

# DECONTAMINATION PROTOCOL IN THE PRODUCTION OF HERBAL MEDICINES: A SHORT COMMUNICATION



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## ABSTRACT

**Background:** The effectiveness and safety of natural products depend not only on the inherent properties of the plant, but also on careful preparation, which includes critical steps such as cleaning to remove impurities and washing to eliminate surface contaminants. Thus, the scientific literature emphasizes the significance of thorough cleaning in ensuring botanical purity, directly impacting the ultimate quality of the product. **Methods:** The study utilized the plant species *Cordia verbenacea*, *Alternanthera brasiliana*, and *Lippia alba*, cultivated in Tatuí - SP, subjected to a rigorous selection process, and processed through a washing and drying protocol. The washing involved a two-stage process, including immersion in clean water and a sodium hypochlorite solution. Microbiological analyses were conducted on washed and unwashed samples, including enumeration of viable colony-forming units (CFUs) and detection of pathogens. Moisture content was determined using the oven drying method, and ash content was assessed following the Brazilian Pharmacopeia guidelines. **Results:** Microbiological results indicate the absence of *Salmonella* sp. and *Staphylococcus aureus* in all analyzed plant drugs, regardless of washing and stabilization conditions. *Pseudomonas aeruginosa* was observed only in unwashed/stabilized *Cordia verbenacea* and *Alternanthera brasiliana* samples. *Escherichia coli* was identified in an unwashed/stabilized *Lippia alba* sample. The washing/stabilization process significantly reduced total bacterial and fungal counts, especially when exceeding the limit set by the Brazilian Pharmacopeia ( $10^3$  CFU/g). Total ash content remained unchanged after the washing/stabilization process in the three plant species, staying within the Brazilian Pharmacopeia limits (14%). Moisture content did not differ statistically between washed/stabilized and unwashed/unstabilized plant species and met Brazilian Pharmacopeia specifications (8-14%). **Conclusion:** The washing/stabilization process effectively reduced microbial load in *Lippia alba*, *Alternanthera brasiliens*, and *Cordia verbenacea*, with no significant changes in ash and moisture content. Preliminary analysis suggests that processing control ensures minimum quality conditions, enhancing overall safety for consumers.

**Key words:** washing, stabilization, microbiological, pathogens, ash, moisture.

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## INTRODUCTION

The herbal drugs, widely utilized in the pharmaceutical industry, culinary arts, and traditional medicine, play a crucial role in various cultures around the world. The effectiveness and safety of these substances depend not only on the intrinsic properties of the plants but also on the careful preparation process, which includes fundamental steps such as cleaning, washing, drying, and stabilization. Cleaning marks the initial stage of the plant-based drug preparation process, a pivotal step to remove impurities such as dirt, stones, and organic debris. Thorough elimination of these contaminants is vital to ensure the botanical purity of the drug. Scientific literature emphasizes the significance of meticulous cleaning in the plant-based drug production process, underscoring its direct impact on the final product quality (1). Following cleaning, the washing step is conducted to remove surface impurities. The use of clean water and, in some cases, specific solvents, plays a crucial role in this phase. Studies, such as Oliveira *et al.* (2015) (2), highlight effective washing methods that ensure complete removal of surface contaminants, thereby promoting the safety and quality of plant-based drugs. Subsequently, proper drying of the plants is essential to preserve their chemical properties and prevent decomposition. Different techniques, such as air drying or controlled greenhouse drying, are employed based on the plant's characteristics. Research by Silva and Santos (2018) (3) emphasizes the importance of controlled temperature and humidity during drying, highlighting their impact on the final properties of the herbal drug. Stabilization is the final stage of the process, where plant-based drugs are treated to preserve their quality over time. Stabilization methods include the use of natural preservatives and specific storage conditions. Studies such as Almeida *et al.* (2019) (4) underscore the relevance of adequate stabilization in maintaining the therapeutic properties of plant-based drugs, emphasizing its importance for the pharmaceutical and medicinal industry. Thus, this study aims to evaluate a method of cleaning and washing herbal drugs and its impact on microbial load, moisture content, and total ash content in *Cordia verbenacea*, *Alternanthera brasiliana*, and *Lippia alba* herbal drugs.

