USING BIOACTIVE COMPOUNDS TO DEVELOP AN ALTERNATIVE TO CONTROL *CANDIDA* SPP.

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1. Introduction

The *Candida* genre are responsible to caused superficial or invasive infections in the human body. [1]. During the last 20 years, the incidence of systemic infections caused by *Candida* genre increased drastically, mainly in premature babies and patients in the Intensive Care Unit [2]. *Candida* is an important infection agent due to the own medicine progress: the appearance of huge numbers of invasive procedures, braking the human natural protections, the intensive use of antibiotics with the capacity to keep alive weakened people and successful to the opportunistic microorganisms infections [2].

2. Research Methods

The research methods was divided in 10 steps. The first one was the bioprospection of microorganisms with activity against, initially, two species of *Candida*: *C. albicans* and *C. glabrata.* The microorganism bioprospected was a bacterium from an orange orchard in Astorga city. The bacterium was isolated and named as LV. With the LV, the next step was the cultivation using 5 L of Nutrient Broth with a 0,01% of CuCl2.2H2O, pH 6.8 in 82.4 ºF, the bacterium grew during 10 days. After this, the production was centrifuged and the evaluation of the supernatant activity was made. The next step was the extraction of these compounds from supernatant. With a partition balloon and an organic solvent (Dichloromethane) the supernatant compounds was extracted in a crude fraction named of FD. With the FD, the procedure of purification had start. A Column Liquid Chromatography divided FD in 13 fractions, and one of them showed activity against *C. albicans* and *C. glabrata*, the fraction F4A. In the next step of the purification was the Column Flash, which divided the F4A in more four compounds: PCA, PCN, OAC and Indolinone. The confirmation of the purification level of the compunds was made with HPLC analysis. All these compounds were tested against *C. albicans* and *C. glabrata*. The next step was Minimum Inhibitory Concentration (MIC) in a 96 wells plate. The 6th methodology part was the molecular identification with spectroscopy NMR, where PCA, PCN and OAC was defined. With these results, the 7th step was the cytotoxicity test, to evaluate the toxicity of the compounds in animal cells. After this, the next procedures was a series of tests to compare the bioactive compounds isolated with the commercial drugs. In this moment, more 2 species of *Candida* was added for the procedures: *C. krusei* and *C. dublinensis*. Another MIC was made with the four *Candida* species and one synergism test with PCA + OAC and OAC + Fluconazole (commercial drug) to try to reduce the MIC value.

3. Results

The compounds isolated from F4A was tested against *C. albicans* and *C. glabrata* that showed activity was PCA and OAC. The OAC MIC was 0,78 μg/mL for *C. albicans* and 156 μg mL^{-1} for *C. glabrata*. The PCA MIC was 50 μg mL^{-1} for *C. albicans* and 25 μg/mL for *C. glabrata*. The NMR identification showed that OAC is an Organometallic compound and PCA is a Carboxylic Phenazine. The cytotoxicity test in monkey kidney cells indicated that in a concentration of 1 μg mL^{-1} of OAC are 90% of cellular viability, showing that the metabolic is not toxic in this concentration for the animal cells. The next result was obtained by the tests of comparison between the commercial drug with the OAC. In a test of agar diffusion was possible to see that the inhibition halo formed by the OAC is two times bigger than the Fluconazole drug. Besides that, new *Candida* species was added in the procedures and a new MIC was made with *C. dublinensis, C. krusei, C. glabrata* and *C. albicans.* The OAC MIC for *C. dublinensis, C. krusei* and *C. glabrata* was 0.156 μg mL^{-1}, for *C. albicans* was 0.078 μg mL^{-1}. The synergism tests between OAC + PCA and OAC + Fluconazole showed an inefficient result, considering not interactions between the drugs.

4. Conclusions

The results show that it's possible to find an alternative from a natural source with a higher activity against the four types of *Candida* genre. In this project was possible to find isolated natural compounds with a better potential than the commercial drug Fluconazole.

5. References

[1] COLOMBO, Arnaldo Lopes; GUIMARÃES, Thaís. Epidemiologia das infecções hematogênicas por Candida spp. **Revista da Sociedade Brasileira de Medicina Tropical**, 2003.

[2] LARONE, Davise Honig; LARONE, **Medically important fungi: a guide to identification**. New York: Elsevier, 1987.