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RESEARCH ARTICLE OPEN ACCESS

Heat Treatment Reduces Bacterial Contamination of Medicinal Plants Grown in A Community Garden

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Abstract

The verification of microorganisms that indicate contamination of medicinal plants cultivated in vegetable gardens is fundamental to contribute and improve the conditions and management of the garden, providing safer and healthier food for the consumer population. The study aims to evaluate the bacteriological quality of *Cymbopogon citratus*, *Aloysia citriodora*, *Plectranthus barbalus*, *Aloe vera (L.) Burm. F.*, *Sedum dendroideum*, and *Peumus boldus* cultivated in community gardens. Six samples were randomly collected from each plant and sent to the Laboratory of Veterinary Preventive Medicine and Public Health at Universidade Paranaense-UNIPAR, for bacteriological analysis (aerobic mesophilic microorganisms, total coliforms and coliforms at 45ºC. The cold extract (room temperature) of the medicinal plants evaluated, the *Plectranthus barbalus* had the highest mean for aerobic mesophiles (1.26 CFU/g x 10^6) and the *Cymbopogon citratus* second highest mean for aerobic mesophiles (9.80 CFU/g x 10^5). In the coliform count at 45°C, the highest mean was found in the *Aloysia citriodora* (2.90 CFU/g x 10^3) and it presented the second lowest mean for coliforms at 45°C (0.63 CFU/g x 10^3) and was zero for total coliforms. Regarding the hot extract (95°C-100°C) of the medicinal plants evaluated, a significantly higher aerobic mesophiles count was found for *Aloysia citriodora* when compared with *Aloe vera (L.) Burm. F.*, *Sedum dendroideum* and *Peumus boldus*, not differing statistically from *Cymbopogon citratus* and *Plectranthus barbalus*. It was found that medicinal plants are contaminated but, since they are indicated as teas (infusion), they are safe for their consumers because the risk of bacteriological contamination can be considerably eliminated or reduced due to the boiling process.

Keywords: Aerobic Mesophiles, Food Safety, Microbiology, Public Health, Total Coliforms

INTRODUCTION

The cultivation of food species in home gardens favors access to fresh food in quantity and quality, which contributes to food and nutritional security[1]. These characteristics of vegetables contribute positively to health, as they act as functional/nutraceutical foods, helping to improve health and well-being and/or reduce the risk of diseases, in addition to providing pleasure/taste of planting, cultivar, occupation and therapy[2].

With the growing search for natural products and their derivatives, a more careful assessment of the quality of the products offered to the consumer must be carried out, based on specific legislation, controlling all stages of production. The control and evaluation of the microbiological and parasitological load of medicinal plants are determinant for quality assurance and, if they are not properly complied with, may compromise their use as adjuvant in improving the individual's health status[3-5].

Henz and Alcantara[6] defines a community garden as one that is grown jointly by groups of families or people from a community, by means of production cooperatives, who are responsible for managing production. In general, they are installed in idle urban areas (public and private), used for the cultivation of vegetables, medicinal plants, production of seedlings, vegetables, fruits and other foods, which supply families living near these lands.

Another positive aspect of agricultural production in urban areas is the formation of eating habits, especially as the relationship between food and health is evident. The production and sale of vegetables benefits not only the families involved in the garden, but also the residents around the unit, who generally consume more food free of agrochemicals[7].

The World Health Organization estimates that 80% of people depend on traditional medicine[8] and despite major advances in the pharmaceutical industry, the use of this alternative therapy is growing, which increases the responsibility of regulatory agencies and manufacturers, as they must ensure the quality and therapeutic efficacy of these products[9]. The organization responsible for the safety and efficacy of herbal products in Brazil is the National Health Surveillance Agency, however, these products are not considered with the same vigor as synthetic drugs[10] and yet it is desirable and important that such products are within the minimum quality standards[11].

Nevertheless, investigations of the microbiological quality of vegetable-based drugs and by-products obtained from them,

carried out in other countries, showed rates of microbial contamination in disagreement with internationally accepted standards for drugs[12], and in Brazil, microbiological evaluations of herbal medicines marketed in pharmacies showed similar results[13].

Thus, the verification of microorganisms that indicate contamination of medicinal plants cultivated in vegetable gardens is fundamental to contribute and improve the conditions and management of the garden, providing safer and healthier food for the consumer population. The present work aims to evaluate the bacterial contamination by aerobic mesophiles, total coliforms and 45°C obtained through heat treatment in six medicinal plants grown in a community garden.