## METHODS

### Plant material

The plant species *Cordia verbenacea*, *Alternanthera brasiliana*, and *Lippia alba* were obtained after cultivation in Tatuí - SP, on the Chacara Santo Exedito, Estrada Municipal Joaquim de Campos Vieira s/n°, Bairro Jurumirim - 23°25'48.4"S 47°56'58.1"W. The plants were harvested and transported to the processing site, where they underwent a rigorous selection process, removing all non-relevant parts, including damaged or unsuitable leaves for analysis. After this stage, the selected plants were forwarded to the subsequent steps.

### Washing and drying protocol for plants

The washing of the plants was carried out in two stages. Initially, the plants were placed in a tank containing water from an artesian well, free from chlorine or other impurities, being subjected to manual immersion washing process. Subsequently, the plants were immersed in a tank containing water and a sodium hypochlorite solution at a concentration of 0.04%, for 5 minutes. After immersion in sodium hypochlorite, the plants were washed again in clean water to remove any residual disinfectant. Following the washing, the samples underwent a centrifugation process to eliminate excess water. Finally, the plants were dried at a controlled temperature between 40 and 45°C for a variable time established according to the characteristics of each species, to achieve total drying of the plant material.

### Microbiological analysis

The samples were dry and packed in plastic bags, properly sealed and labeled with the manufacturing date and lot number. Subsequently, they were sent to the Multidisciplinary Laboratory of Anhembi Morumbi University, São José dos Campos - SP campus, and to the Pharmaceutical Processing Laboratory of GAP Biotech, located in São José dos Campos - SP. The microbiological analyses of the plant material included direct plate count methodologies per colony-forming unit per gram (CFU/g), as well as the detection of viable forms of mesophilic aerobes, molds, and yeasts, total coliforms, fecal coliforms, *Salmonella* sp., and *Staphylococcus aureus*. Microbiological analyses were conducted in triplicate.

### Sample Preparation

Thirty grams of each plant species (washed and unwashed), previously homogenized, were weighed. Each sample consisted of leaves from *Cordia verbenacea*, *Alternanthera brasiliana*, and *Lippia alba*. These samples were homogenized separately with 300 mL of 0.1% sterile peptone water. Vigorous shaking was followed by one-hour incubation at room temperature. These treated samples corresponded to a 10<sup>-1</sup> dilution. Subsequently, they were immediately used for the necessary subsequent dilutions for all microbiological analyses.

### Enumeration of Viable Colony-Forming Units (CFUs)

Mesophilic aerobic counts were determined using 0.1 mL of each sample at dilutions of 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup>. The samples were seeded by pour plating in Petri dishes containing Soybean Casein Digest Agar. Subsequently, the plates were inverted and incubated at 35°C for 48 hours in a controlled temperature incubator. For molds and yeasts, fractions of 0.1 mL from each sample at dilutions of 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup> were similarly plated. These samples were incubated at 20-25°C for five days. After the incubation period, colonies were counted using a colony counter. The mean count obtained from the sample and its corresponding triplicate at a specific dilution, multiplied by the dilution factor,

yielded the number of colony-forming units per gram (CFU/g). To count yeasts and filamentous fungi, Yeast agar was used for yeasts and Potato-dextrose agar for filamentous fungi.

### Pathogens detection

Pathogen detection was performed through cultivation on selective media, following enrichment in selective broth or otherwise. For the detection of *Salmonella* sp, samples were enriched in Tetrathionate Broth at 35-37°C for 24 hours. Colonies were then isolated on Petri dishes using *Salmonella-Shigella* Agar and incubated at 35-37°C for 24 hours. Detection of *Staphylococcus aureus* involved sample enrichment in casein-soy broth, followed by incubation at 35-37°C. Identification was carried out using Mannitol Agar as a selective medium and incubation at 35-37°C for 48 hours. Lastly, *Pseudomonas aeruginosa* detection was conducted with enrichment in casein-soy broth, incubated at 35-37°C for 48 hours, followed by identification using Cetrimide Agar as a selective medium and incubation at 35-37°C for 48 hours.

