japhac.wix.com/japhac
ISSN 2358-3495

Journal of Applied Pharmaceutical Sciences

Submitted: 25-12-17
Corrected Version: 17-01-18
Accepted: 22-01-18

Artigo Original/ Original Article

Evaluation of Physicochemical and Microbiological Quality of two Phytoterapic Formulations Dispensed in a Brazilian Public Health Care Program

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Abstract: Phytotherapics are formulations obtained exclusively from raw materials of vegetable origin, with constant and reproducible quality. Farmácia Viva (literally *Alive Pharmacy*) is a project of social, cultural and economic nature, and the main objective is to provide access to therapies derived from medicinal plants to patients in economic vulnerability. From this perspective, it is essential to evaluate the quality of phytotherapics from physicochemical and microbiological points of view. This study aimed to conduct physicol-chemical and microbiological quality control of cumaru syrup and aroeira topical cream, produced in the Farmácia Viva Project. Three batches of each phytotherapic were used to evaluate sensorial characteristics, physicochemical quality control (pH, relative density, viscosity and rheological behavior) and microbiological quality control (count of aerobic mesophilic microorganisms, fungi, yeasts and pathogens). Regarding the sensorial analysis and physicochemical characteristics, the samples were considered satisfactory. For the microbiological tests, only fungi and yeast counts were within the limits specified by the Brazilian Pharmacopoeia 5th edition, with the count of mesophilic and pathogenic aerobic microorganisms above the limit allowed by this official compendium, therefore unfit for human consumption. More aseptic care is required in the phytotherapeutic manufacturing stages in the Farmácia Viva Project in order to set the microbial load within the allowed limits physicochemical.

Keywords: Phytotherapeutics, Farmácia Viva Project, Quality Control, Physicochemical Analysis, Microbiological Analysis.

Introduction

Phytotherapy has acquired an increasingly important role in health care. This picture is associated to the fact that patients often have doubts on the dangers concerning the use of synthetic drugs, due to its indiscriminate use and high costs, thus seeking to replace these by herbal medicines [1-5]. The Farmácia Viva Project started in 1983, by the pharmacist and professor Francisco José de Abreu Matos, whom proposed an initiative of pharmaceutical and social assistance based on the use of medicinal plants for the production of phytotherapics [6,7]. In this project, different formulations are produced, including cumaru syrup (*Amburana cearensis*), with pharmacotherapeutic activities bronchodilator and expectorant, and the aroeira topical cream (*Myracrodruom urundeuva*), with pharmacotherapeutic indication in inflammatory processes and for wound healing [8,9]. Due to the inclusion of phytotherapics in the therapeutic arsenal of the Brazilian Unified Health System (UHS), it is essential to conduct quality control of these. Plant-derived raw materials have a greater probability of physical and chemical interactions between the different components of the formulation, as these are of heterogeneous composition, and of microbial contamination as well [10].

These alterations may affect the purpose of the product, change in the appearance, the physical and chemical characteristics of the product and impel to the inactivation of the active principles and excipients used, so the physical, chemical and microbiological tests need to be rigorous in the produced phytotherapics [11]. In this way, this study has as main objective the accomplishment of the physicochemical and microbiological quality control of the syrup of cumaru and the cream of aroeira, produced in the Farmácia Viva Project, following the main official compendiums described. The sensorial analysis and physicochemical characteristics, the samples were considered satisfactory. For the microbiological tests, only fungi and yeast counts were within the limits specified by the Brazilian Pharmacopoeia 5th edition, with the count of mesophilic and pathogenic aerobic microorganisms above the limit allowed by this official compendium, therefore unfit for human consumption.

Materials and Methods

Sensorial Characteristics

The sensorial characteristics used for the macroscopic analysis of the cumaru syrup samples were color, homogeneity, odor and flavor. For the aroeira cream, we analyzed color, homogeneity and odor.

The color classification was: normal, altered, slightly altered and unchanged. The homogeneous classification was: homogeneous or nonhomogeneous. The odor was classified as: odorless, strong odor or slight odor. As for the flavor, it was classified as: sour taste, sweet taste, acid taste or bitter taste.

Physicochemical tests

Hydrogenionic Potential (pH)

For the aroeira cream samples, a 10 % suspension was made in purified water, by reverse osmosis. For the cumaru syrup samples, the electrode was immersed directly into the sample. The pH of the samples was determined by pH meter (MB10 Scientific Mars, BRAZIL). The test was performed in triplicate.

Relative Density (RD)

The metal pycnometer of 25 mL capacity was used to evaluate the relative density of aroeira cream. For the cumaru syrup the glass pycnometer of 25 mL capacity was used. The test was performed in triplicate. For this procedure, the weight of the sample individually and the weight of the purified water, both contained in the respective pycnometer, were found. The relative density was calculated by the coefficient between the weight of the sample and the weight of the purified water.

Viscosity and Rheological Behavior

The viscosity and rheological behavior of the samples were determined using the Brookfield viscometer (Quimis, BRAZIL). Samples were transferred to an appropriate beaker, spindle 3 or 4 were used for samples of aroeira cream, and spindle 1 or 2 for samples of cumaru syrup. The results were expressed in millipascal per second (mPa.s).

Microbiological assays

The samples were submitted to the standard plaque count (SPC) of aerobic mesophilic microorganisms (AMM), fungi and yeasts and pathogenic microorganisms. The SPC for the samples was performed in duplicate. Each sample was diluted in 0.9 % sterile saline, initially at 1:10 dilution and at serial dilutions 1: 100 and 1: 1000.

To prepare the 1:10 concentration, 10 mL of the cumaru syrup was pipetted into 90 mL of saline contained in the test tube, given the first dilution; and for the 1: 100 dilution 1 mL of the 1:10 concentration was pipetted into 9 mL of saline contained in the test tube, given the second dilution; and for 1: 1000 dilution, 1 mL of the 1: 100 concentrations was pipetted into 9 mL of saline contained in the test tube. The above procedure was repeated using the aroeira cream, 10 g of each sample being weighed, diluted in 90 mL of sterile 0.9 % saline.

According to previous studies [12], it was necessary to use substances capable of neutralizing the action of these components, one of the most commonly used is to avoid false-negative effects on microorganism analysis due to the presence of antimicrobial preservatives is polysorbate 80 (0.4 % w/v), which was added at each dilution. The procedure was performed using the spread plate method, according to the recommendations of the Brazilian Pharmacopoeia [3].

Standard Plate Count (SPC) of Aerobic Mesophile Microorganisms (AMM)

From each dilution 200 µL was taken and plated with Drigalky's loop containing Soy Casein Agar (SCA) incubated at 36 °C ± 2 °C for five days (Figure 01). As a negative control, a plate containing SCA alone was incubated, without addition of the samples.

