International Journal of Phytocosmetics and Natural Ingredients 2021;8:6

doi 10.15171/ijpni.2021.06

Original Article

The microbiological evaluation of cumarú syrup, *Dipteryx odorata* (Aubl) sold at public fairs of Macapá-Amapá

Gabriele Gonçalves e Gonçalves[1], Iasmin dos Santos Oliveira[1], Paulo Vinicius Ferreira Lima Oliveira[1], Antonio Carlos Sousa Silva Júnior[2*]

[1]Pharmacy Student, Macapaense Institute of Higher Education – IMMES
[2]Researcher at the Food Science and Technology Center, Institute of Scientific and Technological Research of the State of Amapá – IEPA

***Correspondence to**
Antonio Carlos Sousa Silva Júnior, Email: acsilva.junior@gmail.com

Received 22 Feb. 2020
Revised 14 May 2021
Accepted 9 Jun. 2021
ePublished 1 Jul. 2021

Abstract

Background: Cumarú, *Dipteryx odorata* (Aubl) is a monospheric drupaceous pod with a fibrous and edible pulp, typical of the Amazon region, with great phytotherapeutic power, stimulated the manufacture of cumarú syrup based on traditional popular knowledge. The manner in which this syrup is handled indicates the extent to which oxidative changes are presented, as well as the possibility of contamination by bacteria, fungi and yeast. The objective of this work was to evaluate the microbiological quality of cumarú syrup commercialized in Macapá – Amapá's public fairs.

Materials and Methods: In March 2019, three random samples of cumarú syrup were collected from three different markets, sold without refrigeration and sent to the Macapaense Institute of Higher Education Microscopy Laboratory. 1:10 dilution in peptone saline was performed. For the total mesophil count, 100 µL of the initial dilution was inoculated into plates containing PCA agar and Nutrient agar, incubated at 35 ± 1°C/48 hours. For mold and yeast, the inoculum was seeded on potato dextrose agar incubated at 25 ± 1°C/7 days. For *Escherichia coli*, MacConkey agar was used, for *Staphylococcus aureus*, mannitol salt agar and *Salmonella*, *Salmonella-Shigella* Agar, all incubated at 35 ± 1°C/48 hours.

Results: It was found from the label analysis that only one sample had a different formulation, and all were within the expiration date. Of the 3 samples evaluated, 2 were non-standard for total mesophylls, with counts ranging from 9.3 x 10 ± 5.5 x 10 to 1.7 x 10^3 ± 1.9 x 10^3 CFU/mL. For molds and yeasts 100% of the samples that were out of the legislation, with counts ranging from 2 x 10 to 2 x 10^2 CFU/mL. It was also possible to verify the presence of *S. aureus* and *E. coli* in one of the samples and the total absence of Salmonella in the analyzed syrups, which would make its use unfeasible.

Conclusions: Because it is an industrialized product rich in phytotherapeutic substances, errors in handling cause changes in its therapeutic efficacy, in addition to the risk to the consumer. These products must be handled carefully, with inputs of controlled origin, avoiding conditions favorable to the development of pathogenic microorganisms.

Keywords: Pathogenic Microorganisms, Phytotherapeutic, Quality control

Background

Even though there is a huge scientific pharmaceutical development and the great availability of various medicines for sale in pharmacies, a large part of the Brazilian population still prefer to make use of roots, leaves, bark and medicinal syrups made, in general, in a handcrafted way, following a regional popular tradition. Consumers claim that natural medicinal products are effective, have a lower purchase cost and some treat diseases such as rheumatism, arthritis, lung problems, for which it is not yet accessible to pharmacies, with these characteristics.[1]

In this context, Cumarú (*Dipteryx odorata*), a tree native to the tropical forests, belonging to the *Leguminosidae* family, can be found in greater numbers throughout the Brazilian Amazon, Maranhão, Mato Grosso and southern Pará. It is considered a grid tree, about 30 m high. Its commercial value is used for its shells, almonds and leaves.[2]