### Determination of Moisture Content

The moisture content of each sample was determined in duplicate using the oven drying method, following the procedure established by the Adolfo Lutz Institute (5). Approximately 2g of each sample was placed in pre-dried crucibles and then heated in an oven at 105°C for 6 hours. Afterward, the sets (weight of the empty crucible plus the weight of the dried sample) were removed from the oven, allowed to cool inside a desiccator, and then weighed. The calculation for the percentage of moisture in each sample was performed using the following equation: % Moisture =  $(P_i - P_f) \times 100 / \text{Sample Weight}$

Where:  $P_i$  = Weight of the empty crucible plus the initial sample weight;  $P_f$  = Weight of the empty crucible plus the weight of the dried sample; Sample Weight = Initial weight of the sample.

### Determination of Ash Content

To ascertain the total ash content, we followed the procedure outlined in the Brazilian Pharmacopeia (1998) (6). About 3 g of each powdered plant material were carefully weighed and then placed into porcelain crucibles that had been pre-calcined, cooled, and re-weighed. The samples were subsequently incinerated at 600 °C. The ash percentages were computed in relation to the dried powder. All analyses were performed in triplicate, and the ash content was calculated based on the plant material, with the findings presented as a percentage.

### Statistical analysis

The unpaired Student's t-test was employed to compare the parameters between plant materials that underwent prior treatment (stabilization) and those without treatment. Statistical significance was defined as a p-value  $\leq 0.05$ . The results were presented as the mean  $\pm$  standard error of the mean from a minimum of three independent experiments.

## RESULTS

The microbiological results have been detailed in Tables 1 and 2. In the identification of pathogens, the absence of *Salmonella* sp. and *Staphylococcus aureus* was observed in all the plant drugs analyzed, regardless of the proposed conditions in the study, i.e., both in washed/stabilized and unwashed/stabilized plant drugs. Regarding *Pseudomonas aeruginosa*, its growth was observed only in the plant drugs *Cordia verbenacea* and *Alternanthera brasilianas*; however, this occurred exclusively in the unwashed/stabilized samples. Lastly, *Escherichia coli* was identified in an unwashed/stabilized sample of *Lippia alba*. (Table 1).

Table 1

Herbals drugs	Presence							
	<i>E. coli</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>		<i>Salmonella</i> sp.	
	N W/S	W/S	N W/S	W/S	N W/S	W/S	N W/S	W/S
<i>C. verbenacea</i>	Absent	Absent	Absent	Absent	<b>Present</b>	Absent	Absent	Absent
<i>A. brasilianas</i>	Absent	Absent	Absent	Absent	<b>Present</b>	Absent	Absent	Absent
<i>L. alba</i>	<b>Present</b>	Absent	Absent	Absent	Absent	Absent	Absent	Absent

W/S = Washed/Stabilized; N W/S = Not Washed/Stabilized; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *L. alba* = *Lippia alba*; *A. brasilianas* = *Alternanthera brasilianas*; *C. verbenacea* = *Cordia verbenacea*.

Regarding the growth of bacteria and fungi, the analysis of the total bacteria count revealed a significant reduction after the washing/stabilization process. This procedure led to a decrease

in the count of these microorganisms, especially in cases where aerobic bacterial growth exceeded the limit established by the Brazilian Pharmacopeia of  $10^3$  CFU/g, as shown in Table 2.

Table 2

Herbals drugs	CFU/g count					
	Bacteria		Yeast		Filamentous fungi	
	N W/S	W/S	N W/S	W/S	N W/S	W/S
<i>C. verbenacea</i>	$2.0 \times 10^4$ ( $\pm 2.1$ )	$0.9 \times 10^3$ ( $\pm 3.2$ )	$1.0 \times 10^1$ ( $\pm 1.7$ )	$0.2 \times 10^1$ ( $\pm 2.9$ )	Absent	Absent
<i>A. brasilianas</i>	<b><math>3.0 \times 10^4</math> (<math>\pm 1.5</math>)</b>	$1.9 \times 10^3$ ( $\pm 3.7$ )	$1.5 \times 10^2$ ( $\pm 2.4$ )	$0.6 \times 10^2$ ( $\pm 3.1$ )	$2.6 \times 10^2$ ( $\pm 3.7$ )	$1.8 \times 10^2$ ( $\pm 2.2$ )
<i>L. alba</i>	<b><math>2.3 \times 10^4</math> (<math>\pm 2.9</math>)</b>	$1.1 \times 10^3$ ( $\pm 1.7$ )	$0.1 \times 10^3$ ( $\pm 3.9$ )	$1.9 \times 10^2$ ( $\pm 2.5$ )	Absent	Absent

W/S = Washed/Stabilized; N W/S = Not Washed/Stabilized; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *L. alba* = *Lippia alba*; *A. brasilianas* = *Alternanthera brasilianas*; *C. verbenacea* = *Cordia verbenacea*. Bold = Values above the limits established by the Brazilian Pharmacopeia. The results represent the average of three independent outcomes.