After the incubation, plates were analyzed by counting the growth of colony forming units (CFU), per gram or milliliter of formultation. The SPC was calculated considering only plates with a number less than 250 CFU/mL or /g. The calculation was made from the mean plate count, multiplied by the dilution and the correction factor (CF), equal to 5, as shown in the formula below:

CFU/g or mL = Average plate count x dilution x CF

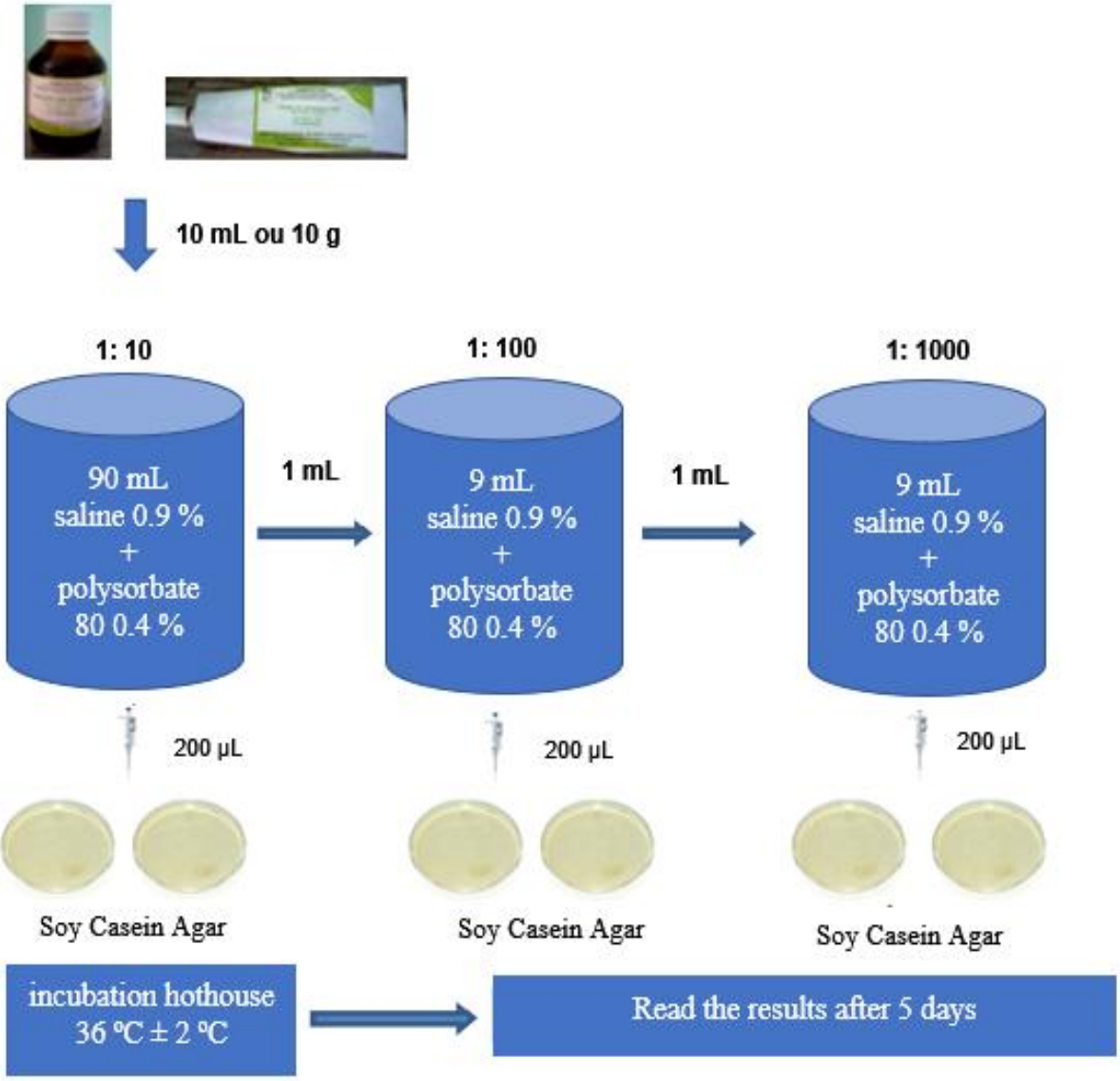

Figure 01 - Scheme of the standard plate count (SPC) of aerobic mesophilic microorganisms (AMM) by the surface scattering method (spread plate).

Fungus and Yeast SPC

A total of 200 µL of each dilution was taken and plated with Drigalky's loop containing the Potato Agar Dextrose medium using the Drigalsky´s loop and incubated at 25 ± 2 °C for a period of 7 days (Figure 02). As a negative control, a plate containing only Potato Agar Dextrose was incubated, without addition of the samples.

The readings of each plate were performed on the 7th day of incubation, by counting the growth of the fungal colony forming units (CFU/g or mL) found. SPC was calculated considering only plaques with a number less than 50 CFU/mL or g. The calculation was made from the mean plate count, multiplied by the corresponding dilution and CF, equal to 5, as shown in the formula below:

CFU/g or mL = Average plate count x dilution x CF

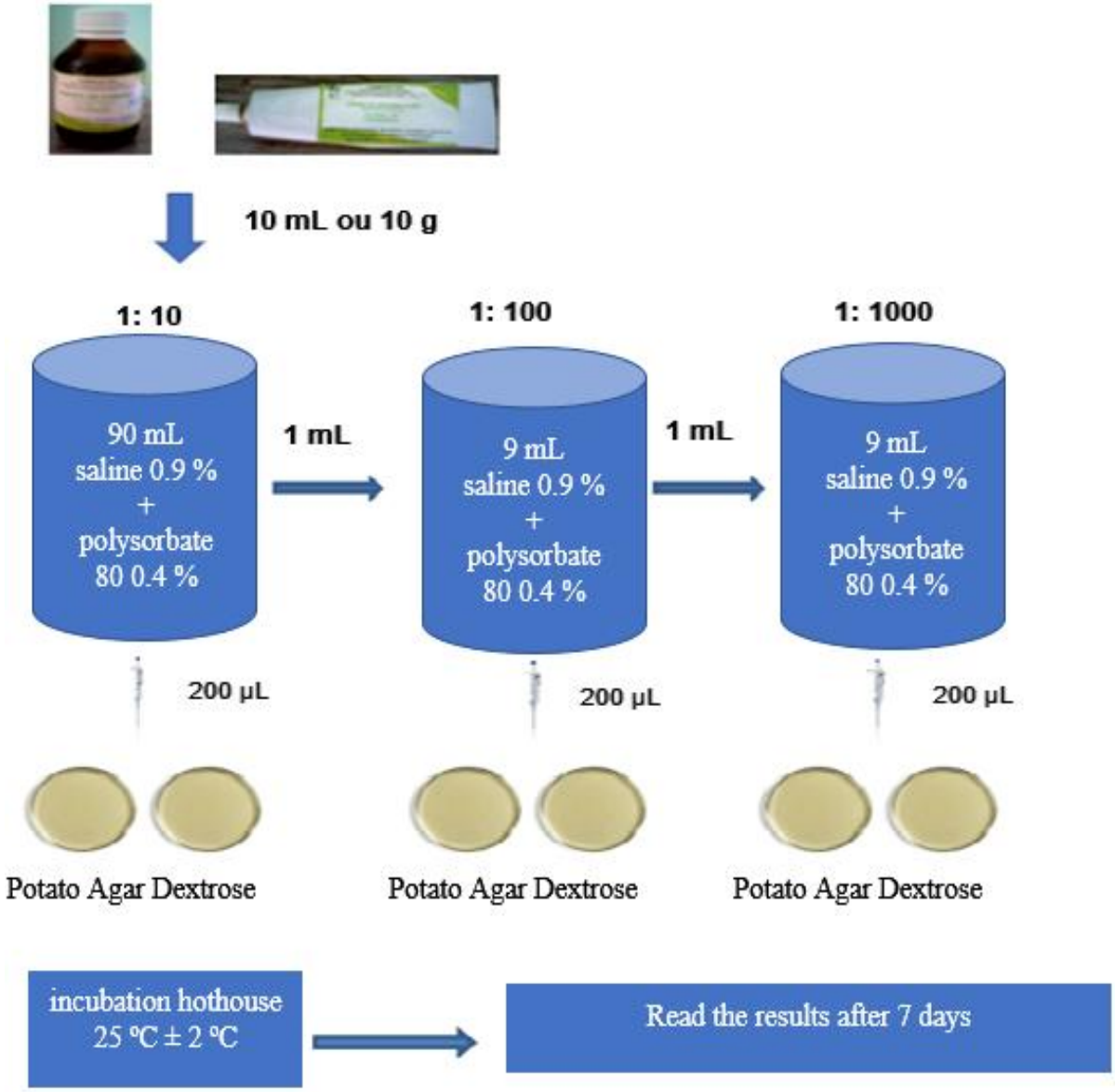

Figure 02 - Scheme of the standard plate count (SPC) of fungi and yeasts by the spread plate method.