Thus, due to the properties of cumarú, it gave rise to the production of syrups based on the plant. With everything, as every product is of vegetable or animal origin, care must be taken in the production management process, since cumarú and other products from the forest may be subject to contamination of microorganisms from their collection to the manufacture of syrup. Therefore, this research sought to analyze the cumarú syrup sold in the

Please cite this paper as: Gonçalves GG, Santos Oliveira LD, Lima Oliveira PVF, Silva Júnior ACS. The microbiological evaluation of *cumarú syrup*, *Dipteryx odorata* (Aubl) sold at public fairs of Macapá-Amapá. Int J Phytocos Nat Ingred. 2020;8:6. doi:10.15171/ijpni.2021.06.

city of Macapá,[3] to know the presence, quantity, and which microorganisms are these.

Materials and Methods

This study is a microbiological investigation of cumarú syrup analyzed by academics of the third semester of the Pharmacy course of the Macapaense Institute of the Best Higher Education-IMMES, since a qualitative and quantitative analysis based on culture media was made.

The collection of samples at the base of the cumarú plant was collected from three public fairs in Macapá, which were located in the following neighborhoods: Novo horizonte, Buritizal and Centro, from March 9 to 11, 2019, totaling 3 randomly selected samples that were sent to the microscopy laboratory of IMMES. The initial dilution was 1 g of each sample analysed and the samples were placed in three test tubes containing 9 mL of protonated water.

The three dilutions of each sample were used for the standard total mesophilic count, and with the aid of a Pasteur pipette was introduced on the surface of the petri dishes sequentially containing PCA agar and Nutrient agar. The swab had to pass through the ends of the plates making circular movements so that all the sample was distributed over all the plates, these were sequentially incubated at 35°C for 48 hours. After this period, the colonies were counted, summed and checked if they complied with the standard of the Brazilian Pharmacopoeia for aqueous oral product in the TAMC column.

The test for the determination of moulds and yeasts was done by the incorporation of 100 mL of the initial dilution to a plate containing potato dextrose agar, staying in the greenhouse for 7 days. Following the same method, it was made for *Escherichia coli* in a petri dish containing MacConkey agar, which was incubated for 48 hours at 35°C, being later applied for its confirmation the deep biting technique.

For the determination of *Salmonella* and *Staphylococcus* test tubes containing sequentially 1 g of the sample and 9 mL of Rappaport Vassiliadis Soya and brain heart infusion (BHI) broth incubated for 24 hours at 35°C were used, and subsequently the streaking technique was used by depletion on plates with MacConkey agar and *Salmonella-Shigella* agar. The data processing and analysis were performed and verified by three research teams, accompanied by the corresponding author (A.C.S.S.J.) and the Excel program was used for the construction of the table.

Results and Discussion

Microbial contamination of pharmaceuticals, cosmetics and herbal medicines can come from various sources due to the complexity of the production processes.[4] In this research, the microbiological test applied to the herbal medicine is a viable and appropriate technique[5] to verify the presence/absence of microorganisms, from such technique will be presented and discussed the results obtained from the samples used.

Microbiological Analysis

In total mesophiles as presented in Table 1 of the three samples evaluated two were not in accordance with Table 2 (Brazilian Pharmacopoeia, 2010) presenting elevated colonies from 8×10^2 to 1.7×10^3 CFU/mL. These results were similar to those observed by the author Fabiana Goncalves et al[6,7] who obtained an overall result in the microbiological analysis of herbal therapies, the presence of mesophylls highlighting that only two exceeded the microbial limit.

Of the samples evaluated 66.6%, the presence of *Staphylococcus* was not found in the plates containing MacConkey agar and Mannitol agar, due to the absence of colony (Figure 1). According to Schlievert et al, Madigan et al, Veras et al quoted by Silva-Junior et al[3] *S. aureus* is the main representative of coagulase positive staphylococci, they are biologically active, producing several proteins, toxins and enzymes that have the capacity to cause food infections and/or intoxications.

