World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Evaluation of irregularly manufactured *garrafadas* and syrups seized in the state of Amapá, Brazil

Amanda Furtado de Almeida [1, 2, *], Clebson de Jesus Macrino [2, 4], Nayara Araújo dos Santos [2, 4], Francy Mendes Nogueira Cardoso [3], Luciana Sampaio Lima [3], Aldo Aparecido Proietti Junior [3], Carolina Miranda de Sousa Lima [1], Henderson Nildom Gomes Barboza [4], Wanderson Romão [2, 5] and Madson Ralide Fonseca Gomes [1, 2]

[1] *Graduate Program in Pharmaceutical Sciences - Federal University of Amapá, Macapá, Amapá, Brazil.*
[2] *National Institute of Forensic Science and Technology, INCT-FORENSE, Porto Alegre, Rio Grande do Sul, Brazil.*
[3] *Special Laboratory of Applied Microbiology, Federal University of Amapá, Macapá, Amapá, Brazil.*
[4] *Technical-Scientific Police of the state of Amapá, Macapá, Amapá, Brazil.*
[5] *Laboratory of Research and Development of Petroleum Analysis Methodology - Federal University of Espírito Santo, Vitória, Espirito Santo, Brazil.*

World Journal of Biology Pharmacy and Health Sciences, 2022, 10(01), 046–060

Publication history: Received on 24 February 2022; revised on 16 April 2022; accepted on 18 April 2022

Article DOI: https://doi.org/10.30574/wjbphs.2022.10.1.0060

Abstract

Introduction: The *garrafadas*, mixtures of plants with medicinal purposes conveyed in alcoholic beverages, do not have regulation and, for being presented with many therapeutic indications and no contraindication, they can be risky for the population.

Methods: For this reason, the objective of this study was to evaluate the Brix degree°, evaluate the presence of pathogenic microorganisms, analyze the chemical characteristics pH and mass spectrometry (FT-ICR MS) and evaluate the label of syrup samples and *garrafadas* suspicious of adulteration seized by police.

Results: The degree Brix° value of the samples was within the standards. The chemical analysis of the pH of the *garrafadas* and syrups samples obtained acid pH values, which can cause chemical instability on the components. Through the mass spectrometry, it was possible to detect some fragments of compounds related to the plants described on the labels, and no synthetic chemicals or drugs of abuse were found. The result of the microbiological evaluation detected *Staphylococcus* spp. in 4 samples, and, in 2 syrups, fungus growth occurred, beeing for fungi the growth were within limits established in the *Farmacopeia Brasileira* for this microorganism. In only four of the twelve samples evaluated, it was possible to detect mass fragments related to chemical substances of the plants described on the labels of the packages and most of them was made up of sugars only.

Conclusions: Based on the results found, these products are not safe for consumers, as there is no way to guarantee their safety and efficacy.

Keywords: Garrafadas; Medicinal preparations; Syrups; Seizure

1. Introduction

The consumption of herbal medicines and phytotherapeutic products has been growing exponentially every year, and it is a therapeutic alternative used in parallel to traditional drug therapy [1]. In the northern region of Brazil, the

* Corresponding author: Amanda Furtado de Almeida
Graduate Program in Pharmaceutical Sciences - Federal University of Amapá, Macapá, Amapá, Brazil.

consumption of medicinal plants is widespread: the difficulty of access to regular treatments, the folk medicine use, and its efficacy attract the interest of the population, as well as of the pharmaceutical industry [2].

The phytotherapeutic products are commonly used by the northern population of Brazil, and the culture of consumption of *garrafadas* is widespread. The "Mercado do Ver-o-Peso" in Belém do Pará, became famous for selling this product and the popular "*banhos de cheiro*" (herbal baths). *Garrafadas* are mixtures of plants with medicinal purposes conveyed in alcoholic beverages packaged in bottles, usually in glass containers (*garrafas* in Portuguese), originating the Brazilian term *"garrafadas"*.

The *garrafadas* are not considered medicines and, therefore, cannot have therapeutic indications or dosage in their packaging [3]. However, the population has a strong belief that these phytotherapeutic products, *garrafadas*, and syrups cure many diseases. As these herbal products aim to treat common diseases, such as the ones related to the digestive, respiratory, and pain systems in general, they have gained popularity, as they have a lower cost when compared to synthetic drugs. Therefore, these preparations, characteristic of folk medicine, became, over the years, a strong cultural product.

The g*arrafadas* became a great challenge for public health because they do not have their legislation. It is hard to inspect these products in Brazil because they are products outside the scope of health regulation. This situation facilitates "irregular" productions of the *garrafadas* and other medicinal preparations. Malicious people can use this cultural element to add synthetic substances to correlate with some therapeutic effect proposed on the packaging label, or even illicit drugs to attract sales and retain the buyer. Adulterants in phytotherapeutic products have become a significant and potentially dangerous problem to public health and have proven to be a common illegal practice. An example occurred in 2017, in the city of Macapá, there was a police operation, after reports of irregular production of these products in factories with precarious hygiene conditions, after investigations, the factory was closed.

In addition, research related to these phytotherapeutic products such as *garrafadas* and syrups from the northern region of Brazil are still incipient and, therefore, it is important to highlight studies that report the quality of these medicinal preparations that are susceptible to various types of contamination, be it by substance chemical or microbiological.

Considering that the *garrafadas* and medicinal preparations are not regulated by the inspection agencies and are products for medicinal purposes, spread in folk medicine due to traditional usage and cultural issues, they are products susceptible to adulteration and unsatisfactory quality results due to, in most cases, not fulfilling the good manufacturing practices. So, the objective of this study was to evaluate the physical, chemical and microbiological characteristics of *garrafadas* and syrups to elucidate the quality of these medicinal products marketed in the State of Amapá and seized by the Technical-Scientific Police of Amapá (POLITEC), to curb this practice.

2. Material and methods

2.1. Samples

For this study, 12 samples of herbal preparations were used, 4 samples from *garrafadas* and 8 samples from syrups. These samples from the seizure, carried out in June 2017 by the civil police of the State of Amapá in partnership with the health surveillance, were provided by the Department of Forensic Chemistry of the Technical and Scientific Police of the State of Amapá (POLITEC), were used in this study. Aliquots of 40 mL were taken from the samples, which were immediately transferred to sterile flasks. These samples were transported, under refrigeration in a styrofoam cooler with reusable artificial ice, to the Clinical Analysis Laboratory of the Federal University of Amapá.

2.2. Label evaluation

The labels of the *garrafadas* and syrups packaging were analyzed and compared with the criteria established by Resolution No. 26, of May 13, 2014, of ANVISA [4], in chapter VIII, subsection II, concerning the packing of phytotherapeutic products[4]. It was observed if the labels on the product packaging contained the following information:

- Commercial name of the phytotherapeutic product;
- Tradicional nomenclature, followed by the botanical nomenclature;
- Concentration;
- Route of administration;

- Name of the holder of the registration or its logo, if it contains the name of the company;
- Telephone number of the Customer Support Center (CSC) of the company that holds the registration or notification;
- Batch number;
- Expiration date.