MATERIAL AND METHODS

The survey was conducted in the community garden of the State School of Campo Benjamim Antonio Motter, located in the district of Central Santa Cruz, Cafelandia, state of Parana.

The Municipality of Cafelandia is located in the western region of Parana, at latitude 24° 36' 12" and longitude 53° 21' 09", and its altitude is 550 meters and coordinates are 24° 37' South Latitude, 53° 20' Longitude W - GK.

Cafelandia is a predominantly agricultural municipality, largely due to the good natural fertility of its soils. Four types of soils are detected in the Municipality (Eutrophic Purple Latoil, Structured Purple Soil, Indiscriminate Hydromorphic Soils and Litholic Soils), with a predominance of Eutrophic Purple Latoil (90.3% of the total area) and Structured Purple Soil (8.0%)[14].

The medicinal plants were collected from May to July 2019, using gloves and previously sterilized material. This work was carried out with six species of medicinal plants, which were chosen for their availability at the time of this work and followed the procedure described in Fig. 1.

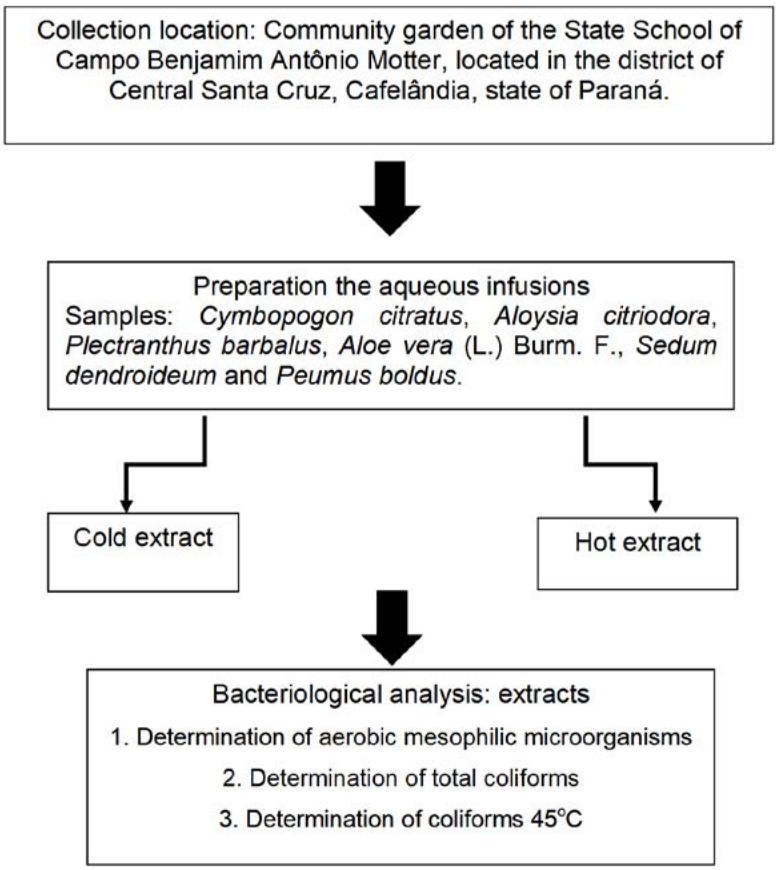

Fig. 1. Scheme of the processing stages of medicinal plants

From the six species, two samples (to cold extract and hot extract) randomly chosen from different points were collected and the samples were packed in sterile Kraft paper bags with the appropriate identifications until processing in the UNIPAR Laboratory of Veterinary Preventive Medicine and Public Health.

In the laboratory, the samples of each species, after being weighed (10 grams of each sample), were transferred aseptically to different plastic bags, with hermetic closure. Then, the technique known as surface washing or rinsing was used to obtain the microorganisms present in medicinal plants[15].

To determine aerobic mesophilic microorganisms, the methodology described by Silva et al.[16] and Barbosa et al.[17] was used with some modifications.

The culture medium used was Plate Count Agar (PCA) together with the surface seeding technique. Samples were analyzed by pipetting 0.1 mL aliquots or 100 microliters of the 10^{-1}, 10^{-2} and 10^{-3} dilutions to sterile petri dishes. The plates were homogenized and incubated inverted in an oven at 35°C for 24-48 hours. After the incubation period, the colony forming units (CFU) were counted, aided by a colony counter where the number of plates between 25 and 250 colonies were considered for counting, multiplying the number of CFU by the respective dilution factor. The results were expressed in CFU/g (colony forming units/gram), considering the initial weight of the samples.

