CYTOTOXIC AND ANTIBACTERIAL ACTIVITY OF CHICHÁ GUM HYDROGEL ASSOCIATED WITH NEROLIDOL

Idglan Sá de Lima[1], Maria Onaira Ferreira Gonçalves[1], Albert Santos Silva[1], Alessandra Braga Ribeiro[2], Humberto Medeiros Barreto[3] Josy Anteveli Osajima Furtini[1], Edson Cavalcanti da Silva Filho[1]

1 LIMAV, Interdisciplinary Laboratory for Advanced Materials, Piaui Federal University, Campus Universitário Ministro Petrônio Portela, Teresina, 64049-550 Piaui, Brazil
corresponding_edsonfilho@ufpi.edu.br
2 Catholic University of Portugal, CBQF – Centre of Biotechnology and Fine Chemistry – Associate Laboratory, Faculty of Biotechnology, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal.
3 Departamento de Parasitologia e Microbiologia, Universidade Federal do Piauí – UFPI, Teresina-PI, Brazil

Abstract Chichá gum is a polysaccharide from the *Sterculia striata* plant, with a chemical structure composed of hydroxyl groups, which favor the formation of hydrogels, enabling the association with other materials to improve their biological properties and as drug delivery systems. Hydrogels were produced from chichá gum associated with nerolidol in concentrations of 1 and 2%, cytotoxicity was evaluated against *Artemia salina*. The hydrogels were tested against the strains *Staphylococcus aureus* and *Escherichia coli*, by the direct contact test. the material showed no toxic activity and an antibacterial effect was observed with 83.6% growth inhibition with the use of the hydrogel with 2% nerolidol. It concludes that the materials produced have the potential to be used in the future as products with good antibacterial properties.
Keywords: Antimicrobial, Polymer and Toxicity

Introduction

Chichá gum is obtained from the exudate of the plant *Sterculia striata*, which is produced when the plant tissue is damaged or suffers some type of injury, in order to prevent dryness, reduce the risk of attack by microorganisms and consequently of avoid possible infections in the plant. Exudates are rich in secondary metabolites and play a fundamental role in the survival of a species, from adapting a plant to its environment, antimicrobial and, antigermicide, phytoalexins and protection against ultraviolet rays, probably due to the presence of active pharmacological substances [1].

Considering the aforementioned characteristics, Chichá gum presents itself as a promising polysaccharide for use in formulations such as hydrogels and nanoparticles, with the possibility of use in controlled drug delivery systems, in addition to the appeal for the valorization of regional raw materials [2]

Among the substances that can be incorporated in formulations based on gum of Chichá, are essential oils, as nerolidol can be found in essential oils of plants, such as pepper, kiwi, strawberry and corn [3] which have many applications in the pharmaceutical, chemical and food fields. However, their use is limited because they are very unstable, requiring their protection in order to preserve their properties [4]. These substances have antimicrobial and antioxidant activity already extensively reported in the scientific literature, with promising biomedical applications [5-6]. Thus, this work aims at the synthesis of hydrogels of Chichá associated with nerolidol at 1 and 2%, to verify the antimicrobial activity and toxicity of the products developed.

Experimental

The pure Chichá hydrogel (HC) was obtained by dispersing Chichá gum in distilled water, 1.5 g of Chichá gum to 98.5 mL of distilled water, the solution was subjected to magnetic rotation for 30 minutes.

Chicha hydrogel associated with 1% nerolidol (HCN1) was synthesized by adding 1 mL of nerolidol (NRL) to 99 mL of HC, and stirred for another 30 minutes. The same procedure was carried out to obtain the hydrogel of Chichá with 2% nerolidol (HCN2), with the proportion adjusted to 2 ml of nerolidol for every 98 ml of HC.

The toxicity test was carried out according to the methodology proposed by [7]. The eggs of *Artemia salina* were hatched in salinity water at 12 ppm and after 48 hours, the larvae were collected to perform the bioassays. Dilutions of samples and blank test were performed in seawater and 0.5 mL of dimethyl sulfoxide. Triplicate solutions of the samples to be tested in concentrations 1000, 100, 10 and 1 µg / mL were prepared, and 10 nauplii of *A. salina* per flask were added, with the survivors being counted after 24 hours.

The direct contact test in solid medium was performed according to [8]. To carry out these tests, 100µg / mL of the material to be tested and 100 µL of the standardized inoculum suspension at 1.5 x 108 colony forming units per mL (CFU / mL) were transferred to Petri dishes containing the Mueller agar medium Hinton and sown with the aid of a Drigalski loop using the spread plate method, followed by incubation at 37 ° C for 24 h. As a positive control, only the bacterial inocula were seeded on the plates. The tests were carried out in triplicate.

