Title: Antifungal activity of fractions and ethanolic extract from *Cymbopogon nardus* against *Candida glabrata*

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Abstract:

Candida glabrata has emerged as the second most prevalent cause of *Candida* infections and is an important fungal pathogen in the clinical practice due to ability of development resistance to azoles. The search for new molecules presenting antifungal activity is necessary. Thus, the aim of this study was to evaluate the antifungal activity of ethanolic extract (EE) and fractions of crude extract from *Cymbopogon nardus* (L.) Rendle against *C. glabrata* species (ATCC 2001 and clinical isolate). A portion of the crude extract was separated by solid phase extraction from silica (60-200 µm) by elution with hexane/ethyl acetate (9:1, v/v), hexane/ ethyl acetate (7:3, v/v), ethyl acetate, ethyl acetate/methanol (9:1, v/v) and methanol. Ten fractions were collected and monitored by thin layer chromatography. For this study were selected the fraction 2 (F2) and fraction 3 (F3). The minimal inhibitory concentration (MIC) of EE, F2 and F3 was determined according to the protocol described by Araújo et al, with modifications. The initial concentration of EE, F2 and F3 was 1000 µg/mL. 0.1 mL was placed in a 96-well microtiter plate containing RPMI 1640 medium. Each well was inoculated with 0.1 mL of a suspension containing $2.5x10^3$ cfu/mL of yeast. Amphotericin-B and fluconazole were used as controls of the antifungal activity. The plates were incubated for 48 h at 37°C. The MIC of sample was detected following the addition of 0.02 mL 2.0% triphenyltetrazolium chloride. The results showed effective antifungal activity for EE, F2 and F3. The MIC (µg/mL) described, for ATCC and clinical isolate, are respectively: EE (500 and 125); F2: (62, 5 and 250); F3: (31, 2 and 62, 5). Fluconazole exhibited a MIC of > 64 µg/mL (ATCC and clinical isolate) and amphotericin-B with a MIC of 1, 0 µg/mL (ATCC), 2.0 µg/mL (clinical isolate). The results are promising considering the clinical importance of *C. glabrata* due to resistance to drugs. EE, F2 and F3 are interesting antifungal agents and probably the chemical constituent responsible for activity against *C. glabrata* can be present into F2 and F3. Further analysis as liquid chromatography high efficiency - mass spectrometry is necessary to collaborate with the results this study.

Key-words: *Candida glabrata*, *Cymbopogon nardus*, ethanolic extract, solid-phase extraction, antifungal activity.

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