For the determination of total coliforms and coliforms at 45°C, the methodology described by Bernadin et al.[18] was used with some modifications.

An initial volume of 0.1 mL of each dilution (10^{-1}, 10^{-2} and 10^{-3}) was used. It was inoculated in Brilliant Green (BG) culture medium for total coliform counts and in *Escherichia coli* (EC) culture medium for coliform analyses at 45°C.

The inoculum was carefully spread over the entire surface until it was completely absorbed with the aid of a "Drigalski" strap. The BG plates were incubated in an incubator at 35°C for 24h and the EC plates were incubated at 45°C for 48h.

Plates containing 15 to 150 colonies were considered for counting and the value corrected according to dilution. Results were expressed in CFU/g (colony forming units/gram), considering the initial weight of the samples.

Bacteriological analysis of medicinal plants (CFU/gram) was performed using the

Table 1. Mean of aerobic mesophilus count (CFU/gram), total coliforms (CFU/gram) and coliforms at 45°C (CFU/gram) of the cold and hot extract of six different plants (*Aloe vera L. Burm. F., Sedum dendroideum, Peumus boldus, Cymbopogon citratus, Aloysia citriodora* and *Plectranthus barbalus*) collected in a community garden

Medicinal plant	Aerobic mesophilesa*	Total coliforms[a]	Coliforms at 45°C[b]
		Cold extract	
Aloe vera L. Burm. F.	2.1×10^{5bc}	1.8×10^{4cd}	0.67×10^{3}
Sedum dendroideum	5.0×10^{4bc}	2.3×10^{3d}	0.70×10^{3}
Peumus boldus	2.6×10^{3cd}	1.1×10^{3d}	1.03×10^{3}
Cymbopogon citratus	9.8×10^{5ab}	1.6×10^{5ab}	0,00
Aloysia citriodora	7.4×10^{5ab}	4.1×10^{4bc}	2.90×10^{3}
Plectranthus barbalus	1.26×10^{6a}	2.6×10^{3cd}	0.63×10^{3}
		Hot extract[a]	
Aloe vera L. Burm. F.	0.07×10^{3c}	---	---
Sedum dendroideum	0.30×10^{3bc}	---	---
Peumus boldus	0.83×10^{3bc}	---	---
Cymbopogon citratus	1.83×10^{3ab}	---	---
Aloysia citriodora	3.17×10^{3a}	---	---
Plectranthus barbalus	1.50×10^{3ab}	---	---

[a]Averages means followed by different letters in the column differ by *Student-Newman-Keuls* test (P<0.05); b Not significant by *Kruskal-wallis* test (P>0.05). *Standard to count of aerobic bacteria in plant drugs submitted to cold extraction process (10^5 CFU/g) and hot extraction process (10^7 CFU/g)

Kruskall-wallis test, using the Bioestat 5.0 statistical program[19]. When relevant, the *Student-Newman-Keuls* test was chosen to compare means, and for all tests a 5% significance level was chosen.

RESULTS

There were variations in the count of the microorganisms evaluated in the present study for the different medicinal plants (Table 1).

For the cold extract, there was a higher ($P < 0.05$) aerobic mesophilic count for *P. barbalus* when compared to *A. vera* L. Burm. F., *P. boldus* and *S. dendroideum*, not differing ($P > 0.05$) from *C. citratus* and *A. citriodora*. On the other hand, in relation to the total coliform count, this plant presented the third lowest count (2.6×10^3 CFU/g) differing ($P < 0.05$) only from *C. citratus*.

In the hot extract, there was a reduction in the aerobic mesophil count for all medicinal plants evaluated, however, *A. citriodora* had a higher ($P < 0.05$) count (1.5×10^3 CFU/g) when compared to *A. vera* (0.07×10^3 CFU/g), *S. dendroideum* (0.3×10^3 CFU/g) and *P. boldus* (0.83×10^3 CFU/g).

Regarding total coliforms and at 45°C, counting was found only for the cold extract (Table 1), with *C. citratus* presenting the highest ($P < 0.05$) total coliform count (1.6×10^5 CFU/g) when compared with *A. vera* (1.8×10^4 CFU/g), *S. dendroideum* (2.3×10^3 CFU/g), *P. boldus* (1.1×10^3 CFU/g) and *P. barbalus* (2.6×10^3 CFU/g). For coliforms at 45°C there was no significant difference ($P > 0.05$) in the count for the different medicinal plants evaluated, and the highest mean was found for *A. citriodora* (2.90×10^3CFU/g).

