TITLE: LMM5 as potential antifungal against *Paracoccidioides brasiliensis.*

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ABSTRACT 1:

Paracoccidioidomycosis (PCM) is a granulomatous systemic mycosis and its etiological agent is *Paracoccidiodes* spp.. Although it does not have a notifiable disease, it is admitted that 10 million people living in endemic regions have had contact with this fungus. Indeed, there are few therapeutic options for PCM treatments. The patients need the treatment for long periods and the drugs available showed high toxicity . In the search for new antifungals, in silico methods were used to optimize the identification and development of new drugs in a more specific way. Thus, the objective of this work was to evaluate the potential antifungal of LMM5 *in vitro* against *P. brasiliensis* (Pb18) at different times. The time–kill assay was performand using Pb18 (1×10^4 CFU/mL) treated with different concentrations of LMM5 8, 16 and 32 μg/mL, corresponding to 0.5xMIC, 1xMIC and 2xMIC (data previously found) for 0, 1, 3, 5, 7 and 14 days at 35°C. At each time, treated and untreated yeasts were diluted in phosphate-buffered saline (PBS), stained with trypan blue for evaluating cell viability. Additionally, Live/Dead fluorescence viable dye FUN1 (Kit LIVE/DEAD Life Technologies) with calcofluor was used. The time–kill assay showed that the lowest survival rate was at 7 days of LMM5 action with 93% dead yeasts in 2xMIC concentration. The 1xMIC and 0.5xMIC were able to kill 85 and 77% of the yeast cells, respectively. The live/dead assay showed the fungicidal ability of LMM5 at concentrations of 2xMIC, the cells show bright diffuse green staining, whereas in the concentration of MIC it is possible to visualize a reduction in the cells number in the fields and metabolic activity only in some yeast cells. Then, virtual screening of small molecules libraries provides an alternative in the search for new agents against human pathogens. In these *in vitro* assays we showed that the antifungal activity of this new compound was in low concentrations and time dependent, showing a promising candidate, a novel antifungal against *P. brasilienesis*.

Keywords: Paracoccidioidomycosis, new molecule, potential antifungal, virtual screening.

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