2.3. Physical Evaluation

For the evaluation of the degree Brix of each sample, the content of total sugars was also evaluated, in a refractometer for sugar 0-32% Brix-unit K52-032.

2.4. Chemical Evaluation

2.4.1. pH measurement

The pH was measured with the digital pH meter (Metrohm model 827 pH lab). The evaluation of the pH of the *garrafadas* and syrups was performed according to the *Farmacopeia Brasileira 5a edição* [5].

2.4.2. Chemical Analysis by Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS)

Sample preparation and chemical analysis

For the injection in the electrospray ionization (ESI) source, methanolic solutions were prepared containing 50 mg of each sample of the *garrafadas* and syrups in 5 mL of methanol. For analysis, aliquots of 10 µL were individually taken from each sample for injection in the mass spectrometer. All samples were also fractionated through the solid phase extraction (SPE) technique to avoid suppression of other signals present in the spectrum, caused by sugars in the syrup samples. Subsequently, the dilutions and fractions of each phytotherapeutic product sample were injected into the FT-ICR MS (Model 9.4 Solarix, Brucker Daltonics, Bremen, Germany). The conditions of the ESI source were: nebulizer gas pressure 1.0 bar, capillary voltage 4.0 kV, and capillary transfer temperature 250°C. The analyses were performed in positive and negative mode in a mass range of 150-1500 m/z. The mass spectrum obtained was processed using the *Compass Data Analysis* software (Brucker Daltonics, Bremen, Germany).

2.5. Research On Pathogenic Microorganisms

The research on pathogenic microorganisms in samples of *garrafadas* and syrups were performed according to the *Farmacopeia Brasileira 5ª edição* [5] for microbiological tests for non-sterile products, with some adjustments.

2.5.1. Fungi

For the quantification of molds and yeasts, 1 mL aliquots of the phytotherapeutic products were added in casein peptone broth for dilution, at a ratio of 1:10. After that, 0.1 µL of the dilution was transferred to the Sabouroud dextrose agar plate. Each sample was sown in triplicate using the *spread plate* technique. Subsequently, all plates were incubated for 2 to 5 days, at a temperature of 25ºC, away from light and excessive heat. Finally, the colony-forming units (CFU) were counted. The counting result was multiplied by the decimal dilution of the count and then was compared to what current legislation recommends.

2.5.2. Research on pathogenic bacteria

For the identification of pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Staphylococcus aureus*, the samples were sown in enrichment broth, tryptic soy broth. Aliquots of 1 mL were added in tubes containing 9 mL of enrichment broth, ratio 1:10, with subsequent incubation in a bacteriological incubator for 24-48h at 35°C. For identification of Gram-negative bacteria *E. coli*, *P. aeruginosa*, and *Salmonella* spp., triplicate sowing of the samples on Macconkey agar was done. Subsequently, the plates were incubated in a bacteriological incubator for 24-48h at 35°C. For the identification of *S. aureus*, samples were sown on mannitol salt agar. All seeding was done using the *spread plate* technique. Biochemical tests of coagulase and catalase were performed according to the manual of clinical microbiology of ANVISA [6] for confirmation of characteristic colonies of *Staphylococcus aureus*, and Gram staining for morphological identification of the bacteria.

3. Results and discussion

3.1. Analysis of the label

After evaluating the labels of the primary packaging of the samples of the seized *garrafadas* and syrups, when compared with Resolution No. 26/2014 [4], they were outside the recommended. Only the items "route of administration" and "expiration date" were present in 100% of the evaluated samples. The other itens of the primary packaging labels evaluation was not present, but there was the description "*product exempt from registration*" in all the labels of the packaging of the evaluated samples.

Concerning the *garrafadas*, no legislation regulates this type of product, making its evaluation and inspection difficult. The labeling of all seized samples contained information on being products of plant origin, with therapeutic indications and self-denomination of "*100% natural products*". The following table shows the composition of the *garrafadas* and syrups with the indication of use shown on the labels evaluated.

Table 1 Composition of the seized *garrafadas* and syrups, ceded by POLITEC-AP, according to the description and indication of use present on the label

Phytotherapeutic Products	Composition	Indication (Adapted Translation)
Saúde do homem	*Stryphnodendron adstringens*, *Symphytum officinale*, *Handroanthus impetiginosus*, *Hura creptans*, *Plumeria lancifolia*, and *Uncaria tomentosa*.	Inflammation treatment, cystitis, urethritis, urinary tract infection, cancer treatment and prevention.
Uxi-amarelo com unha de gato	*Endopleura uchi* and *Uncaria tomentosa*.	Myoma, polycystic ovary syndrome, urinary tract infection, uterus inflammation, cancer prevention, and female fertility stimulator.
Quebra-pedra e boldo	*Phyllanthus niruri*, *Plectranthus barbatus*, *Attalea speciosa* mesocarp, *Attalea speciosa* and *Quassia amara*.	Kidney stone disease, diuretic, diabetes treatment, liver infection prevention.
Xarope cumarú	Honey, *Dipteryx odorata*, *Pterodon emarginatus*, *Punica granatum*, *Mentha spicata*, *Allium sativum*, *Citrus limon*, propolis, *Zingiber officinale*, *Eucalyptus globulus*, *Hymenaea courbaril* L. sap, *Caryocar brasiliense* fruit oil, *Copaifera langisdorffii* oil, *Carapa guianensis* seed oil, *Mentha spicata* leaf extract.	Influenza, bronchitis, sinusitis, pneumonia, asthma, wheezing, phlegm, acute cough, sore throat, and cold.
Tônico dos pulmões	Honey, *Chenopodium ambrosioides*, and *Brosimum parinarioides*.	Cough, bronchitis, and asthma
Elixir de longa vida	*Erythroxylum vacciniifolium*, *Hymenaea courbaril*, *Strychnos pseudoquina*, *Mentha spicata*, *Punica granatum*, *Matricaria chamomilla*, *Prunus dulcis*, *Citrus sinensis*, *Illicium verum*, Quarenta Galhos (unknown botanical nomenclature), *Morus alba*, *Bertholletia excelsa*, honey.	Blood depurative, anemia, dizziness, weakness in legs and bones, blurred vision, and nerve stimulation.
Cura tudo	*Maytenus ilicifolia*, *Cynara scolymus*, *Artemisia annua*, *Baccharis trimera*, *Aesculus hippocastanum*, *Equisetum arvense*, *Echinodorus grandiflorus*, *Handroanthus impetiginosus*, *Solanum paniculatum*, and *Similax* spp.	Gastritis, peptic ulcer, kidney stone disease, liver infection, hepatitis, rheumatism, constipation, hemorrhoids, vermifuges. Diabetes management and weight loss auxiliary.
Saúde da mulher	*Endopleura uchi*, *Uncaria tomentosa*, *Stryphnodendron adstringens*, *Dalbergia monetaria*,	Uterus and ovary inflammation, cyst, myomas, vaginal itching,