SPC of pathogenic microorganisms

In the pathogen analysis the absence or presence of *Staphylococcus aureus* and *Escherichia coli* were investigated. The culture media of Manitol Agar Salt and MacConkey Agar were used to investigate these microorganisms, respectively. A total of 200 µL of each dilution and plated with the help of the Drigalky´s loop, containing the respective media for *S. aureus* and *E. coli*. They were incubated in a greenhouse at 36 °C ± 2 °C for a period of 5 days (Figures 03 and 04).

As a negative control, plates containing only the culture medium were incubated without addition of the samples. The readings of each plate were performed on the 5th day of incubation, by counting the growth of the bacterial CFU found. The SPC was calculated considering only plates with a number less than 250 CFU/mL or g. The calculation was made from the mean plate count, multiplied by the dilution and CF, equal to 5, as demonstrated in the formula below:

CFU/g or mL = Average plate count x dilution x CF

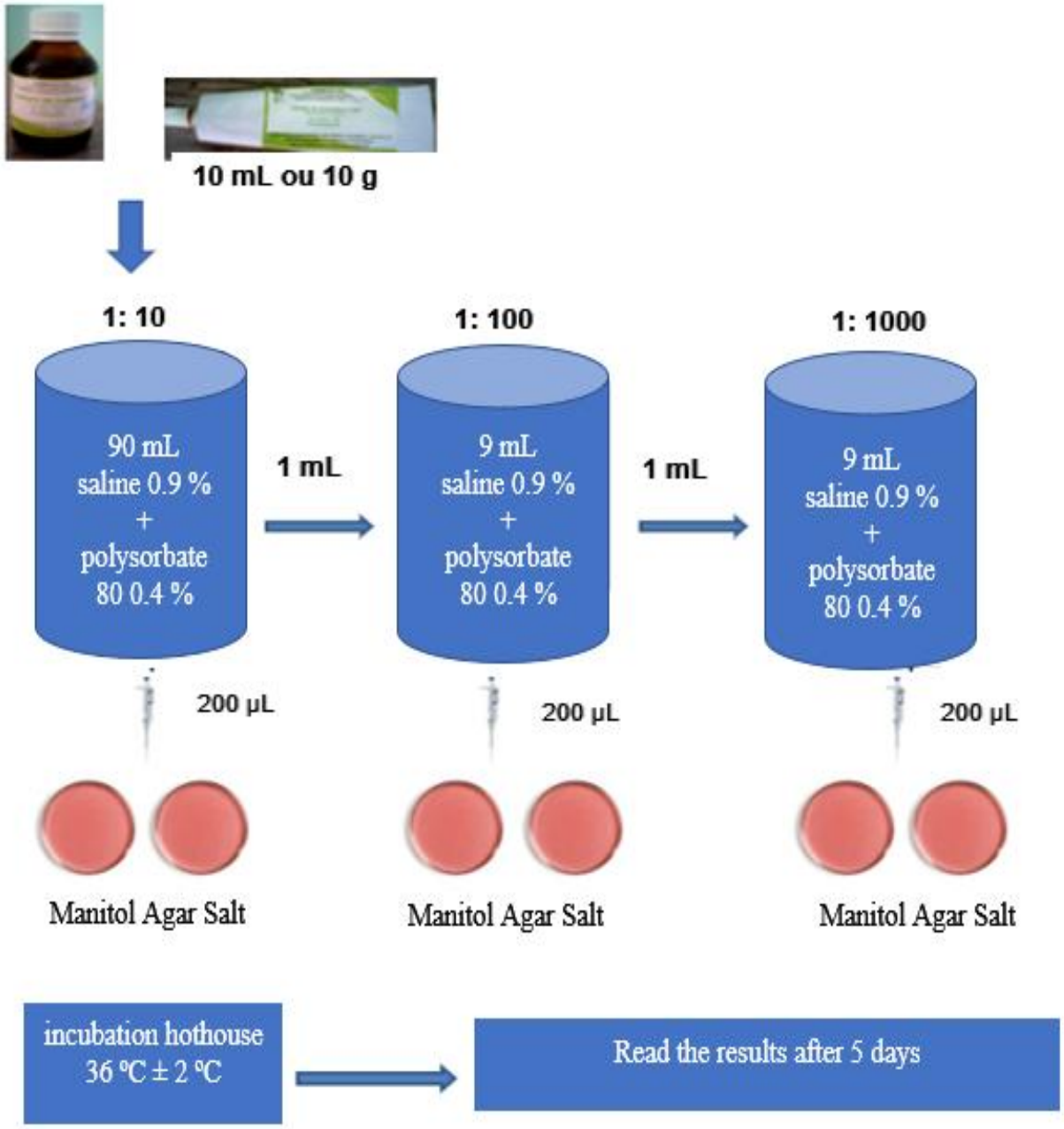

Figure 3 - *Staphylococcus aureus* standard plaque counting (SPC) scheme by the spread plate method.

