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### **Bioactive-Compounds: alternative to control Candida spp.**

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#### **ABSTRACTS**

Fungal Infections by candida is increased drastically, the research finds the possibility to find an alternative, from a natural source with a higher activity against the four types of Candida genre. In this project it was possible to find isolated natural compounds with a better potential than the commercial drug Fluconazole using HPLC and Mass Spectroscopy techniques.

**KEYWORDS-**Candida, Fungal Infections, HPLC, Mass Spectroscopy, natural compounds

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## **1. INTRODUCTION**

A fungal infection typically on the skin or mucous membranes caused by candida<sup>1</sup>. During the last 15 years, the incidence of systemic infections caused by Candida genre increased drastically, mainly in premature babies and patients in the Intensive Care Unit<sup>2</sup>. Candida is an important infection agent due to the own medicine progress: the appearance of huge numbers of invasive procedures, breaking the human natural protections, the intensive use of antibiotics with the capacity to keep alive weakened people and successful to the opportunistic microorganisms infections<sup>3</sup>.

## **2. RESEARCH METHOD**

The research methods was divided in 10 steps.<sup>1</sup> The first one was the bioprospection of microorganisms with activity against, initially, two species of Candida: *C. albicans* and *C. glabrata*. The microorganism bioprospecting was a bacterium from an orange orchard in Punjab.<sup>2</sup> The bacterium was isolated and named as A0. With the A0, the next step was the cultivation using 5 L of Nutrient Broth with a 0,01% of CuCl<sub>2</sub>.2H<sub>2</sub>O, pH 6.8 in 82.4 °F, the bacterium grew during 10 days.<sup>3</sup> After this, the production was centrifuged and the evaluation of the supernatant activity was made.<sup>4</sup> 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 FD0. With the FD0, the procedure of purification had started.<sup>4</sup> A Column Liquid Chromatography divided FD in 13 fractions, and one of them showed activity against *C. albicans* and *C. glabrata*, the fraction F4A.<sup>5</sup> In the next step of the purification was the Column Flash, which divided the F4A in more four compounds: PCA, PCN, OAC and Indolinone.<sup>6</sup> The confirmation of the purification level of the compounds 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.<sup>7</sup> With these results, the 7th step was the cytotoxicity test, to evaluate the toxicity of the compounds in animal cells.<sup>8</sup> After this, the next procedures was a series of tests to compare the bioactive compounds isolated from the commercial drugs.<sup>9</sup> In this moment, more 2 species of Candida was added for the procedures: *C. krusei* and *C. dubliniensis*.<sup>10</sup> 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<sup>-1</sup> for *C. glabrata*. The PCA MIC was 50 µg mL<sup>-1</sup> 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  $\mu\text{g mL}^{-1}$  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  $\mu\text{g mL}^{-1}$ , for *C. albicans* was 0.078  $\mu\text{g mL}^{-1}$ . 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**

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