	Handroanthus impetiginosus, *Hura crepitans*, *Schinus terebinthifolius*, *Caesalpinia ferrea*, *Poincianella pyramidalis* flower, *Bauhinia forficata*, and *Morinda citrifolia*.	abnormal vaginal discharge, irregular periods, cramps and cancer prevention, and painful urination.
Aguardente alemã	*Operculina macrocarpa*, *Operculina hamiltonii*, *Licaria puchury-major*, *Myristica fragrans*, *Illicium verum*, *Bunchosia armeniaca*, *Rheum palmatum*, *Baccharis trimera*, *Pinus elliotti*.	Indigestion, congestion, liver infection, facial palsy, tremors, headache, migraine, menopause, blood depurative, and blood circulation aid.
Mel com mastruz e leite do amapá	Honey, *Chenopodium ambrosioides*, and *Brosimum parinarioides*.	Gastritis, peptic ulcer, pneumonia, prostate cancer prevention, and pulmonary fortifier.
Xarope de cupim	Honey, Cupim (unknown botanical nomenclature), *Pterodon emarginatus*, *Punica granatum*, *Mentha spicata*, *Allium sativum*, *Citrus limon*, propolis, *Zingiber officinale*, *Eucalyptus globulus*, *Hymenaea courbaril* L. sap, *Caryocar brasiliense* fruit oil, *Copaifera langisdorffii* oil, *Carapa guianensis* seed oil.	Influenza, bronchitis, sinusitis, pneumonia, asthma, wheezing, phlegm, acute cough, sore throat, and cold.
Mel com limão e alho	*Eucalyptus globulus*, propolis, menthol, *Pterodon emarginatus* barc extract, *Hymenaea courbaril* seed extract, *Gochnatia polymorpha* leaf extract, *Anadenanthera colubrina* bark extract, *Caryocar brasiliense* fruit oil, *Carapa guianensis* seed oil.	Shortness of breath, influenza, cold, cough, pharyngitis, bronchitis, pneumonia, asthma, hoarseness, sore throat, and amygdalas inflammation.

Considering that the *garrafadas* are not considered medicines but carry the description of therapeutic indication and dosage, the denomination of "safe products" should not be present on their labels. However, these pieces of information are necessary for the regulatory and the correct instruction and safety of their users.

The absence of specifications on the labels may be risky to its users. The unknown concentration can cause damages because it can imply in the administration of a wrong low or high dose, even when specified on the label since this information can be invalid for another batch of the same product. After all, there is uncertainty about the equivalent concentration in grams of each vegetable metabolite produced by the plant species.

The Customer care numbers (CSC) of the company information is needed to contact the manufacturer for guidance, suggestions, or even a possible complaint about a batch produced, and its absence makes the consumer feedback impossible.

The fact that the *garrafadas* are not inspected allows them to inform in their labeling supposed laws or decrees, with the words "*Product Exempt from Registration according to Article 28 - Decree No. 79094 - Law No. 6360 of 23/09/1976*". In this decree, besides having been repealed by Decree No. 8077/13, there is no mention of *garrafadas*, especially its registration. Making false or misleading statements of products or services is provided for in Law No. 8078 of September 11, 1990, in its Article 66 of the Consumer Protection Code, with criminal detention of up to one year and payment of a fine [7].

In addition, on their labels, is mentioned a mixture of plants in each product. This information is dubious for the consumers because there is no proof of it since they are not registered products, and there is no botanical identification. In other words, there is no way to prove their presence.

The use of these products should be judicious, even for those who have no contraindications. Moreover, risk groups such as pregnant women, the elderly, and those with chronic diseases should not self-medicate, or even replace the treatment prescribed by a medical professional, for an already known health problem, for this type of product. Accordingly, in many syrups with high sugar concentrations, the contraindication for diabetic users must be explicit on their label.

The negligence in the inspection of these products and the traditional use increase the inherent risks to the *garrafadas* because they are mixtures of several complex active substances in unknown concentrations. Besides that, there is no scientific evidence of use or the presence of plants or parts of them in its composition and, still, do not guarantee security and effectiveness to its consumers. The manufacturing process is another relevant factor because, when not governed by good manufacturing and handling practices, chemical degradation, enzymatic actions, and microbial contamination can occur [3].

3.2. Determination of degree Brix (°Bx)

The degree Brix value was evaluated in all samples of syrups to verify the number of soluble solids (sugars) present in the analyzed samples. The amount of sugar present in these products is related to the storage of the product and its viscosity.

According to Andrade and Silva [8] the determination of sugar content was given by the degree Brix (°Bx), a method that consists in the analysis of light refraction by a refractometer, which considers the soluble solids present in a sample, which can generate false positives for sugar contents, because the method covers all soluble solids (sugars and salts).

For syrups, the sugar content in their formulation should not be less than 45% weight/weight (w/w) of sucrose or other sugars. Syrups may still contain flavoring agents, adding a characteristic flavor to them [9]. The sucrose content on the Brix scale varies from 50 to 70°Bx in artificial syrups [10].

The syrups "*Mel com mastruz e leite do amapá*", "*Mel com limão e alho*", "*Xarope de cumaru*", "*Xarope de cupim*" and "*Tônico dos pulmões*" were diluted in the proportion 1:2 for the reading in the refractometer. After reading, the diluted results of the samples were multiplied by 3x the 1:2 dilution (sample: water). Obtening the following results 72°, 60.9°, 66.9°, 69.3° Brix degree for "*Mel com mastruz e leite do Amapá*", "*Xarope de cumaru*", "*Tônico dos pulmões*" and "*Xarope de cupim*", respectively. Only the "*Mel com limão e alho*" (87.3° Bx) syrup that not was in according. The desirable sucrose range for syrups is usually 60 to 80%, which provides the syrup with flavor and viscosity [11]. Therefore, the syrups evaluated are within the sucrose concentration range.

Although the degree Brix approximates the sugar concentration in the sample, it is a method of low specificity because, besides sugar, it can identify other soluble solids and generate an inaccurate result for the analysis of carbohydrates of interest in a sugar sample.

3.3. Chemical Evaluation

3.3.1. pH measurement

Among the tests used for physical evaluation, the verification of the pH of all *garrafadas* and medicinal preparations was performed, which showed low values, with a pH media value of 2.15 in a temperature range from 14 to 21 ° C.

A POLITEC expert gave all the samples for the study. There is no information about the storage of these samples.

The appropriate pH of liquid formulations is related to the stability of the medicine. The medicine may present chemical instability when present at inadequate pH, thus causing its decomposition [12].

The growth of microorganisms in compounded pharmaceutical preparations can modify the physical stability of these products, causing reactions of degradation, such as the hydrolysis of fats, alterations in pH, color, and odor. The inadequate storage temperature and the altered pH can also influence the solubility of the active ingredient [13].

According to Bruno *et al.* [14] low pH values facilitate the growth of fungi and, depending on how the product is stored, it may reduce its shelf life and cause toxi-infection in the consumer.

Rodrigues *et al*. [15] evaluated the physical-chemical properties of *"xarope de cupim"* and found average pH values of 4.33 in the temperature range of 15 to 20°C. In this study, the same syrup showed a lower pH value at a temperature above 21.5°C.

Silva *et al*. [12] points out that syrups with low pH values, associated with inadequate oral hygiene, cause tooth enamel erosion, creating a favorable environment for the growth of dental cavities.