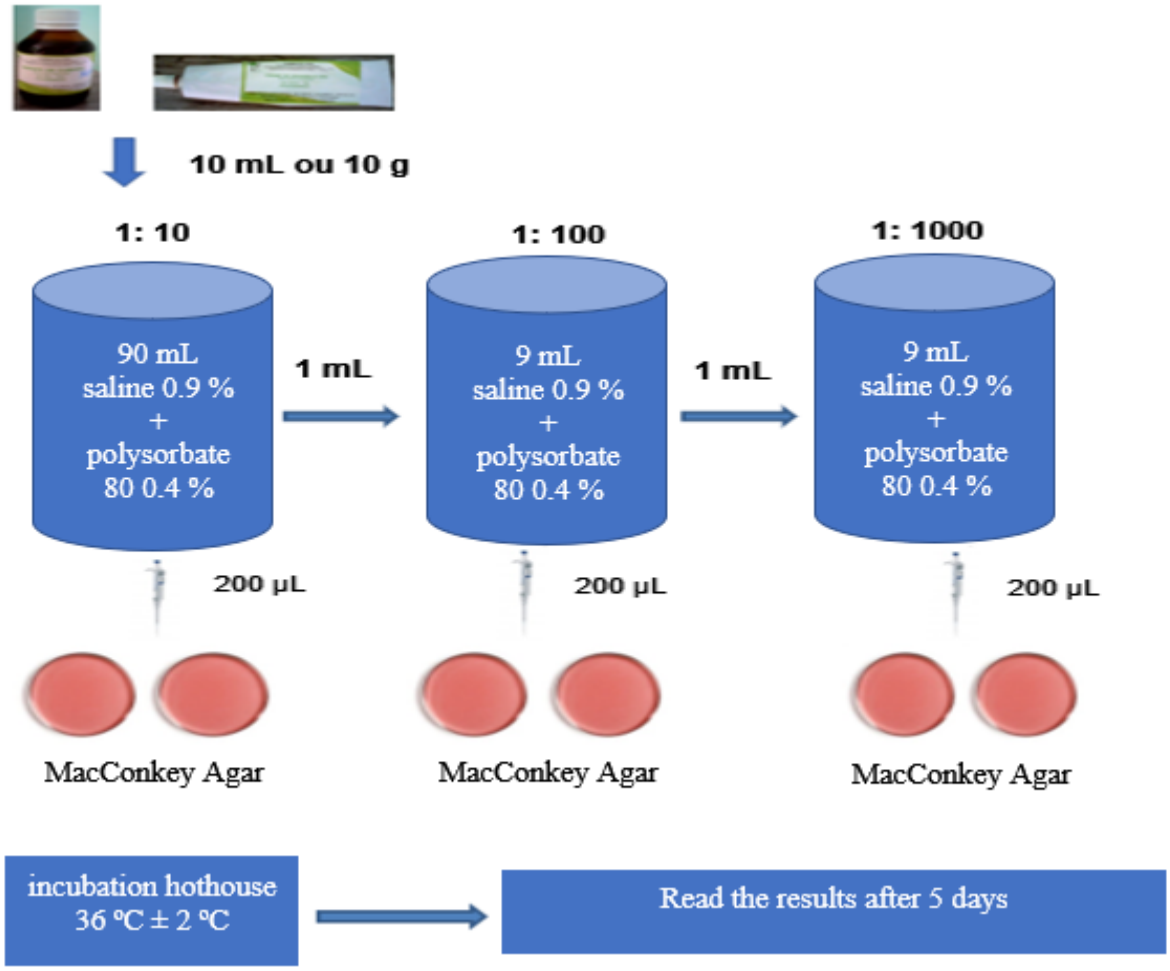

Figure 4 - *Escherichia coli* standard plate count (SPC) scheme by the spread plate method.

Statistical analysis of data

The data and results obtained were analyzed and calculated using the Microsoft Excel® and Microsoft Word® software, version 2016. Only the average and standard deviation of the samples were performed.

Ethical aspects

No animal or human specimens were necessary for this study, and questionnaires were not used, which justifies the non-submission to the CEUA (Committee for Ethics in the Use of Animals) and the CEP (Research Ethics Committee). The project was sent to the Secretary of Health of Sobral city via the online platform through the Integrated System of Scientific Commission (ISSC), which gave the favorable decision to start the research.

Results and Discussion

Sensorial Analysis

The cumaru syrup samples showed characteristic odor of the liquid extract of the medicinal plant, with sweet taste, normal color and homogeneous appearance (Table 01). The sensorial characteristics, such as color, odor and taste are relevant for patient adherence to treatment. The nauseous reflex caused by a taste or smell can cause the secretion of digestive juices, making it difficult to absorb the drug [13]. However, in cases of taste correction, it is necessary to use a flavoring or flavoring agent or to perform other techniques to cover the odor and taste, thus making the preparation palatable and thereby increasing treatment adherence to the patient [14].

Table 01 - Sensorial characteristics of cumaru syrup and aroeira cream produced in the Farmácia Viva Project.

Cumaru Syrup			
	Lot		
Characteristics	**1**	**2**	**3**
Color	Normal	Normal	Normal
Homogeneity	Homogeneous	Homogeneous	Homogeneous
Odor	Characteristic	Characteristic	Characteristic
Flavor	Candy	Candy	Candy
Cream of Aroeira			
	Lot		
Characteristics	**1**	**2**	**3**
Color	Normal	Normal	Normal
Homogeneity	Homogeneous	Homogeneous	Homogeneous
Odor	Characteristic	Characteristic	Characteristic

In the present study, the aroeira cream samples were homogeneous, with a characteristic odor of the liquid extract of the medicinal plant and with a shiny appearance. It was also observed that the cream formulations showed no variation in coloration. No precipitates were observed in the samples during the analyzes. In the work developed by Vasconcelos [38], in two semi-solid formulations, no alteration in the sensorial characteristics of the preparations was also identified. In relation to a phytocosmetic product, the homogeneity and color of a product are important from a commercial point of view and may influence consumer purchase if they are not attracted by the appearance of a biphasic product or color which may not be characteristic of that product [15]. The sensorial characteristics of liquid and semi-solid formulations relate to the integrity and quality of the samples, but can not be used alone for analytical purposes of product approval [16].

Physical and Chemical Tests

Hydrogenionic Potential (pH)

Based on the Table 02, the pH values were slightly acidic for both the cumaru syrup samples and the aroeira cream samples. For Thompson and Davidow [17] and Ferreira [18], oral liquid formulations have values of hydrogenation potential around 4.5 to 5.5, and these results are similar to the present study.

In analyzes of synthetic pediatric syrups, it was verified that the mean pH of these samples was 5.37 [19]. Although the samples mentioned above are not totally similar with the samples of the present study, we can compare them both to the physicochemical parameter of pH. In the studies of Mendes [40] and Guimarães [41], evaluating the pH of eucalyptus syrups and pomegranate syrups, respectively, obtaining mean values of 3.47 and 3.59, respectively, being necessary correction with alkalinizing solutions in these samples before of the dispensation.

Table 02 - pH of samples of cumaru syrup and aroeira cream produced in the Farmácia Viva Project.

Cumaru Syrup			
	Lot		
	1	**2**	**3**
pH	5.65	5.59	5.60
	5.73	5.66	5.64
	5.75	5.73	5.59
Average (± SD)	5.71 (± 0,0832)	5.66 (± 0.1272)	5.61 (± 0.0216)
Cream of Aroeira			
	Lot		
	1	**2**	**3**
pH	4.94	4.59	4.46
	4.91	4.50	4.51
	4.95	4.40	4.47
Average (± SD)	4.93 (± 0.0208)	4.49 (± 0.0950)	4.48 (± 0.0264)

SD = Standard deviation

Temperature and pH are elements that affect the stability of drugs prone to hydrolytic decomposition. Refrigeration is recommended for many preparations subject to hydrolysis. Therefore, the optimal stability of syrups is in the slightly acid pH range, between 4.0 and 6.0 [20,40,41], corroborating with the results of this study.

Ferreira [16] and Shimabuku [20] state that pH values between 5 and 7 are suitable for topical formulations, avoiding possible skin irritations, necessitating therefore that the samples of the aroeira cream of this study need to be alkalized after production. In the work of Vasconcelos [38], in two semi-solid formulations, one of these presented pH below the references cited previously, and also its correction with alkalinizing solutions.

Relative Density (RD)

The values in Table 03 show the results obtained from the relative density of cumaru syrup and aroeira cream, analyzed in this study.

Table 03 - Relative density (RD) of the samples of the syrup of cumaru and cream of aroeira produced in the Farmácia Viva Project.

