

TITLE: In Vitro Interactions of Terpinen-4-ol and Nystatin on *Candida* spp.

AUTHORS: Tonon C.C.¹, Francisconi R.S.¹, Huacho P.M.M.¹, Bordini E.A.F.¹, Sardi J.C.O.², Spolidorio D.M.P.¹

INSTITUTIONS:

¹Faculdade de Odontologia de Araraquara, UNESP, Araraquara- São Paulo, BRAZIL

²Faculdade de Odontologia de Piracicaba - UNICAMP, Piracicaba - Sao Paulo, BRAZIL

ABSTRACT:

Terpinen-4-ol is a kind of main components of essential oil from *Melaleuca alternifolia*, and its antifungal action makes it a potential agent against oral candidiasis. The objectives of the investigation were to evaluate the synergistic antifungal effect, the adhesion in oral cells and the change in virulence factors of *Candida albicans* and *Candida tropicalis* after treatment with terpinen-4-ol. In the experimental methods, the minimum inhibitory concentrations (MICs) and minimum fungicide concentrations (CFMs) of terpinen-4-ol and nystatin on *Candida albicans* (ATCC 90028) and *Candida tropicalis* (ATCC4563) were determined using the microdilution broth method, along with their synergistic activity ("checkerboard" method). Mono and dual-species biofilms were prepared using the static microtiter plate model, culture medium RPMI 1640, and quantified by colony forming units (CFU/mL). The effect of Terpinen-4-ol in adhesion of *C. tropicalis* and *C. albicans* in co-culture with oral cells (keratinocytes) as well as virulence factors (proteases, phospholipases and hemolysin) were evaluated in vitro. The MIC for terpinen-4-ol was 2.31 mg/ml for both *Candida* species, and the CFM was 0.002 mg/ml and 0.004 mg/ml for *Candida albicans* and *Candida tropicalis*, respectively. When tested in combination, there was additive and synergistic effect for *Candida albicans*. In biofilm for both species only additism was observed. Terpinen-4-ol showed a decrease in the adhesion of *Candida tropicalis* in keratinocytes cells and nystatin had a greater effect for both species. For enzymatic activity, terpinen-4-ol and nystatin showed no action compared to the untreated group. Thus, in conclusion, Terpinen-4-ol has an antifungal effect against both yeasts, and in combination with nystatin, the effect of terpinen-4-ol is potentiated while reducing the adhesion of *Candida tropicalis* in vitro.

KEY WORDS: *Candida albicans*, *Candida tropicalis*, Biofilm, Terpinen-4-ol, Nystatin.

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