The pH control of the formulations has great relevance on the stability of the products: providing ideal conditions during the handling, storage, and transport of the final product, protecting from extrinsic factors such as light, heat, and humidity ensure long shelf life.

3.3.2. Chemical Analysis by ESI (-) FT-ICR MS

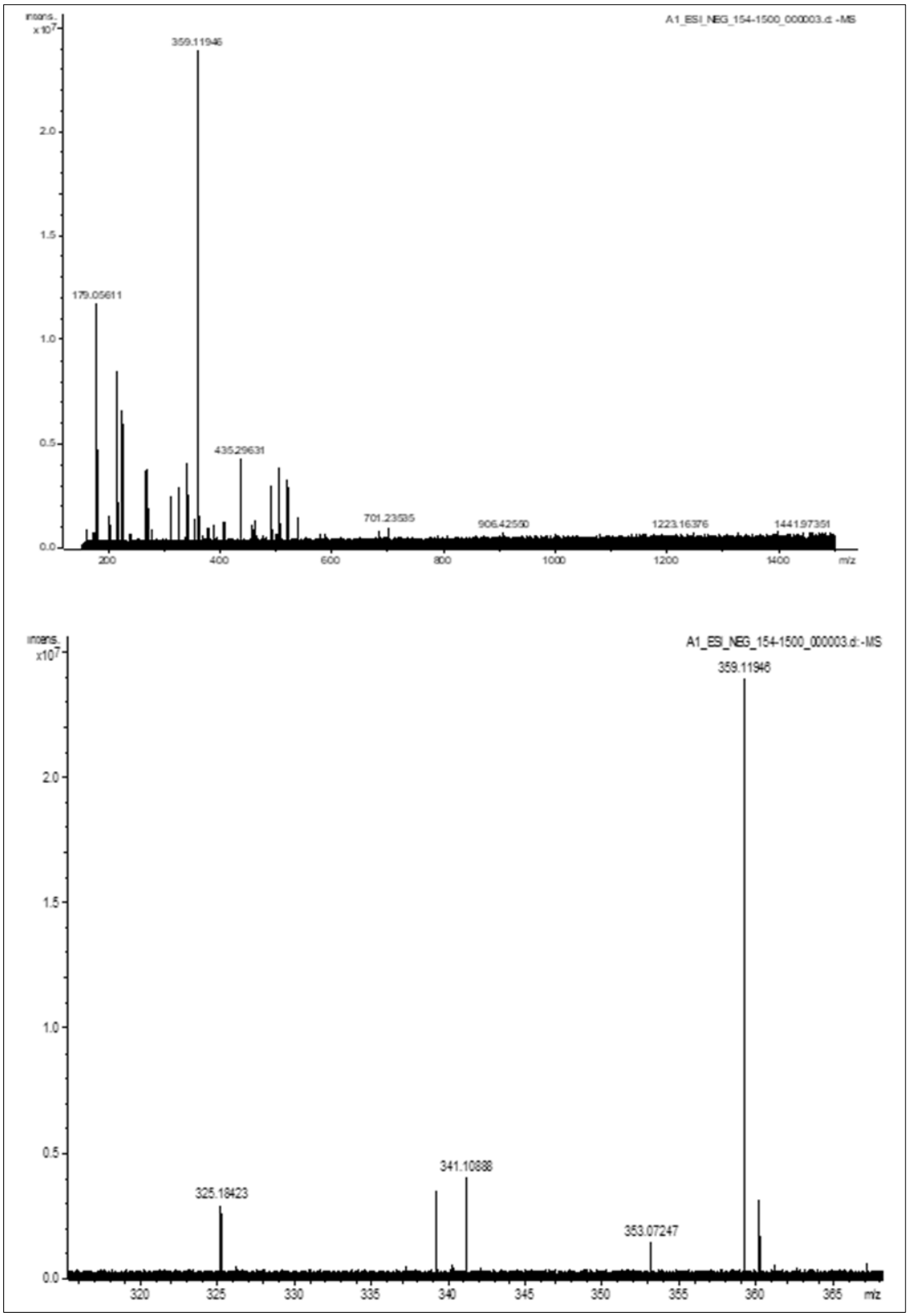

Figure 1 Spectrum of the *garrafada* "Mel com mastruz e leite do Amapá"

Adulterating in *garrafadas* is a matter of concern for public health since they are potentially risky for those who consume, since certain people may be sensitive to different components and, in extreme cases, can cause intoxications and be hazardous to health. The addition of adulterant substances is a crime, foreseen in law No. 9677 of July 2, 1998 [16]. However, interpretation of article 273 of this law is still subjective, making it difficult to resolve these cases. That said, forensic chemistry helps in crime-solving using the knowledge of chemistry in judicial resolution [17].

