization; (h) *Tinospora* plant powder after 1 hour of UV ray sterilization; and (i) *Tinospora* plant powder after 2 hours of UV ray sterilization.

Figure 3

Sterility of Eclipta plant powder

Note. (a) 'C' denotes *Eclipta* before sterilization and 'EA' denotes *Eclipta* after 3 minutes of microwave pasteurization on NA; (b) 'EA' denotes *E. alba* after 4 minutes of microwave pasteurization on NA; (c) *Eclipta* after 70 °C of hot air oven sterilization on NA; (d) *Eclipta* after 80 °C of hot air oven sterilization on NA; (e) *Eclipta* after 30 minutes of UV ray sterilization on NA; (f) *Eclipta* after 1 hour of UV ray sterilization on NA; (g) 'EA' denotes *Eclipta* after 2 hours of UV ray sterilization on NA; (h) 'C' denotes *Eclipta* before sterilization and 'EA' denotes *Eclipta* after 3 minutes of microwave pasteurization on SDA; (i) 'EA' denotes *Eclipta* after 4 minutes of microwave pasteurization on SDA; (j) *Eclipta* after 70 °C of hot air oven sterilization on SDA. (k) *Eclipta* after 80 °C of hot air oven sterilization on SDA; (l) *Eclipta* after 30 minutes of UV ray sterilization on SDA; (m) *Eclipta* after 1 hour of UV ray sterilization on SDA; and (n) 'EA' denotes *Eclipta* after 2 hours of UV ray sterilization on SDA.



