

**Title:** *Candida tropicalis*: antifungal activity and time kill assay of essential oil and ethanolic extract from *Cymbopogon nardus*

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**Abstract:**

*Candida tropicalis* is the one of most commonly non-*albicans* species isolated, mainly, in Latina America and Asia. The incidence of candidemia for *C. tropicalis* has increased over the years. The increased isolation of *C. tropicalis* has been associated with the ability to develop resistance to fluconazole. Thus, the aim of this study was to evaluate the antifungal activity of essential oil (EO) and ethanolic extract (EE) from *Cymbopogon nardus* (L.) Rendle against *C. tropicalis* strains (ATCC 13803 and clinical isolate). The minimal inhibitory concentration (MIC) of EO and EE was determined according to the protocol described by Araújo et al, with modifications. The initial concentration of EO 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<sup>3</sup> 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 time-kill assay was performed according to Zore et al., with modifications. In brief, Sabouraud broth medium (10 mL) containing 2.5 x 10<sup>3</sup> cfu/mL of *C. tropicalis* and 2 x MIC (EO, EE) were incubated and aliquots of 0.4 mL were removed at different time intervals (30 min, and 1, 2, 4, 8, 12, 24 and 36 h), and re-suspended in Sabouraud broth medium and 100 µL were inoculated on Sabouraud agar plates. All plates were incubated at 37°C for 48 h. Amphotericin-B (32 µg/mL) was used as control. The number of colonies was counted and compared with controls. The results showed effective antifungal activity for EO with a MIC of 1000 µg/mL (ATCC and clinical isolate), and EE with a MIC of 500 µg/mL (ATCC and clinical isolate), while fluconazole and amphotericin-B with a MIC of > 64 µg/mL (resistant) and 8.0 µg/mL (resistant), respectively. The time-kill assay curve showed that EO killed 100% of the ATCC strain and clinical isolate within 24 h of exposure. EE exhibited a fungistatic profile for ATCC and clinical isolate. In conclusion, EO and EE are promising antifungal agents, however the EO show a fungicidal profile and EE exhibit a fungistatic profile.

**Key-words:** antifungal activity, *Candida tropicalis*, *Cymbopogon nardus*, essential oil, ethanolic extract.

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