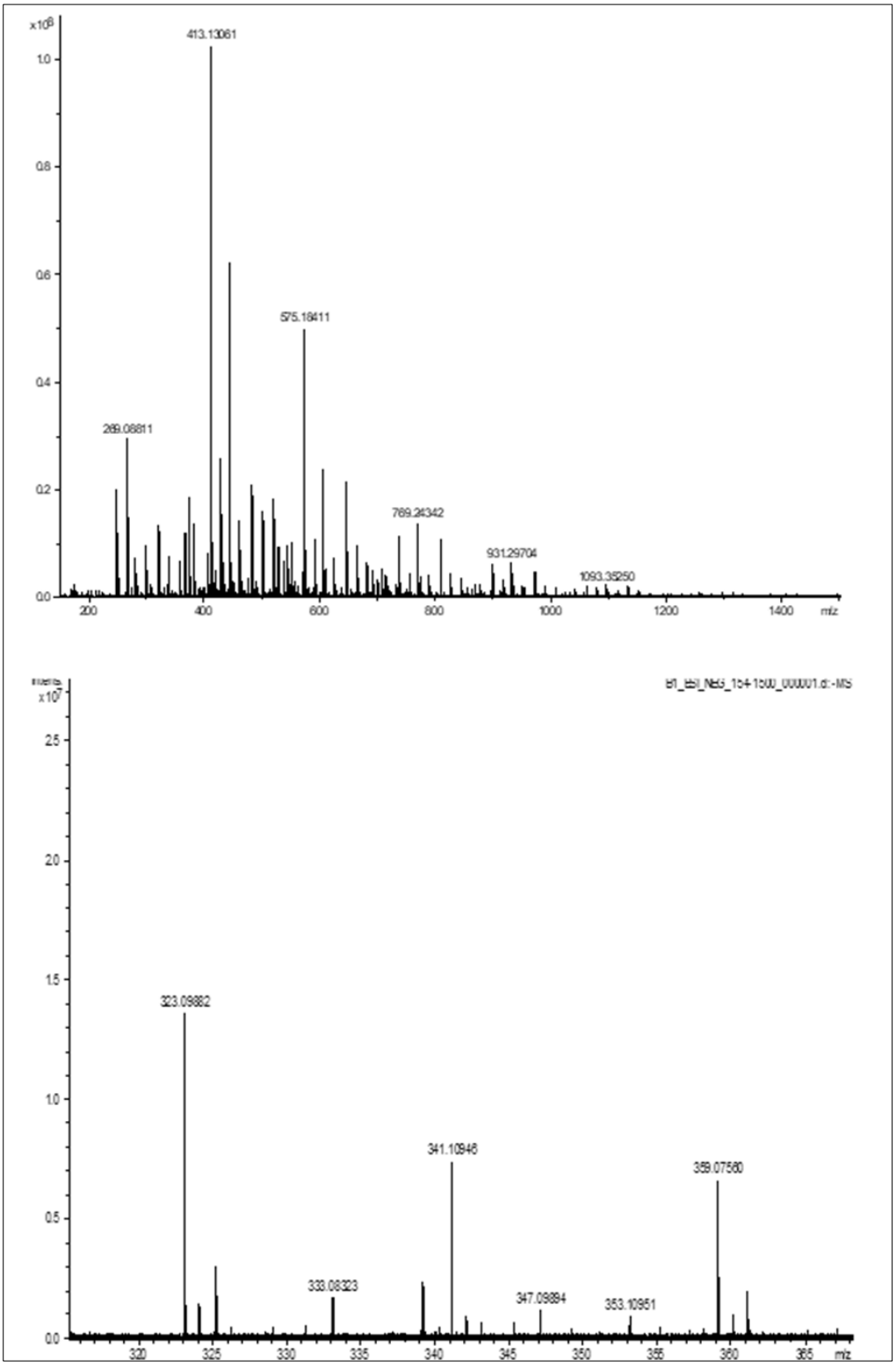

Figure 2 Mass spectrum of fraction 1 of the *garrafada* "*Saúde da mulher*"

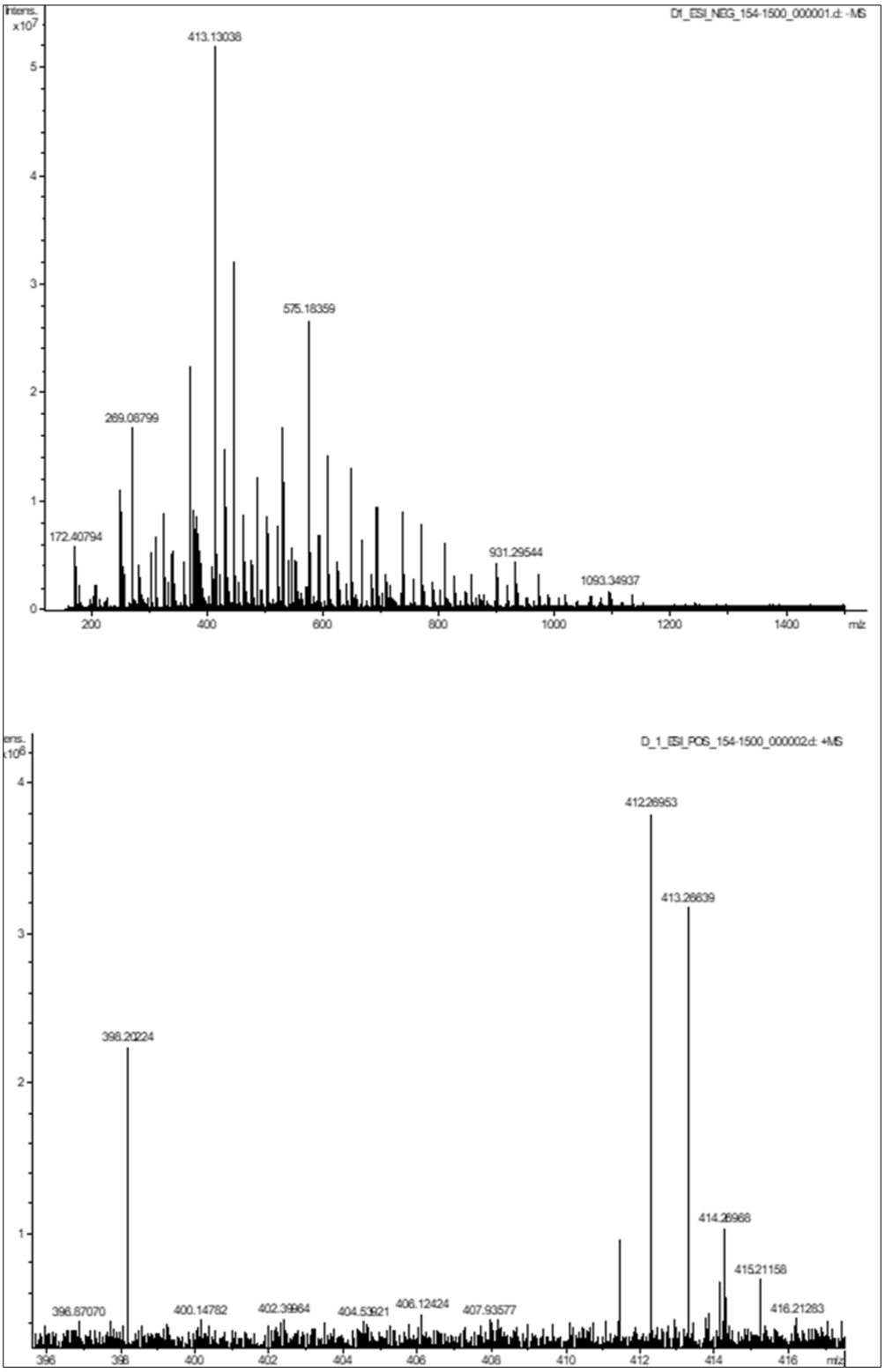

Figure 3 Spectrum of the *garrafada* "*Saúde do homem*" negative and positive mode, respectively

The chemical analysis aimed to identify possible adulterants, substances not described on the label of the seized products that could be related to the effects described. The chemical evaluation of the samples of *garrafadas* and syrups found no masses of adulterant substances such as synthetic drugs (anti-inflammatory, phosphodiesterase inhibitors, or bronchodilators) or drugs of abuse. Those masses were researched in the spectra according to the SAFS-FORENDEX database [18].

The main signals identification in the spectrum revealed some substances that suggest the presence of the plants described on the labels. This could be observed in the spectrum of the *garrafadas* "*Mel com mastruz e leite do Amapá*" (figure 1), "*Saúde da mulher*" (figure 2) and "*Saúde do homem*" (figure 3).

In some syrups, signs of sugars with *m/z* 341.109 $[M+H]^-$, *m/z* 359.119 $[M+H]^-$ e *m/z* 521.172 $[M+H]^-$ were found due to the prevalence of these and suppression caused in other less intense signals. The mass-to-charge ratio (*m/z*) of the masses found in the spectra was created based on MS images and literature data, according to table 2.