In only one plate containing MacConkey were colonies

Table 1:

Critério de aceitabilidade para qualidade microbiológica de formas de dose não estéril(Capítulo <1111>, Farmacopeia

Rotas de Administração	TAMC – UFC/g ou UFC/mL	TYMC – UFC/g ou UFC/mL	Ausência de microrganismos específicos
Oral (não aquoso)	10^3	10^2	*E. coli*
Oral (aquoso)	10^2	10^1	*E. coli*
Retal	10^3	10^2	Não designado
Oromucosa	10^2	10^1	*S. aureus, P.aeruginosa*
Gengival	10^2	10^1	*S. aureus, P.aeruginosa*
Cutâneo	10^2	10^1	*S. aureus, P.aeruginosa*
Nasal	10^2	10^1	*S. aureus, P.aeruginosa*
Auricular	10^2	10^1	*S. aureus, P.aeruginosa*
Vaginal	10^2	10^1	*S. aureus, P.aeruginosa,Candida albicans*

Source: Farmacopeia Brasileira.

Table 2. General Results

Total Mesophiles	1F	2F	3F
	720	20	70
	700	70	20
	730	170	4560
	1030	110	2080
	795	92.5	1682.5
	136.1066	54.94315	1857.557
Moulds and yeasts (Figure 3)	200	20	40
Staphylococcus	Neg	Pos	Neg
E. coli	Neg	Pos	Neg
Salmonella			

Source: Data obtained from microbiological analyses.[8-11]

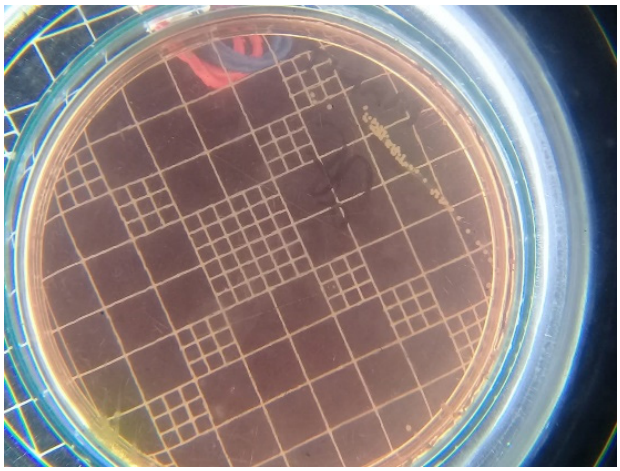

Figure 1. *Staphylococcus* Colony.

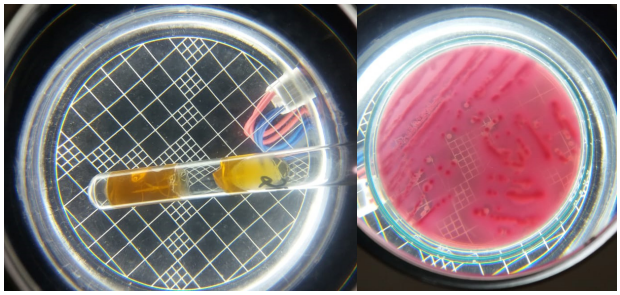

Figure 2. *Escherichia coli* Identification.

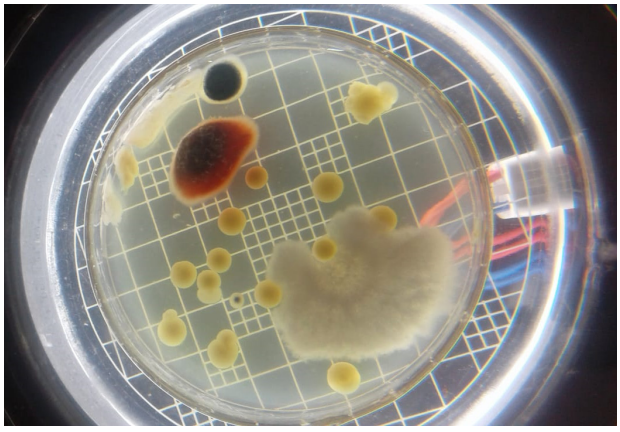

Figure 3. Mould and Yeast Register Corresponding to Sample 1.

with reddish characteristics similar to the microorganism *E. coli* observed (Figure 2), which was later confirmed to be present by a change in colour and rupture of the culture medium contained in the test tube. And among the most relevant general aspects observed in this work are that 100% of the samples tested on the plate containing *Salmonella-Shigella* Agar were found to be Salmonella-free, due to the lack of colony growth.