Tests were conducted in triplicates.

The results obtained after determining the total ash content showed that the washing/stabilization process did not significantly alter ( $p > 0.05$ ) the levels found in the three evaluated plant species (Table 3). Furthermore, the total ash content did not exceed the limits established by the Brazilian Pharmacopeia (14% for total ash). Similarly, the determination

of moisture content by desiccation of the washed/stabilized plant species did not show statistically different values when compared to the unwashed/unstabilized plant species ( $p > 0.05$ ) (Table 3). The moisture content results also did not exceed the specified limits of the Brazilian Pharmacopeia (range of 8 - 14% for plant drugs).

Table 3

Herbals drugs	Ash Content (%)		Moisture Content (%)	
	N W/S	W/S	N W/S	W/S
<i>C. verbenacea</i>	8.54 ( $\pm 1.6$ )	9.02 ( $\pm 2.9$ )	8.21 ( $\pm 3.4$ )	8.96 ( $\pm 2.9$ )
<i>A. brasilianas</i>	9.32 ( $\pm 3.5$ )	8.96 ( $\pm 3.7$ )	10.4 ( $\pm 4.1$ )	9.21 ( $\pm 2.9$ )
<i>L. alba</i>	7.86 ( $\pm 2.1$ )	8.22 ( $\pm 1.7$ )	10.1 ( $\pm 3.9$ )	11.2 ( $\pm 2.5$ )

## DISCUSSION

The increasing use of herbal remedies by the population has raised concerns about the quality of these products. Challenges

related to the authenticity, purity, and chemical composition of plant-based raw materials are common issues that can result in subpar herbal remedies (7). This situation generates a need for efficient methods to control the quality of its inputs. Therefore,

establishing the identity and quality of the plant-based raw material can be regarded as a preliminary step in ensuring the final product's quality (8). The results obtained from the microbiological analyses revealed that the proposed washing/stabilization process effectively reduced the microbial load, particularly when the values exceeded the limits recommended by the 5th edition of the Brazilian Pharmacopeia. These specifications set the maximum allowable limits at  $10^3$  CFU/g or mL for bacteria and  $10^2$  CFU/g or mL for fungi and yeasts. The purpose of microbiological control is to ascertain the total number of microorganisms in non-sterile preparations, cosmetics, and herbal medicines. It also involves identifying pathogens such as *Salmonella* sp., *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, which should not be present (9). The moisture content and total ash tests revealed that none of the analyzed herbal materials exceeded the limits specified in the 5th edition of the Brazilian Pharmacopeia. According to the Pharmacopeia, herbal materials should have a moisture content ranging from 8% to 14%, and the total ash content should not exceed 14% (6). An excess of water in the samples is detrimental to their quality as it promotes enzymatic activity and the proliferation of microorganisms, which can break down the plant's active compounds and produce substances that, if ingested, may lead to intoxication (9). The determination of total ash content is crucial for quality control, aiming to identify the presence of non-volatile inorganic impurities that might be contaminating the herbal material (10, 11). Finally, this short communication aimed to implement a protocol for washing and stabilizing plant-based drugs. The goal was to ensure the quality of these plant-based drugs, certifying that they adhere to pharmacopeial standards, free from pathogenic microorganisms or potentially harmful substances. This ensures the microbiological and physicochemical quality of the plant-based drug, promoting safe and reliable consumption.

## CONCLUSIONS

Based on the results obtained, it is possible to conclude that the proposed washing/stabilization process in this study, to which samples of *Lippia alba*, *Alternanthera brasiliensis*, and *Cordia verbenacea* were subjected, proved to be effective in reducing the microbial load of these plants. No significant differences were observed in ash and moisture content levels. Additionally, it can be inferred that the processing control, in a preliminary analysis, ensures minimum quality conditions for the analyzed plant species, enhancing the quality and safety for consumers.

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