Table 2 Mass-to-charge ratio (*m/z*) of the main signals identified in the spectra of the analyzed samples

Mel com mastruz e leite do Amapá	**Mass observed $[M-H]^-$ *m/z***	**Identification**	**Reference**
	325.184	Pentosidic feruloyl acid	19
	367.216	Feruloylquinic acid isomer	20
	359.119	$C_{12}H_{23}O_{12}$	
	637.342	Isorhamnetin O-rhamnosyl-glucorinide	19
Saúde da mulher	**Mass observed $[M-H]^-$ *m/z***	**Identification**	**Reference**
	255.233	Fatty acid (palmitic acid)	20
	593.4	Robinetinidol-(epi)galocatechin	21
	607.3	Robinetinidol-4′-O-methyl(epi)galocatechin	21
Aguardente alemã	**Mass observed $[M-H]^-$ *m/z***	**Identification**	
	345.283	Unknown	
	347.094	Unknown	
	659.547	Unknown	
Saúde do homem	**Mass observed $[M-H]^+$ *m/z***	**Identification**	
	398.202	Echimidine	22
	412.269	Lasiocarpine	22
Uxi-amarelo com unha de gato	**Mass observed $[M-H]^-$ *m/z***	**Identification**	
	255.233	Fatty acid (palmitic acid)	20
	653.301	Unknown	
Longa vida	**Mass observed $[M-H]^-$ *m/z***	**Identification**	
	445.135	Tannin	
Mel com limão e alho	**Mass observed $[M-H]^-$ *m/z***	**Identification**	
	341.109	Sucrose	
	359.119	Sucrose	
	521.172	Sucrose	

Xarope de cumaru	**Mass observed [M-H]⁻ *m/z***	**Identification**	
	215.032	Glucose+Cl	
	341.109	Sucrose	
Quebra-pedra	**Mass observed [M-H]⁻ *m/z***	**Identification**	
	445.135	Tannin	
Xarope cura tudo	**Mass observed [M-H]⁻ *m/z***	**Identification**	
	445.135	Tannin	
Tônico dos pulmões	**Mass observed [M-H]⁻ *m/z***	**Identification**	
	215.033	Glucose+Cl	
	341.109	Sucrose	
Xarope de cupim	**Mass observed [M-H]⁻ *m/z***	**Identification**	
	341.109	Sucrose	

In all syrups, the presence of sugars, in simple form, as monomers and dimers of sucrose was observed, corroborating the results of degree Brixº.

Fatty acids such as palmitic acid, *m/z* 255.233 [M+H] ⁻, flavonoids and their phenolic derivatives, such as pentosidic feruloyl acid *m/z* 325.184 [M+H] ⁻, feruloylquinic acid isomer *m/z* 367.216 [M+H]⁻, Isorhamnetin O-rhamnosyl-glucorinide *m/z* 637.342 [M+H] ⁻ and gallontannin were present in the samples of the *garrafadas* and syrups analyzed, which suggests the presence of the plant or parts thereof. However, it was not possible to confirm the presence of its phytochemical markers since there is a mixture of plants, and many substances, as observed in the spectrum and specified on the label of the products.

Phenolic compounds and their derivatives can be found in plants on a wide range of structures. These substances have antioxidant, antimicrobial, anti-inflammatory, and vasodilator properties. Its chemical structure has at least one aromatic ring and one or more hydroxyl radical [23].

Studies by El-Askary *et al*. [24] on the quinic acid derivatives of the leaves of *Artemisia annua* L. have shown that these derivatives have a strong correlation with the antioxidant and hepatoprotective potential described in the genus properties of the plant species. The quinic acid derivatives can increase glutathione secretion and normalize hepatic marker levels.

Through the analysis by electrospray ionization mass spectrometry (ESI-MS) in positive mode, it was possible to identify, in the sample of the *garrafada* "*Saúde do homem*", the pyrrolizidine alkaloids lasiocarpine and echimidine. The masses corresponding signals in the spectrum are *m/z* 398.202 $[M+H]^+$ and *m/z* 412.269 $[M+H]^+$, respectively. In this *garrafada*, there is a description of the comfrey plant on their label, corroborating its chemical finding with the presence of parts of the plant.

Pyrrolizidine alkaloids are secondary metabolites resulting from the metabolism of some plant species, such as comfrey (*Symphytum officinalis* L.), which metabolizes echimidine and lasiocarpine. The hepatotoxic effect of these alkaloids is due to the action of their metabolites as alkylating agents, which cause a reaction of oxidation in the α-carbon to the N-carbon, catalyzed by monooxygenases of the cytochrome P-450 [25].

Echimidine is related to several cases of veno-occlusive liver disease when used as a tea or herbal drug. Lasiocarpine caused hepatocellular carcinoma in 61% of rats used in a study: squamous cell carcinoma of the skin in 33%, and lung

adenomas in 28%. In another study, with rats fed lasiocarpine at a dietary concentration of 50 ppm for 55 weeks, 45% of these animals developed liver angiosarcomas, and 35% had hepatocellular carcinomas [26].

Although most labels of *garrafadas* and syrups describe a wide range of plants, during the chemical analysis, it was not possible to confirm the presence of all the phytochemical markers, due to the sample being of plant origin and containing many complex substances, which makes it tough to interpret the signals. This can be observed in the inconclusive signals generated in the spectra of the "*Aguardente alemã*" and "*uxi-amarelo*" because the masses referring to the signals of the spectrum found are not compatible with the literature and, possibly, the substances may have suffered cleavage, formed cations and ions, and consequent formation of adducts in the spectrum, which cause inconsistencies during interpretation [27].

A study carried out in the state of Parana by Indras *et al*. [28], in which he evaluated the toxic effects of the *garrafadas* in rats, observed in 30 days of treatment that the alcohol present in the *garrafadas* changed biochemical and hematological parameters ($p < 0.05$) in rats. Thus, *garrafadas* are products that should be used with great care, especially if this is used exclusively for a disease treatment over a long period.

3.4. Microbiological Evaluation

No presence of microorganisms of the genus *E. coli, P. aeruginosa*, and *Salmonella* was detected. However, colonies suggestive of *S. aureus* grew in 4 samples of the syrups "*Mel com limão e alho*", "*Tônico dos pulmões*", and "*Xarope de cupim*". In the research of fungi and yeasts, growth was observed in 2 samples of the "*Xarope de cumaru*" and "*Xarope de quebra pedra e boldo*" less than <10 CFU/mL (est.).

Microorganisms may be naturally present in plants [29]. Herbal pharmaceutical products may be contaminated with pathogenic organisms such as *Salmonella*, *Pseudomonas*, *Enterobacter*, and others [30]. This contamination by microorganisms, observed in the plating of the samples, harms not only the consumer and the product but also causes financial losses to the food, cosmetic, and pharmaceutical sectors. Pharmaceutical products offer a favorable environment for microbial growth. If the harvesting, drying, or storage process of plant material is not performed properly [31], these microorganisms can exponentially grow and therefore provide unsatisfactory quality in pharmaceutical products. In addition, due to the constituents of some formulations being sources of nutrients for these microorganisms, although the product has a preservative in its formulation, the degradation of the product is possible when there is a high presence of microbial load in it [32].

The presence of microorganisms can cause instability in the product, leading to a decrease or even loss of its effectiveness with consequent degradation of the active ingredient. The pH of the medicinal preparation can also suffer alterations due to the microbial presence. These physical and chemical alterations compromise the bioavailability and acceptance by the consumer [33].

Also, the low quality and the non-compliance with good manufacturing practices bring severe consequences for health. There is no guarantee that the drug or phytotherapeutic preparation is safe and effective during treatment. In cases of antimicrobial drugs, it may also lead to antimicrobial resistance and produce side effects, consequently increasing public expenditure with the health care system [34].