Cumaru Syrup			
	Lot		
RD	**1**	**2**	**3**
	1.34	1.27	1.20
	1.32	1.20	1.23
	1.31	1.25	1.25
Average (± SD)	1.32 (± 0.0236)	1.24 (± 0.0655)	1.23 (± 0.0205)
Cream of Aroeira			
	Lot		
RD	**1**	**2**	**3**
	0.98	0.96	0.94
	0.99	0.95	0.95
	0.97	0.97	0.96
Average (± SD)	0.98 (± 0.0149)	0.96 (± 0.0187)	0.95 (± 0.0082)

SD = Standard deviation

The relative density is a quantity that varies according to the temperature change and is used to specify the purity of the products. According to Allen Jr., Popovich and Ansel [21], the relative density suitable for syrups would be around 1.33, similar to the relative density of glycerin. In the work of Mendes [40], evaluating DR of eucalyptus syrups, obtained an average value of 1.23, similar to our study. However, in the work of Guimarães [41], evaluating DR of pomegranate syrups, obtained an average value of 1.0, different from our study. A study by Ouchemoukh [22] and Manfio [23] in samples of syrups and honey showed similar results to the present study, with density variations between 1.31 and 1.45.

In the study of Parente [24], when analyzing creams containing noni fluid extract, showed density results equal to 0.94, 0.95 and 0.99, similar to the present study. In the research by Vasconcelos [38], the relative density of the emulsified systems did not show any variation among the evaluated formulations. However, there are no comparisons with this study in the Brazilian Pharmacopoeia 5th edition [3] or any other national official compendium, relative density specification for these preparations, making comparisons difficult with this study.

Viscosity and Rheological Behavior

In relation to the viscosity and rheological behavior, Table 04 shows the results obtained for the samples of the syrup of cumaru and aroeira cream, analyzed in this study.

Table 04 - Viscosity values and rheological behavior for cumaru syrup and aroeira cream produced in the Farmácia Viva Project.

Cumaru Syrup			
	Lot		
RPM	**1 (mPa.s)**	**2 (mPa.s)**	**3 (mPa.s)**
40	8.25	10.5	8.25
45	9.33	10.6	8.66
50	9.00	10.8	9.0
55	9.27	10.9	12.0
60	9.50	11.0	14.5
Cream of Aroeira			
	Lot		
RPM	**1 (mPa.s)**	**2 (mPa.s)**	**3 (mPa.s)**
1	367,200	184,800	199,200
2	238,500	115,200	123,300
3	188,400	89,800	97,400
4	86,250	75,450	84,900
5	37,720	66,720	73,800

mPa.s – Milipascal per second; RPM - Rotation per minute

Newtonian systems always have the same viscosity values regardless of the shear force. Non-Newtonian systems have viscosity values dependent on the composition of the formulation, with the ratio of force to shear velocity not constant. Non-Newtonian systems can be classified into: plastics, pseudoplastics and dilatants [17,18,21,25].

In this study, for cumaru syrup, these samples showed a Newtonian rheological behavior. In the studies by Billany [14], Mendes [40] and Guimarães [41] have reported that the presence of sucrose as part of the excipients in the formulation is responsible for ensuring viscosity increase, since it depicts the resistance of this solution to the movement. For the authors cited above, liquid oral formulations containing sucrose are classified with Newtonian rheological behavior, as they do not vary their viscosity with the shear rate.

In the study of Mendes [40], evaluating the viscosity of eucalyptus syrups, the Newtonian behavior was observed, with viscosity values ranging from 1,500 mPa.s, 1,666 mPa.s and 1,845 mPa.s, at different shear rates. Similar to the previous and our study, in the research of Guimarães [41], it was also observed the Newtonian behavior in the samples of pomegranate syrup, with values of viscosity ranging from 1,250 mPa.s, 1,385 mPa.s and 1,143 mPa.s.

The results contained in Table 04 revealed that the viscosity of the aroeira cream had great variation, presenting values between 37,720 to 367,200 mPa.s, expected behavior for these samples, since they are non-Newtonian pseudoplastic fluids. The viscosity analysis assists in the determination of product quality, demonstrating the consistency or fluidity of the cream, and may indicate the stability of the sample, defining whether it is suitable for use or not [26]. In the work of Vasconcelos [38], in two cosmetic emulsions, both also showed non-Newtonian pseudoplastic behavior, with viscosity values between 35,450 mPa.s and 549,600 mPa.s.

Microbiological Testing

Standard Plate Count (SPC) of Aerobic Mesophile Microorganisms (AMM)

For the standard plate count (SPC) of aerobic mesophilic microorganisms (AMM), microbial growth was observed, but within the specifications of the Brazilian Pharmacopoeia 5th edition [3], both in cumaru syrup samples and in the cream samples of aroeira. After reading the plates, the results for the AMM SPC of the syrup of cumaru and aroeira cream are detailed in Table 05.

Table 05 - Count of the number of aerobic mesophilic microorganisms (in CFU/mL and CFU/g) after incubation of the plates, containing samples of cumaru syrup and aroeira cream.

Cumaru Syrup			
SPC para AMM (average x dilution x CF)			
	Dilution (CFU/mL)		
Lot	**1:10**	**1:100**	**1:1000**
01	Absence	Absence	50,000
02	Absence	5,000	Absence
03	5,000	Absence	50,000
Cream of Aroeira			
SPC para AMM (average x dilution x CF)			
	Dilution (CFU/g)		
Lot	**1:10**	**1:100**	**1:1000**
01	Absence	Absence	Absence
02	250	2,500	100,000
03	Absence	Absence	Absence

SPC – Standard plate count; AMM – Aerobic mesophilic microorganisms; CFU – Colony forming unit; CF – Correction factor

According to the Brazilian Pharmacopoeia 5th edition [3], cumaru syrup is included in non-sterile vegetable products for oral use, assuming microbial load equivalent to 10^4 CFU/mL of mesophilic aerobic bacteria. The samples had growth ranging from 5.0 x 10^3 to 5.0 x 10^4 CFU/mL, it was observed that in lot 01 and lot 03, in the dilutions of 1:1000 were outside the established by this official compendium, with growth of 5.0 x 10^4 CFU/mL, therefore, unfit for human consumption, being these lots failed in this question.

According to the Brazilian Pharmacopoeia 5th edition [3], aroeira cream is included in the non-sterile products of vegetal origin produced by hot extraction processes and should not exceed 10^7 CFU of mesophilic aerobic bacteria per gram of sample, therefore, the three batches analyzed are within the permitted value, ranging from 2.5 x 10^2 to 10^5 CFU/g.

In the study of Carline [27], there was a growth of AMM in all plates of the dilutions of the phytotherapics studied in their research, and these were all above the limit established by the Brazilian Pharmacopoeia 5th edition [3], having some plaques with colonies uncountable, differing from the present study, where there was growth only in some plaques.

Freitas [28] analyzed thirty phytotherapics marketed in pharmacies and drugstores located in the state of Rio de Janeiro, in different pharmaceutical forms, and found that 22 samples had contamination by total aerobic bacteria, ranging from 10^4 CFU/g or mL to 10^9 CFU/g or mL, that is, in disagreement with the official compendium quoted above, different from the results of this study when compared to the aroeira cream. In the study of Vasconcelos [38], in the two emulsion systems evaluated, the nonionic emulsion presented growth above that referenced by the Brazilian Pharmacopoeia 5th edition [3], being therefore considered to be faulty in this respect. Still on this study, the main reason for this nonconformity was the inadequate site where the test was performed.