The molds and yeasts developed in variations in the plates containing potato dextrose agar, which according to Maria Cecilia this group fits the class of microorganisms that are not able to transmit health risks directly, as reported by International Commission on Microbiological Specifications for Foods.

In a general analysis of the results obtained, it is also shown that only one sample obtained the presence of a pathogenic microorganism, which is unsuitable for consumption, a microbiological quality control is of utmost importance to prevent possible degrees of contamination of such a herbal product, ensuring greater safety and quality for the consumer population.

Conclusions

The results acquired in the analysis point to errors in the management process in the manufacture of the product, even in trade fairs, where it is sold. Among the aspects of the results and necessary highlighting the great importance that the cumarú syrup, for being an industrialized product, rich in phytotherapeutic substances, must be produced in a properly hygienized environment, manipulated by people capable in the manufacture who carry out the production in a carefully correct way, with sterile materials, as well as the product already ready, must be provided with an ideal thermal conditioning, in order to avoid favorable conditions in the development of microorganisms that harm their quality.[8,9] Cumarú syrup is consumed by a large number of citizens in the city, which can compromise the health of the population.

It should be noted that this study aims to provide useful information to the local population on the possible risks of contamination of microorganisms that cumarú syrup may suffer, in the same way that it informs producers and traders of the care needed to be used both in the production process and its sale; in order to seek to guarantee the quality and the desired effects of the product, without any complication that any microorganism may cause.

Competing Interests

None.

Acknowledgements

The authors would like to thank the teacher of the Macapaense Institute of Higher Education Antonio Carlos Souza da Silva Júnior and the coordination of the Pharmacy Course, for the guidance and assistance to the research.

Founding Sources

None.

References

1. Shanley P, Medina G. F*ruit Trees and Plants Useful in Amazonian Life.* Cifor; 2018.
2. Clay JW, Sampaio PTB, Clement CR. A*mazonian Biodiversity: Examples and Strategies for Use.* Manaus: Ministry of Science and Technology, National Institute of Amazonian Research; 2000.
3. Júnior-Silva. Antimicrobial Resistance Profile of Staphylococcus aureus isolated cheese commercialized in Public Fair of the city of Macapá, AP. Hig Aliment. 2017;

31(274/275).
4. Passion FG, Olive Tree DP. Microbiological control of herbal products. Hig Aliment. 2004;18,:55-7.
5. Silva MC da. Evaluation of the microbiological quality of food using conventional methodologies and the simplate system [Thesis]. São Paulo: University of São Paulo; 2002.
6. Yamamoto CH, et al. Microbiological Quality Control of Pharmaceuticals, Cosmetics and Phytotherapic Products produced in Zona da Mata, MG, Brazil. In: Brazilian Congress of University Extension, 2, 2004, Belo Horizonte. Annals II Brazilian Congress of University Extension, Belo Horizonte; Sept.ember 2004.
7. Fabiana Golçalves P, de Oliveira DP, da Silva PB, Nascimento GGF. Controle microbiológico de produtos fitoterápicos. In 14º Congresso de Iniciação Científica; 2006.
8. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. *Clin Microbiol Rev.* 2000;13(1):16-34. doi:10.1128/CMR.13.1.16
9. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer Epidemiol Biomarkers Prev.* 2004;13(10):1558-1568.
10. Veras JF, do Carmo LS, Tong LC, et al. A study of the enterotoxigenicity of coagulase-negative and coagulase-positive staphylococcal isolates from food poisoning outbreaks in Minas Gerais, Brazil. *Int J Infect Dis.* 2008;12(4):410-415. doi:10.1016/j.ijid.2007.09.018
11. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. *Clin Microbiol Rev.* 2000;13(1):16-34. doi:10.1128/CMR.13.1.16