The Gram staining performed on the samples revealed Gram-positive cocci in the following syrups "*Mel com mastruz e leite do Amapá*", "*Mel com limão e alho*", "*Tônico dos pulmões*", and "*Xarope de cupim*" characteristic of *S. aureus*. Thus, it was necessary to verify the species through phenotypic tests such as coagulase and catalase. Given the result, the presence of catalase-producing coagulase-negative *Staphylococcus* was confirmed.

According to the *Farmacopeia Brasileira 5ª edição* [5], in non-sterile products for oral use containing raw material of natural origin, *Escherichia coli* and *Staphylococcus aureus* should be absent in 1 g or 1 mL; for bacteria of the genus *Salmonella*, absence in 10 g or 10 mL; and for fungi and yeasts, the colony forming units (CFU) count should be less than 10^2 CFU/g or mL. Therefore, all samples of seized medicinal preparations evaluated were within the limits established for these microorganisms, and are in accordance with the official Brazilian compendium. However, four samples were disapproved, because there was coagulase-negative *Staphylococcus* growth.

Although most samples were within the microbial limits allowed, it is necessary that these products for medicinal purposes, such as *garrafadas*, teas, and other types of preparations, are manufactured in good hygiene conditions since the manufacturing process directly influences the quality of these products.

The coagulase-negative *Staphylococcus* (SCN) have lower pathogenicity compared to the *S. aureus*. However, due to adaptive factors such as the ability to form biofilms and carry genes that encode antimicrobial resistance, and the indiscriminate use of antibiotics, it can cause more severe infections [35].

The *S. aureus* is a facultative anaerobe, Gram-positive, coagulase-positive bacteria and can appear microscopically as a single coccus, in pairs or clusters of cocci. The *Staphylococcus* spp. can be differentiated from streptococci by the catalase test [36]. It has different habitats, and various species act as a commensal of the human microbiota. Among these species, *S. aureus* is the most relevant human pathogen in the genus, considered an opportunistic microorganism, very common in nosocomial infections. It requires a minimum pH of 4.5 for its proliferation and optimal temperature around 37°C for its growth. However, at higher temperatures, they are capable of producing a toxin (>15°C), but in cases of products contaminated by this microorganism, these factors can vary [37].

Silva *et al*.[33] evaluated the microbiological quality of *lambedores* (homemade syrups) sold in the city of Cuité, Pernambuco. The researchers found pathogenic microorganisms such as *S. aureus* in two samples and *Salmonella* spp. in another.

Studies conducted by Braz *et al*. [38] evaluated medicinal preparations obtained from a *raizeiro* (traditional healer) in the city of Sanclerlândia, Goiás. They found, in samples of *garrafadas*, high fungal contamination, $3{,}5x10^3$ CFU/mL, above the limit established by the *Farmacopeia Brasileira 5ª edição* [5].

Therefore, the microbiological quality of a pharmaceutical product depends on the provision of hygiene and aseptic conditions of the location, tools, equipment, and the manipulator. These measures prevent and considerably reduce the risk of contamination by microorganisms.

4. Conclusion

No synthetic chemicals or drugs of abuse were found in the samples analyzed by the FT-ICR MS. However, in only four of the twelve samples evaluated, it was possible to detect mass fragments related to chemical substances of the plants described on the labels of the packages. Most of them were made up of sugars only, which could compromise their therapeutic effectiveness. The microbiological evaluation detected negative-coagulase *S. aureus* bacteria in four syrup samples ("*Mel com mastruz e leite do Amapá*", "*Mel com limão e alho*", "*Tônico dos pulmões*", and "*Xarope de cupim*"), making them unfit for consumption. This result is associated with the low pH value of the samples, which hinders microbial growth. All the products of this study were commercialized in the state of Amapá. After evaluation, the samples proved to be unsuitable for commercialization or consumption. The *garrafadas*, homemade syrups, and other preparations have great cultural importance in the northern region of Brazil and therefore deserve attention. This paper reinforces the importance of controlling risks associated with low-quality control in the manufacturing of these products. The *garrafadas* and syrups must be inspected to assure adequate hygiene conditions in all the stages of production. The solution to this regulatory aspect should include the creation of public policies to coordinate the production and marketing chain for these products.

Compliance with ethical standards

Acknowledgments

Our thanks go to Ryan da Silva Ramos for their technical assistance with the mass spectrum interpretations and to INCT - Forense for their support of this research.

Disclosure of conflict of interest

The authors declare that they have no competing interests.

References

[1] Van Rooyen RM, Pretorius B, Tembani NM, Ham-Baloyi WT. Evidence-based Recommendations To Facilitate Professional Collaboration Between Allopathic And Traditional Health Practitioners. Health Sa Ge Sondheid. 2017; 22: 291-299.

[2] Yang Y, Deng J. Analysis Of Pharmaceutical Products And Herbal Medicines Using Ambient Mass Spectrometry. TrAC. 2016; 82: 68–88.

[3] Passos MMB, Albino RC, Silva MF, Oliveira DR. A disseminação cultural das garrafadas no Brasil: um paralelo entre medicina popular e legislação sanitária. Saúde debate. 2018; 42(116): 248-262.

[4] ANVISA. Agência Nacional de Vigilância Sanitária. Resolução da diretoria colegiada-RDC nº 26, de 13 de maio de 2014. Dispõe sobre o registro de medicamentos fitoterápicos e o registro e a notificação de produtos tradicionais fitoterápicos.

[5] ANVISA. Agência Nacional De Vigilância Sanitária. Farmacopeia Brasileira, volume 1. 5ª Ed. Brasília. 2010.

[6] ANVISA. Agência Nacional de Vigilância Sanitária. Microbiologia Clínica para o Controle de Infecção Relacionada à Assistência à Saúde. Módulo 5: Tecnologias em Serviços de Saúde: descrição dos meios de cultura empregados nos exames microbiológicos/Agência Nacional de Vigilância Sanitária.– Brasília: Anvisa. 2013.

[7] BRASIL. Lei nº 8.078, de 11 de Setembro de 1990. Dispõe sobre a proteção do consumidor e dá outras providências. [Accessed 06 ago 2019] Available at: http://www.planalto.gov.br/ccivil_03/LEIS/L8078.htm

[8] Andrade ND, Silva DM. Análise dos métodos brix e cromatografia para a determinação de açúcares redutores residuais totais. Faculdade de Engenharia de Produção, Universidade do Rio Verde, 2016.

[9] ANVISA. Agência Nacional De Vigilância Sanitária. Formulário de fitoterápicos da farmacopeia brasileira. Brasília: ANVISA, 2011.

[10] Monteiro MAA, Vaz ELS, Samapaio MM, Codaro EM, Acciari HA. Determinação de sacarose no xarope artificial de groselha por medidas de viscosidade: Uma abordagem interdisciplinar. Cad Bras Ens Fís. 2013;30(3):566-578.

[11] Allen Jr LV, Popovich NG, Ansel HC. Formas farmacêuticas e sistemas de liberação de fármacos. 9. ed. Artmed, 2013.