The results obtained from the analyzes performed on 6 lots of chambá syrups and 5 lots of comfrey ointments by Silva [29], indicated that they were within the values established by the Brazilian Pharmacopoeia 5th edition [3], where the values varied, for the ointment, from 6.7 x 10^2 to 2.0 x 10^4 CFU/g and for syrups ranged from 4.0 x 10^2 to 8.3 x 10^3 CFU/mL, demonstrating a similarity to our work, although there was a higher microbial load in some samples in our research. In the study conducted by Guimarães [41], evaluating SPC of AMM in pomegranate syrups, it observed a growth below that recommended by the Brazilian Pharmacopoeia 5th edition [3], with values ranging from 25 to 5,500 CFU/mL, being these samples approved according to this parameter. However, in the Mendes study [40], evaluating AMM SPC in eucalyptus syrups, it was observed a growth above that recommended by the Brazilian Pharmacopoeia 5th edition [3], with interlot values ranging from 25 to 125,000 CFU/mL, these samples being disapproved according to this parameter.

The microbial load above the allowed compromises the stability of the product, consequently, the therapeutic efficacy can be impaired, by degradation of the active principle or by alteration of the pH. In addition, contamination by microorganisms may lead to the development of pathologies for phytotherapeutic users [37,38].

Fungus and Yeast SPC

For fungal and yeast SPC, after the plates were read, growth was observed in some plates, but within the allowed values for these types of samples, according to the Brazilian Farnacopeia 5th edition [3]. The results of reading the yeast and fungal counts of cumaru syrup and aroeira cream are given in Table 06.

Table 06 - Number of fungi and yeasts (in CFU/mL and CFU/g), after incubation of the plates, containing the samples of the syrup of cumaru and cream of aroeira.

Cumaru Syrup			
SPC of fungi and yeasts (average x dilution x CF)			
	Dilution (CFU/mL)		
Lot	**1:10**	**1:100**	**1:1000**
01	Absence	Absence	Absence
02	Absence	Absence	Absence
03	Absence	Absence	Absence
Cream of Aroeira			
SPC of fungi and yeasts (average x dilution x CF)			
	Dilution (CFU/g)		
Lot	**1:10**	**1:100**	**1:1000**
01	2,000	Absence	Absence
02	Absence	Absence	Absence
03	1,000	Absence	Absence

SPC – Standard plate count; CFU – Colony forming unit; CF – Correction factor

According to the Brazilian Pharmacopoeia 5th edition [3], cumaru syrup is included in the non-sterile products of plant origin, admitting the growth of fungal colonies, for preparations of oral use of natural origin, at a maximum of 10^2 CFU/mL. As observed Table 06, it is verified that there was no growth of fungi and yeasts in the three batches of cumaru syrup, and the samples were approved in this case for human consumption.

The aroeira cream is included in non-sterile products of vegetable origin produced by hot extractive processes, so the microbial load should not exceed 10^4 CFU/g of fungi and yeasts, so in the present study, the plates are within the allowed value by the Brazilian Pharmacopoeia 5th edition [3], varying between 10^3 and 2.0 x 10^3 CFU/g.

In the work of Mendes [40], evaluating the SPC of fungi and yeasts in samples of eucalyptus syrups, it was observed a growth of less than 25 CFU/mL. However, in the study conducted by Guimarães [41], evaluating the SPC of fungi and yeasts in samples of pomegranate syrups, of the lots analyzed, only one was considered unsatisfactory, with growth above 1,000 CFU/mL. This result may be related to some possible mistake in the preparation of the raw material, in the handling or even in the storage and/or storage, distribution and commercialization stages of the product, since plant raw materials and phytoterapics are sources more propitious to the growth of microorganisms, therefore, care with the raw materials, preparation of the samples and storage of the products are fundamental [40,41].

In the work of Vasconcelos [38], two topical emulsified formulations were evaluated: SPC of fungi and yeasts was performed, finding values within the limits established by the Brazilian Pharmacopoeia 5th edition [3], with values lower than 25 CFU/g.

Fungi and yeasts are microorganisms with a significant pathogenic character for human health, resulting in physical and chemical alterations in the phytotherapics obtained from them, as well as loss of their action or toxicity [30,31]. The possible contamination by fungi is due to the environmental conditions related mainly to the cultivation and storage of the inputs. The contact of the plants with the soil that sometimes leads to the contamination, and, therefore, it is necessary a correct practice of the manipulators, from the primary processing to the moment of the quality control performed in the herbal medicines [32, 33].

Pathogenic Microorganisms SPC

In the counting of pathogenic microorganisms, for the species *Staphylococcus aureus*, the microbial growth was observed, in both samples of cumaru syrup and in samples of the aroeira cream, where the color change of the medium, from pink to yellowish was verified, being an indicative presence of this bacterium. The results regarding the counting of this pathogenic microorganism of cumaru syrup and aroeira cream are detailed in Table 07.

Table 07 - Count of *S. aureus* number (in CFU/mL and CFU/g), after incubation of the plates, containing the samples of the syrup of cumaru and cream of aroeira.

Cumaru Syrup			
SPC of *S. aureus* (average x dilution x CF)			
	Dilution (CFU/mL)		
Lot	**1:10**	**1:100**	**1:1000**
01	Absence	Absence	Absence
02	Absence	Absence	Absence
03	500	5,000	Absence
Cream of Aroeira			
SPC of *S. aureus* (average x dilution x CF)			
	Dilution (CFU/g)		
Lot	**1:10**	**1:100**	**1:1000**
01	Absence	5,000	Absence
02	Absence	5,000	Absence
03	Absence	5,000	Absence

SPC – Standard plate count; CFU – Colony forming unit; CF – Correction factor

Cumaru syrup is included in non-sterile plant products for oral use, therefore the Brazilian Pharmacopoeia 5th edition [3] recommends that there is no presence of *S. aureus* in 1 mL of analyzed sample, and the values found ranged from 5.0×10^2 to 5.0×10^3 per milliliter of the sample only for lot 03, therefore, it is unfit for human consumption, due to the contamination by this pathogen, possibly due to improper handling, which was either originated in the fabrication of the herbal medicine or during the process of inoculation of these samples to perform the quality control.

The Brazilian Pharmacopoeia 5th edition [3] does not recommend a limiting amount of *S. aureus* for plant drugs that will undergo hot extractive processes, in which the aroeira cream fits, having, in this case, a difficult comparison with the main official compendium Brazilian.

Medeiros [33] in a study of 9 samples, including syrups and cosmetics, marketed at a drugstore in Campina Grande-PB, reported a contamination by *S. aureus* in the cosmetic sample. The possible contamination is due to the manipulation by asymptomatic carriers of this bacterium, as it is part of the resident microbiota of the human skin, and may have been originated due to the loss of scales on the skin, being found in the nasal, skin, intestine and throat. Therefore, it is essential to use personal protective equipment (PPE) during the handling of these products.

Regarding the counting of pathogenic microorganisms, for the species *Escherichia coli*, the microbial growth was observed, only in the samples of the aroeira cream, where it was verified a change in the color of the medium, showing a pink color, indicative of presence of this bacterium. The results regarding the count of this pathogen of cumaru syrup and aroeira cream are in Table 08.

Table 08 - Number of *E. coli* counts (in CFU/mL and CFU/g), after incubation of the plates, containing samples of cumaru syrup and aroeira cream.