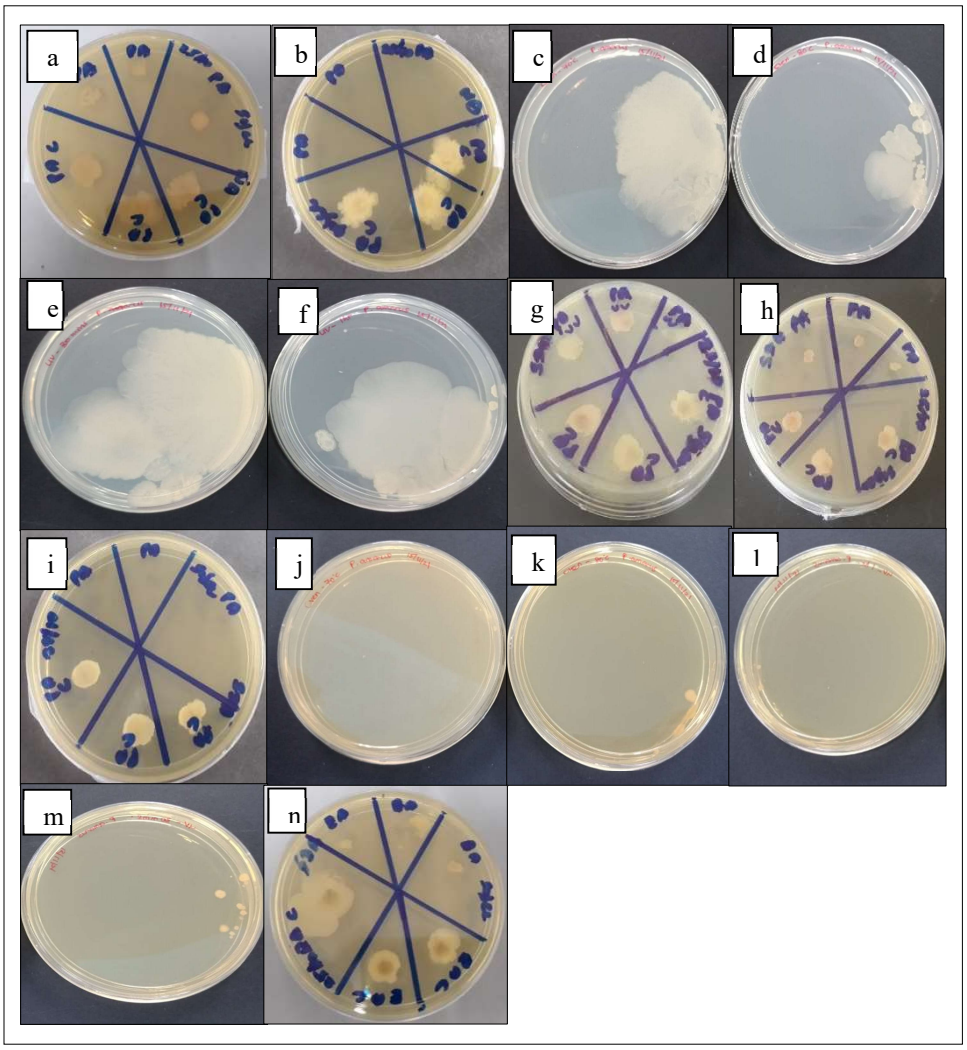


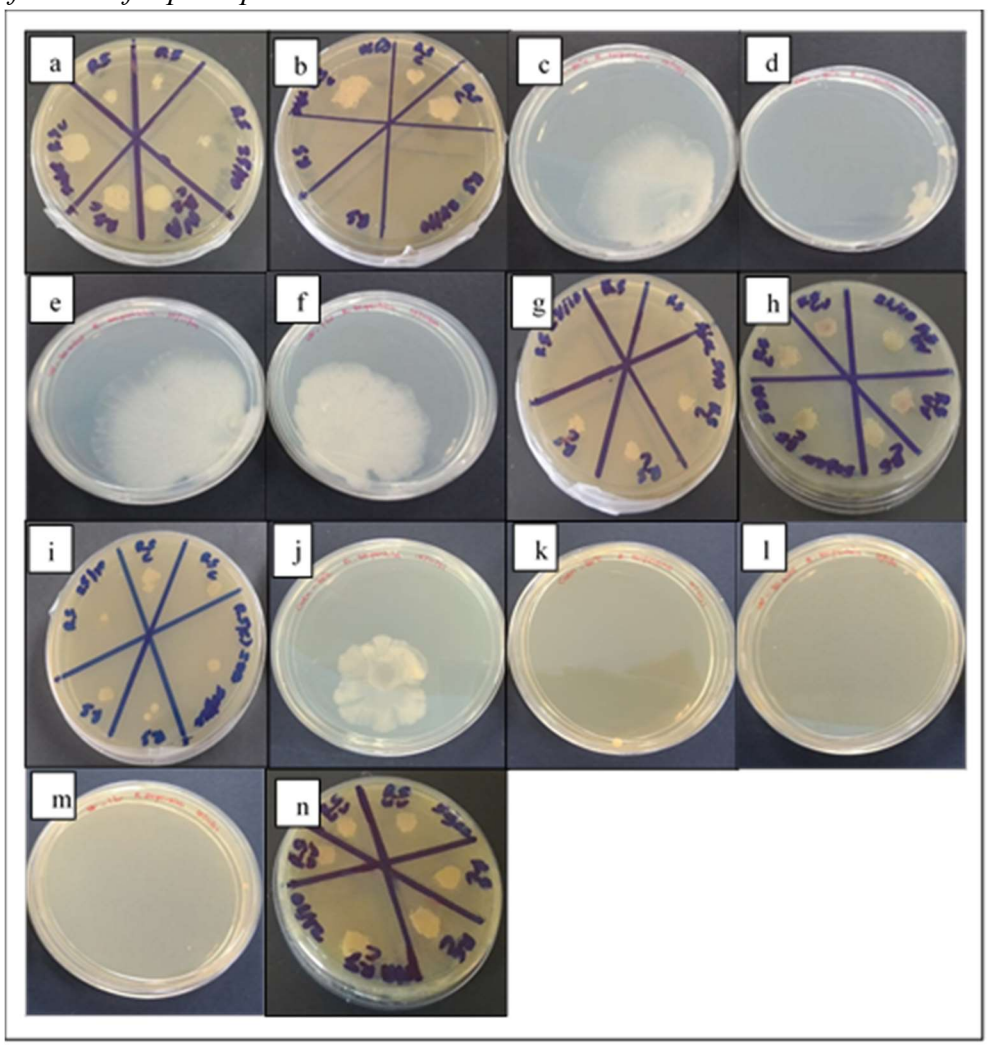
Figure 4
Sterility of Phyllanthus plant powder

Note. (a) 'C' denotes *Phyllanthus* before sterilization and 'PA' denotes *Phyllanthus* after 3 minutes of microwave pasteurization on NA; (b) 'PA' denotes *Phyllanthus* after 4 minutes of microwave pasteurization on NA; (c) *Phyllanthus* after 70 °C of hot air oven sterilization on NA; (d) *Phyllanthus* after 80 °C of hot air oven sterilization on NA; (e) *Phyllanthus* after 30 minutes of UV ray sterilization on NA; (f) *Phyllanthus* after 1 hour of UV ray sterilization on NA; (g) 'PA' denotes *Phyllanthus* after 2 hours of UV ray sterilization on NA; (h) 'C' denotes *Phyllanthus* before sterilization and 'PA' denotes *Phyllanthus* after 3 minutes of microwave pasteurization on SDA; (i) 'PA' denotes *Phyllanthus* after 4 minutes of microwave pasteurization on SDA; (j) *Phyllanthus* after 70 °C of hot air oven sterilization on SDA. (k)

Phyllanthus after 80 °C of hot air oven sterilization on SDA; (l) *Phyllanthus* after 30 minutes of UV ray sterilization on SDA; (m) *Phyllanthus* after 1 hour of UV ray sterilization on SDA; and (n) 'PA' denotes *Phyllanthus* after 2 hours of UV ray sterilization on SDA.