The inhibitory effect produced by each test solution was calculated according to the following equation:

$$\eta = \frac{N_1 - N_2}{N_1} \ x\,100\%$$

Equation (01)

Where η is defined as the inhibitory effect, N1 is the arithmetic mean of the colony-forming units of the control plates and N2 is the arithmetic mean of the colony-forming units of each of the solutions tested.

Results and Discussion

The toxicity test of pure chichá hydrogels associated with nerolidol made it possible to verify that the pure chicha hydrogel has LD_{50} >1000 µg / mL, which indicates that the hydrogel produced from the chichá gum is not toxic. Chicha hydrogels associated with nerolidol demonstrated a LD_{50} of 100 µg/mL, and were therefore considered to have moderate toxicity (Table 1).

Table 1 – Toxicity against *Artemia saline* hydrogels for samples of nerolidol (NRL), chicha hydrogel (HC), nerolidol chicha hydrogel (HCN).

Mortality (%)				
Concentration (µg/mL)				
Samples	1000	100	10	1
NRL	100	100	0	0
HC	0	0	0	0

HCN	100	100	0	0

Table 2 shows the results of the antibacterial activity for the direct contact test of the pure hickory hydrogel (HC), 1% nerolidol (NRL1), 2% nerolidol (NRL2), 1% nerolidol (HCN1) and Chichá hydrogel with 2% nerolidol (HCN2) against the species *S. aureus* and *E. coli.*

Table 2 - Antibacterial activity of HC, NRL1, NRL2, HCN1 and HCN2, against *Staphylococcus aureus* (ATCC 25.923) and *Escherichia coli (*ATCC 10536).

	Inhibitory effect (%)	
Samples	*S. aureus*	*E. coli*
NRL1	40,1 ± 4,2	19,2 ± 3,5
NRL2	56,4 ± 3,5	29,1 ± 5,3
HC	36,6 ± 7,3	32 ± 3,2
HCN1	66,6 ± 3,9	24 ± 3,1
HCN2	83,6 ± 5,5	37,4± 3,2

HC exhibited an antibacterial effect against *S. aureus* strain of 36.6%, NRL1 of 40.1% and for NRL2 an inhibitory effect of 56.4% was observed. After the combination of the materials and formation of the hydrogels of Chichá with 1% nerolidol (HCN1) and of Chichá with 2% nerolidol (HCN2), there is an increase in the inhibitory effect of these materials by 26.5% for HCN1 and 27.2% for HCN2 compared to pure nerolidol. Against *E. coli* from HC, NRL1 and NRL2, they exhibited a lesser inhibitory effect compared to S. aureus. However, after combining the materials and forming the hydrogels HCN1 and HCN2, an inhibitory effect of these materials increased by approximately 5.0% for HCN1 and -9.0% for HCN2.
The incorporation of nerolidol in the HC, improved the antibacterial activity compared to pure nerolidol and Chichá hydrogel, against the tested strains, and synergism between the properties of nerolidol and chicha hydrogel was observed.

Conclusions

The synthesis of the materials was carried out in a simple and efficient way Although the toxicity test showed a moderately toxic profile of nerolidol, the hydrogels associated with nerolidol have adequate inhibitory activity against *S. aureus*, being viable its use as an antimicrobial agent for use topic.

Acknowledgements

The authors thank CAPES, CNPq, and UFPI, for financial and/or structural support.

References

1. E. Fumagali, R. A. C. Gonçalves, M. de F. P. S. Machado, G. J. Vidoti, and A. J. B. de Oliveira, *Rev. Bras. Farmacogn.*1995, *18*,. 627–641.
2. M. O. G. Ferreira. *J. Drug Deliv. Sci. Technol.* 2019, *49*, 375–382.
3. A. M. Marques, A. L. S. Barreto, J. A. da R. Curvelo, M. T. V. Romanos, R. M. de A. Soares, and M. A. C. Kaplan. *Rev. Bras. Farmacogn.*2011, *21*, 908–914.
4. H. C. B. Paula, E. F. Oliveira, M. J. M. Carneiro, and R. C. M. De Paula. *Planta Med.* 2017, *83*, 392–397.

5. F. Bakkali, S. Averbeck, D. Averbeck, and M. Idaomar. *Food Chem. Toxicol.* 2008, *46*, 446–475.
6. S. Burt. *Int. J. Food Microbiol*. 2004, *94*, 223–253.
7. B. N. Meyer, N. R. Ferrigni, and J. E. Putnam. *Planta Med.*1982, *45*, 31–34.
8. L.-Y. Zheng and J.-F. Zhu. *Carbohydr. Polym.* 2003, *54*, 527–530.