Cumaru Syrup			
SPC of *E. coli* (average x dilution x CF)			
	Dilution (CFU/mL)		
Lot	**1:10**	**1:100**	**1:1000**
01	Absence	Absence	Absence
02	Absence	Absence	Absence
03	Absence	Absence	Absence
Cream of Aroeira			
SPC of *E. coli* (average x dilution x CF)			
	Dilution (CFU/g)		
Lot	**1:10**	**1:100**	**1:1000**
01	500	Absence	Absence
02	500	Absence	Absence
03	500	Absence	Absence

SPC – Standard plate count; CFU – Colony forming unit; CF – Correction factor

According to the results inserted in Table 08, it can be seen that there was no colony growth in the samples of cumaru syrup. The Brazilian Pharmacopoeia 5th edition [3] recommends that there is no presence of this bacterium in 1 mL of analyzed sample, for non-sterile products of vegetable origin for oral use, therefore, the cumaru syrup is in agreement with the official compendia, for this parameter, being approved for human consumption.

According to the Brazilian Pharmacopoeia 5th edition [3], a limit of 10^2 CFU/g of *E. coli* is accepted in samples containing vegetal drugs that will be submitted to hot extractive processes, therefore, the three lots of aroeira cream are reproved for consumption, since there was growth of 5.0 x 10^2 CFU/g, outside the allowed values.

In the research done by Silva [34], with the comfrey ointment and chambá syrup, a difference was verified between its results with the semi-solid samples of the present study. Of the 11 samples analyzed, all showed absence of the *E. coli* bacteria, although a limit of 10^2 CFU/g of this bacterium was allowed in samples submitted to hot extractive processes, where they fit the phytotherapeutic creams and ointments, being therefore, the samples of such study approved for human use.

In the study of Gonçalves [35], three phytotherapeutic preparations were studied: capsules containing the dried extract of guarana, bottles of ipecacuanha and chambá syrup purchased from merchants in the city of Sanclerlândia-GO, where the results presented by these authors are similar to the results of the present study, where they reported the presence of total and fecal coliforms, possibly having to be present in the plant used, contaminated water or under poor conditions of preparation of the samples, group where *E. coli* belongs, these samples having values higher than allowed by the Pharmacopoeia Brazilian 5th edition [3], and is therefore disapproved for human consumption.

Contamination by *Staphylococcus, Escherichia, Salmonella, Klebsiella* and *Serratia* is due to the manipulation of products by possibly sick individuals, who are common in the areas of manufacture of herbal medicines, which can sometimes contaminate the environment [36]. For pharmaceutical products to achieve a good level of microbiological quality, it is essential that the sources and mechanisms responsible for this contamination are known. Among the most common are: water, air, environment, raw material, packaging material, equipment and especially the manipulator [39].

The presence of these microorganisms in the analyzed samples may possibly be due to a poor hygiene of the manipulators, as well as inadequate use of PPE during some stage of the manufacture of phytotherapics of the Farmácia Viva Project.

Conclusion

The quality control of phytotherapic preparations is essential for the generation of products physically-chemically and microbiologically acceptable for the patient. All batches of both cumaru syrup and aroeira cream showed good performance in all the physicochemical tests performed. About the microbiological tests, to verify the mesophilic aerobic microorganisms, it was verified that some counts are outside the limits established by the main Brazilian official compendium, having only lot 02 of the syrup of cumaru and the three batches of the cream of aroeira being considered approved.

On fungi and yeast research, only lot 02 of the aroeira cream and the three batches of cumaru syrup were approved according to the compendium mentioned above. Regarding pathogen research, there were presumptive growth of these bacteria in some samples, therefore, these are unfit for human use, with only 01 and 02 batches of cumaru syrup considered approved.

More aseptic care is necessary in the phytotherapeutic production stages produced in the Farmácia Viva Project in order to reduce the microbial load found. Toxicological and efficacy tests of the phytotherapics analyzed are necessary to this study in order to complement the present information.

This study aimed to improve the planting, collection, production stages and quality control of phytotherapeutics for the Farmácia Viva Project. Therefore, the quality control of phytotherapics is of great relevance, in order to discover their physicochemical and microbiological performance, for greater safety, quality and efficacy to the users of herbal medicines.

Acknowledgments

We are thankful to the Pharmacy School of the University Center INTA (UNINTA) and to the Farmácia Viva Project, as well as to its professors, students and collaborators, for generating scientific knowledge of great relevance for the academic community and scientific environment. This research was funded by the Pharmacy School of the University Center INTA (UNINTA) and to the Farmácia Viva Project.

Contribution of the Authors

AAN: Project supervisor, assistance during the execution of the researches and in the writing of this manuscript.
RNPF: Execution of the research and writing of this manuscript.
EMSAS: Execution of the research and writing of this manuscript.
FVBSM: Intellectual and scientific contribution, research execution and writing of this manuscript.
WMCN: Intellectual and scientific contribution, writing, revision and translation of this manuscript.
MVAA:Intellectual and scientific contribution, research execution writing and revision of this manuscript.

All authors declared no conflict of interest.

References

1. Brasil. Ministério da Saúde. Secretaria de Ciência, Tecnologia e Insumos Estratégicos. Departamento de Assistência Farmacêutica e Insumos Estratégicos. Política Nacional de Plantas Medicinais e Fitoterápicos. Brasília: Ministério da Saúde; 2006.

2. Klein T, Longhini R, Bruschi ML, Mello JCP. Fitoterápicos: um mercado promissor. Rev Ciênc Farm Básica Apl. 2009; 30(3): 241-248.

3. Brasil. Farmacopeia Brasileira. 5ª ed. Agência Nacional de Vigilância Sanitária. Brasília: Anvisa; 2010.

4. Santos RL, Guimarães GP, Nobre MSC, Portela AS. Análise sobre a fitoterapia como prática integrativa no Sistema Único de Saúde. Rev. Bras. Plantas Med. 2011; 4(13): 486-491.

5. Caccia-Bava MCGG, Bertoni BW, Pereira AMS, Martinez EZ. Disponibilidade de medicamentos fitoterápicos e plantas medicinais nas unidades de atenção básica do estado de São Paulo: resultados do Programa Nacional de Melhoria do Acesso e da Qualidade da Atenção Básica (PMAQ). Ciênc. Saúde Coletiva. 2017; 5(22): 1651-1659.

6. Matos FJA. Farmácia Viva: Sistema de utilização de plantas medicinais projetado para pequenas comunidades. 3ª ed. Fortaleza: Imprensa Universitária; 1998.

7. Randal VB, Behrens M, Pereira AMS. Farmácia da natureza: Um modelo eficiente de Farmácia Viva. Revista Fitos. 2016; 10(1): 1-93.

8. Matos FJA. Plantas medicinais: Guia de seleção e emprego das plantas usadas em fitoterapia no nordeste do Brasil. 3ª ed. Fortaleza: Imprensa Universitária; 2007.

9. Secchi P, Virtuoso S. O efeito da valeriana no tratamento da insônia. Visão Acadêmica. 2012; 1(13): 85-107.

10. Santos IMR, Ferreira SMO, Araújo TP, Ferreira SA, Brandão RS, Miranda LCB, Nogueira VS, et al. Análise microbiológica de matérias-primas vegetais comercializadas em farmácias magistrais e ervanarias do município de Goiânia/GO. Revista Eletrônica FMB. 2015; 1(8): 1-9.