[12] Silva CO, Reis MÊS, Santana KFP, Maestri FP, Saatkamp CJ, Maestri RP. Avaliação do potencial cariogênico de anti-histamínicos de uso pediátrico. Eletronic Journal of Pharmacy. 2015;12(3):15-22.

[13] Pombal R, Barata P, Oliveira R. Estabilidade dos medicamentos manipulados. Revista Faculdade de Ciências da Saúde. 2010;7:330-341.

[14] Bruno LM, Queiroz AAM, Andrade APC, Vasconcelos NM, Borges MF. Avaliação microbiológica de hortaliças e frutas minimamente processadas comercializadas em Fortaleza (CE). Boletim do Centro de Pesquisa de Processamento de Alimentos. 2005;23(1):75-84.

[15] Rodrigues APF, Barbosa MEO, Kobayashi NHC, Farias SV, Barbosa ICC, Souza EC, et al. Caracterização físico-química de xarope de cupim. In: CONGRESSO BRASILEIRO DE QUÍMICA, 2018, São Luíz. [Accessed 02 dez 2019] Available at: http://www.abq.org.br/cbq/2018/trabalhos/7/855-26342.html

[16] BRASIL. Ministério da Saúde. Lei n° 9.677 de 02 de julho de 1998. Altera dispositivos do capítulo III do Título VIII do Código Penal, incluindo na classificação dos delitos considerados hediondos crimes contra a saúde pública, e dá outras providências. Brasília, 1998. [Accessed 06 ago 2019] Available at: http://www.planalto.gov.br/ccivil_03/LEIS/L9677.htm

[17] Ferreira AG. Química forense e técnicas utilizadas em resoluções de crimes. Acta de Ciências e Saúde. 2016;2(5).

[18] ASSOCIAÇÃO SUL DE CIENTISTAS FORENSES. 2020 [Accessed 30 jun 2020]. Available at: http://forendex.safs1966.org/index.php/holdings/index

[19] Barros L, Pereira E, Calhelha RC, Dueñas M, Carvalho AM, Santos-buelga C, et al. Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of Chenopodium ambrosioides L. J Funct Foods. 2013;5:1732-1740.

[20] Simirgiotis M, Benites J, Areche C, Sepúlveda B. Antioxidant Capacities and Analysis of Phenolic Compounds in Three Endemic Nolana Species by HPLC-PDA-ESI-MS. Molecules. 2015;20(6):11490–11507.

[21] Pinto SCG, Bueno FG, Panizzon GP, Morais G, Dos Santos PVP, Baesso ML, et al. Stryphnodendron adstringens: Clarifying Wound Healing in Streptozotocin-Induced Diabetic Rats. Planta Med. 2015;81:1090–1096.

[22] Liu F, Wan SY, Jiang Z, Li SFY, Ong ES, Osorio JCC. Determination of pyrrolizidine alkaloids in comfrey by liquid chromatography–electrospray ionization mass spectrometry. Talanta. 2009;80(2):916–923.

[23] Ferrera TS, Heldwein AB, Dos Santos CO, Somavilla JC, Sautter CK. Substâncias fenólicas, flavonoides e capacidade antioxidante em erveiras sob diferentes coberturas do solo e sombreamentos. Rev Bras Pl Med. 2016;18(2):588-596.

[24] El-Askary HI, Mohamed SS, El-Gohari HMA, Ezzat SM, Meselhy MR. Quinic acid derivatives from Artemisia annua L. leaves; biological activities and seasonal variation. S Afr J Bot. 2020;128:200–208.

[25] Silva CM, Bolzan AA, Heinzmann BM. Alcaloides pirrolizidínicos em espécies do gênero Senecio. Quím Nova. 2006;29(5):1047-1053.

[26] Mei N, Guo L, Fu PP, Fuscoe JC, Luan Y, Chen T. Metabolism, genotoxicity and carcinogenicity of comfrey. J Toxicol Env Heal B. 2010;13:509–526.

[27] Bonancêa CE. Estudo dos mecanismos de fotodegradação de corantes sobre dióxido de titânio através de técnicas de espectroscopia Raman intensificadas. 2005. [Dissertação]. São Paulo: Instituto de Química, Universidade de São Paulo; 2005.

[28] Indras D.M, Marek CB, Penteado AJ, Ferreira FS, Silva FC, Itinose AM. Evaluation of the toxic effects of the bottled medicine (garrafada) containing the Echinacea purpurea, Annona muricata, Tabebuia avellanedae, Pterodon emarginatus and Uncaria tomentosa in rats. J. Med. Plants Res. 2020;14(3), 105–117. doi:10.5897/jmpr2019.6862

[29] Nafiu MO, Hamid AA, Muritala HF, Adeyemi SB. Preparation, Standardization, and Quality Control of Medicinal Plants in Africa. In: Medicinal Spices and Vegetables from Africa, Camarões: Academic Press; 2017. p. 171–204.

[30] Jan S, Abbas N. Quality Assurance and Quality Control of Medicinal and Aromatic Herbs. In: Himalayan Phytochemicals, SPi Global: India. 2018. p. 217–246.

[31] Lima CMS, Fujishima MAT, Lima BP, Mastroianni PC, Sousa FFO, Silva JO. Microbial contamination in herbal medicines: a serious health hazard to elderly consumers. BMC Complement Med Ther. 2020;20(1):1-9.

[32] Oliveira LS, Rossato LG, Bertol CD. Análise da contaminação microbiológica de diferentes dentifrícios. Rev odontol UNESP. 2016;2(45):85-89.

[33] Silva BR, Lima IO, Carmo ES, Souza JBP. Avaliação microbiológica de Lambedores. Revista Saúde e Ciência. 2016;5(1):5-22.

[34] Kovacs S, Hawes SE, Maley SN, Mosites E, Wong L, Stergachis A. Technologies for detecting falsified and substandard drugs in low and middle-income countries. PLoS One. 2014;9(3): e90601. https://doi.org/10.1371/journal.pone.0090601

[35] Araújo RLS. Avaliação genotípica e fenotípica de cepas de Staphylococcus ssp. isoladas em hospitais do Distrito Federal no período de 2009 a 2016. 2019. 69 f. [Dissertação]. Brasília: Universidade Católica de Brasília, Programa Stricto Sensu em Ciências Genômicas e Biotecnologia; 2019.

[36] Khan SUH, Khan SM, Majeed A, Ashraf A, Habib A, Wani JM, et al. Antimicrobial effect of colloidal argentum colloid on ampicillin resistant Staphylococcus aureus. J Entomol Zool Stud. 2019;7(1):181-183.

[37] Araújo AS, Almeida AF. Caracterização fenotípica de bactérias isoladas de peixes e da água em tanques de piscicultura no município de Macapá-Ap entre o período chuvoso e seco. 2017. 52f. [Trabalho de Conclusão de Curso]. Amapá: Universidade Federal do Amapá; 2017.

[38] Braz PH, Melo TL, Brandão RS, Pinto MV, Gonçalves VS. Análise microbiológica de preparações medicinais adquiridas em raizeiro na cidade de Sanclerlândia, Goiás. Revista Faculdade Montes Belos. 2015; 8(1): 2-10.