DISCUSSION

Analyzing the results obtained, it was possible to observe that, with the exception of *P. boldus* and *S. dendroideum*, the other medicinal plants showed contamination above that established by Anvisa[20], as published in Normative Instruction No. 4 of June 18, 2014, for the total aerobic bacteria count for the cold extract (Table 1).

According to this normative instruction, the total count of aerobic bacteria in plant drugs submitted to cold extraction process should be 10^5 CFU/g, and to hot extraction process should be 10^7 CFU/g[20]. The count of aerobic bacteria is used to obtain general information on product quality because products with high bacterial rates may indicate deficiency or low sanitary condition, but they may also be pathogenic[16].

A similar work conducted by Marcondes and Esmerino[15], showed that 82% of medicinal plants grown in domestic gardens in a district of Ponta Grossa (PR), analyzed before heat treatment, had an aerobic mesophilic count within the recommended by Normative Instruction No. 4 of June 18, 2014[20].

Similarly, Zaroni et al.[21] evaluating 72 samples of medicinal plants without submitting them to the hot extraction process found that 41.67% of the samples met ANVISA's recommendation for aerobic mesophiles[20].

In the present study, it was found that the count of aerobic mesophiles for *C. citratus* submitted to the cold extraction process was 9.8×10^5 CFU/g, similar to that found by Zaroni et al.[21] who verified counts varying between 8.5×10^5 and 1.7×10^7 CFU/g for the same extraction process.

Although there is no specific Brazilian legislation for plant drugs subjected to the cold or hot extraction process, the analysis of total coliforms and at 45°C is important, as they are microorganisms that indicate contamination, and the group of coliforms at 45°C it is most often used as an indicator of faecal contamination, however, it also inhabits environments such as the soil[22]. Thus, its presence in medicinal plants submitted to the cold extraction process in the present study, may be associated with soil or water contamination.

On the other hand, it should be noted that differences in the degree of contamination may be related to the distance from the soil surface on which the plant grows, presenting greater contamination the greater the proximity to the soil[23]. Furthermore, according to the same authors the difference in the degree of contamination between medicinal plants can be explained by the fact that certain plants contain natural barriers and antimicrobial substances that have an effect on microbial growth.

Marcondes and Esmerino[15] evaluating different medicinal plants grown in home gardens found that the contamination by total coliforms varied between 2.85×10^1 and 1.25×10^3 NMP/g of coliforms at 45°C from 4.3×10^1 to 1.71×10^2 NMP/g. Similarly, Bernadin et al.[18] found contamination of total coliforms and coliforms

at 45°C in medicinal plants subjected to cold extraction, varying between 4 x 10^3 to 3.4 x 10^7 CFU/ g and 1 x 10^3 to 6.6 x 10^6 CFU/g, being slightly higher than found in the present study (Table 1).

One of the important points to be taken into consideration was that after the heat treatment, coliforms were absent in all samples surveyed.

According to Alterthum and Carvalhal[24], heat is one of the most widely used methods for inactivating microorganisms, because high temperatures cause them to be inactivated and irreversibly lose their ability to multiply. When a microbial population is heated, the reduction in the number of viable organisms occurs at high rates, justifying what was found in the present work.

With the considerable increase in medicinal plants or derived products, there is always a concern about their quality, which may lead to public health problems, due to the possibility of access to products without adequate conditions of consumption, resulting from the great potential for contamination linked to these products[25].

Microbiological quality control is one of the essential steps within this process, since microbiological contamination becomes a problem when it results in undesirable effects. Therefore, before using them *in natura* or commercialization, they must go through a rigorous control from collection and storage to final production, so that the quality and safety of the plants can be guaranteed[26]. With these results, it is important to use medicinal plants *in natura*, as infusion or tea, to reduce or eliminate the microorganisms present.

CONCLUSION

Under the conditions in which the present research was carried out, it is concluded that in relation to the count of aerobic mesophiles, only two plants (*P. boldus* and *S. dendroideum*) met the Brazilian legislation in force for the process of cold extraction of plant drugs.

In relation to the total coliforms and at 45°C, counting was verified only in the plants submitted to the cold extraction process. The hot extraction process (infusion or tea) reduced the count of aerobic mesophiles and did not allow the detection of coliforms by the microbiological method, demonstrating that this is a safe way to use plant drugs.

Therefore, it is recommended that the quality control of vegetable drugs be more rigorous in addition to the adoption of good practices in the management of community gardens in order to comply with the relevant legislation and offer a safe product to the population.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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