11. Yamamoto CH, Pinto TJA, Meurer VM, Carvalho AM, Rezende P. Controle de qualidade microbiológico de produtos farmacêuticos, cosméticos e fitoterápicos produzidos na zona da mata, MG. Anais do 2º Congresso Brasileiro de Extensão Universitária; 12-15 set. 2004; Universidade Federal de Juiz de Fora (UFJF). Belo Horizonte: Editora UFJF; 2004. p. 81-87.

12. Vasconcelos TYL, Medeiros, DPF, Nascimento AA. A inibição do sistema conservante de duas emulsões O/A por polissorbato 80. Infarma - Ciências Farmacêuticas. 2015; 4(27): 221-225.

13. Prista LN, Alves AC, Morgado R. Técnica farmacêutica e farmácia galênica. 3ª ed. Lisboa: Fundação Calouste Gulbenkian; 1990.

14. Billany G. Formas farmacêuticas líquidas. In: Aulton, ME. Delineamento de formas farmacêuticas. 2 ª ed. Porto Alegre: Artmed; 2005.

15. Isaac VLB, Cefali LC, Chiari BG, Oliveira CCLG, Salgado HRN, Corrêa MA. Protocolo para ensaios físico-químicos de estabilidade de fitocosméticos. Rev. Ciênc. Farm. Básica Apl. 2008; 1(29): 81-96.

16. Ferreira AO. Guia prático da farmácia magistral. 3ª ed. São Paulo: Pharmabooks Editora; 2008.

17. Thompson JE, Davidow LW. A prática farmacêutica na manipulação de medicamentos. 3ª ed. Porto Alegre: Artmed; 2013.

18. Ferreira AO. Guia prático da farmácia magistral. 4ª ed. São Paulo: Pharmabooks Editora; 2010.

19. Santinho AJP, Waldow C, Santos SB. Estudo sobre a correlação do potencial cariogênico e do pH de xaropes pediátricos. Rev Bras Farm. 2008; 2(89): 88-90.

20. Shimabuku PS, Zilotti LMA, Cunha ARC, Rigato LAB, Zocoler MA. Avaliação da qualidade de cremes dermatológicos manipulados na cidade de Marília (SP). Colloquium Vitae. 2009; 1(1): 30-37.

21. Allen Jr. LV, Popovich NG, Ansel HC. Formas farmacêuticas e sistemas de liberação de fármacos. 9ª ed. Porto Alegre: Artmed; 2013.

22. Ouchemoukh S, Louaileche H, Schweitzer P. Physicochemical characteristics and pollen spectrum of some algerian honeys. Food Control. 2007; 18(1): 52-58.

23. Manfio LJ. Determinação do prazo de validade do medicamento carbocisteína xarope [dissertation]. Porto Alegre: Faculdade de Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul; 2005.

24. Parente SD. Desenvolvimento de fitoterápicos de uso tópico a partir do extrato hidroalcoólico das folhas do noni *(Morinda citrifolia L.*) e realização do controle de qualidade físico-químico [trabalho de conclusão de curso]. Sobral: Faculdade de Farmácia, Centro Universitário INTA; 2015.

25. Senffi L, Hotza D, Repette WL. Comportamento reológico de pastas de cimento com adição de sílica ativa, nanossílica e dispersante policarboxílico. Revista Matéria. 2010; 1(15): 12 –20.

26. Franco LT, Bochi LCS. Produção e caracterização de diferentes formulações tópicas semissólidas contendo meloxicam na forma nanoestruturada. Vivências. 2013; 9(16): 209-231.

27. Carlini EA, Duarte-Almeida JM, Tabach R. Assessment of the toxicity of the brazilian pepper trees Schinus terebinthifolius Raddi (aroeira-da-praia) and Myracrodruon urundeuva Allemão (aroeira-do-sertão). Phytother Res. 2013; 27(5): 692-698.

28. *Freitas AVL, Coelho MFB, Azevedo RAB, Maia SSS*. Os raizeiros e a comercialização de plantas medicinais. Rev Bras Bioci. 2012; 10(2): 147-156.

29. Silva RBLA. A etnobotânica de plantas medicinais da comunidade quilombola de Curiaú, Macapá- AP [dissertation]. Manaus: Faculdade de Ciências Farmacêuticas, Universidade Federal Rural da Amazônia; 2002.

30. Nascimento SA. Análise físico-química e microbiológica de amostras de mel de abelhas obtidas em comércios varejistas na cidade de Sobral-Ce [trabalho de conclusão de curso]. Sobral: Faculdade de Farmácia, Centro Universitário INTA; 2015.

31. Brasil. Ministério da Saúde. Secretaria de Ciência, Tecnologia e Insumos Estratégicos. Departamento de Assistência Farmacêutica e Insumos Estratégicos. Guia de orientação para registro de medicamento fitoterápico e registro e notificação de produto tradicional fitoterápico. Brasília: Ministério da Saúde; 2014.

32. Santos MG, Fonseca SGC. Farmácias Vivas [internet]. Fortaleza: Impressa Universitária; 2014 [acesso em: 09 ago. 2016]. Disponível em: https://cursos.atencaobasica.org.br/sites/default/files/farmacias_vivas_0.pdf.

33. Medeiros ACD, Porto KL, Paiva AVR, Procópio JVV. Análise de contaminantes microbiológicos em produtos comercializados em farmácia de manipulação. Rev Bio Farm. 2007; 1(1): 1-12.

34. Silva AMRC. Estudo de utilização de fitoterápicos dispensados em um centro de saúde em Fortaleza: xarope de chambá (*Justicia pectoralis* Jacq Var.*Stenophylla* Leonard) 5 % e pomada de confrei (*Symphytum officinale* L.) 5 % [doutorado]. Fortaleza: Faculdade de Ciências Farmacêuticas, Universidade Federal do Ceará; 2015.

35. *Gonçalves VS, Braz PH, Melo TL, Brandão RS, Pinto MV*. Análise microbiológica de preparações medicinais adquiridas em raizeiro na cidade de Sanclerlândia, Goiás. Revista Eletrônica FMB. 2015; 1(8): 1-10.

36. Silva BR, Lima IO, Carmo ES, Souza JBP. Avaliação microbiológica de lambedores comercializados no município de Cuité-PB. Revista Saúde & Ciência Online. 2016; 1(5): 5–22.

37. Silva MF, Silva LL. Análise microbiológica de três formulações magistrais. Cadernos da Escola de Saúde. 2011; 6(2): 117-130.

38. Vasconcelos TYL. Avaliação da estabilidade físico-química e microbiológica acelerada dos cremes base aniônico e não-iônico formulados em um laboratório escola em Sobral-Ce [trabalho de conclusão de curso]. Sobral: Faculdade de Farmácia, Centro Universitário INTA; 2015.

39. Brasil. Guia de Estabilidade de Produtos Cosméticos. 1ª ed. Agência Nacional de Vigilância Sanitária. Brasilia: Anvisa; 2004.

40. Mendes SPF. Avaliação da qualidade físico-química e microbiológica de amostras de xaropes de eucalipto comercializadas na cidade de Sobral-CE [Undergrad Monography]. Sobral: Faculdade de Farmácia, Centro Universitário INTA; 2015.

41. Guimarães GAC. Análise físico-química e microbiológica de amostras do xarope de romã comercializados no município de Sobral-Ce [Undergrad Monography]. Sobral: Faculdade de Farmácia, Centro Universitário INTA; 2015.