Figure 5

Sterility of Rauwolfia plant powder



Note. (a) 'C' denotes *Rauwolfia* before sterilization and 'RS' denotes *Rauwolfia* after 3 minutes of microwave pasteurization on NA; (b) 'RS' denotes *Rauwolfia* after 4 minutes of microwave pasteurization on NA; (c) *Rauwolfia* after 70 °C of hot air oven sterilization on NA; (d) *Rauwolfia* after 80 °C of hot air oven sterilization on NA; (e) *Rauwolfia* after 30 minutes of UV ray sterilization on NA; (f) *Rauwolfia* after 1 hour of UV ray sterilization on NA; (g) 'RS' denotes *Rauwolfia* after 2 hours of UV ray sterilization on NA; (h) 'C' denotes *Rauwolfia* before sterilization and 'RS' denotes *Rauwolfia* after 3 minutes of microwave pasteurization on SDA; (i) 'RS' denotes *Rauwolfia* after 4 minutes of microwave pasteurization on SDA; (j) *Rauwolfia*

after 70 °C of hot air oven sterilization on SDA. (k) *Rauwolfia* after 80 °C of hot air oven sterilization on SDA; (l) *Rauwolfia* after 30 minutes of UV ray sterilization on SDA; (m) *Rauwolfia* after 1 hour of UV ray sterilization on SDA; and (n) 'RS' denotes *Rauwolfia* after 2 hours of UV ray sterilization on SDA.

DISCUSSION

Sterilization of plant-based products is the method to completely eliminate and kill the microorganisms that are present in the plant powders. Sterilization of plant-based products is essential to ensure the plant powders are sterile and safe to be consumed. Plant-based products that are used in the pharmaceutical field must be sterile to avoid the likelihood of microbial infection or degradation as a result of their usage [11]. The sterility of plant-based products is of utmost importance to prevent the fatal infections caused by pathogenic and non-pathogenic microorganisms present in plant-based products [11]. In the present study, *Tinospora*, *Eclipta*, *Phyllanthus*, and *Rauwolfia* plant powders were subjected to different methods of sterilization. The study was done in order to standardize and choose the best sterilization method that completely eradicates and kills the microorganisms present in selected plant powders.

All the four plant powders were sterilized by microwave pasteurization for 3 minutes, 4 minutes and 9 minutes. Immense microbial growth was observed when all four plant powders were subjected to 3 minutes of microwave pasteurization. As the duration of microwave pasteurization increased to 4 minutes, massive growth of bacterial and fungal growth was observed on NA and SDA plates. This shows that microorganisms present in *Tinospora* plant powder were not eliminated by 4 minutes of microwave pasteurization. However, there was no bacterial and fungal growth observed on NA and SDA plates of *Eclipta*, *Phyllanthus* and *Rauwolfia* plant powders that were sterilized by microwave pasteurization for 4 minutes. Thus, sterilization by microwave pasteurization for 4 minutes at 78 °C and rapid cooling at 4 °C for 4 consecutive cycles, after which the plant powders were kept under UV light for 30 minutes worked the best for *Eclipta*, *Phyllanthus*, and *Rauwolfia* plant powders.

Since there were microbial growth observed for *Tinospora* plant powder after 4 minutes of microwave pasteurization, the plant powder was subjected to 9 minutes of microwave pasteurization at 78 °C, followed by rapid cooling at 4 °C for 4 consecutive cycles. The plant powder was then kept under UV light for 30 minutes. There were no bacterial and fungal growth observed on NA and SDA plates after the *Tinospora* plant powder was sterilized by microwave pasteurization for 9 minutes at 78 °C and rapid cooling at 4 °C for 4 consecutive cycles, followed by 30 minutes of UV light exposure.

Besides microwave pasteurization, all the plant powders were subjected to hot air oven sterilization at 70 °C and 80 °C, and UV rays sterilization for 30 minutes, 1 hour and 2 hours. The sterilization by hot air oven and UV rays had immense bacterial and fungal growth. However, there were lesser microbial colonies observed for hot air oven sterilization as compared to UV rays sterilization. Therefore, the method that worked the best for sterilizing the

plant powders were microwave pasteurization. The sterilization of plant powders by microwave pasteurization is a safe and convenient method. The technology improvements allow the active control of microwave energy to minimize the over processing of plant-based products during sterilization [12]. The best parameters to sterilize *Eclipta*, *Phyllanthus*, and *Rauwolfia* plant powders were pasteurization at 78 °C for 4 minutes and rapid cooling at 4 °C for 4 consecutive cycles followed by 30 minutes of UV rays exposure and parameter to sterilize *Tinospora* plant powder was pasteurization at 78 °C for 9 minutes and rapid cooling at 4 °C for 4 consecutive cycles followed by 30 minutes of UV rays exposure, respectively.

Conclusion:

Tinospora, *Eclipta*, *Phyllanthus*, and *Rauwolfia* plants have been used for generations by medical practitioners for their curative properties. Sterilization of these plant-based products is a necessary process for many studies as contaminations can alter the result. Many different sterilization methods were applied in this study for the sterilization of all four plant powders. The method that worked the best for all the plant powders was the microwave pasteurization method. Prior to the sterilization of the plant powders, microbial contamination screening was done. The results showed both microbial and fungal contaminations. However, after the plants were sterilized by the microwave pasteurization method, there were no contaminations observed. This indicates that the microwave pasteurization method was successful in completely sterilizing *Tinospora*, *Eclipta*, *Phyllanthus*, and *Rauwolfia* powder powders.

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CONFLICTS OF INTEREST
The authors have no conflicts of interest to declare.

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