Combined effect of *Cinnamomum zeylanicum* Blume essential oil and miconazole against *Candida* spp

**Resumo**

Objetivo – Avaliar o efeito combinado do óleo essencial (OE) de *Cinnamomum zeylanicum* Blume e miconazol sobre cepas de *Candida* spp. Métodos – Foi determinada a concentração inibitória mínima (CIM) de ambos os produtos e o índice de concentração inibitória mínima fracionada (FIC) – checkerboard test. Os dados foram avaliados descritivamente. Resultados – Quando avaliados isolados, *C. zeylanicum* e miconazol apresentaram CIM de, respectivamente, 312.5 μg/mL e 32 μg/mL sobre todas as cepas testadas. Após a associação dos produtos, foi observado que o OE de *C. zeylanicum* inibiu o crescimento das leveduras na concentração de 39 μg/mL. Por outro lado, o miconazol, quando associado, apresentou CIM de, respectivamente, 128, 32 e 32 μg/mL, sobre cepas de *C. albicans*, *C. tropicalis* e *C. krusei*. Conclusão – A associação do óleo essencial de *C. zeylanicum* ao miconazol não constitui em uma possibilidade vantajosa para inibição de crescimento de *Candida* spp. A combinação desse OE com outros antifúngicos padrão deve ser considerada em outros estudos.

**Descritores:** *Cinnamomum zeylanicum*; Miconazol; Sinergismo farmacológico; *Candida albicans*

**Introdução**

*C. albicans* is an opportunistic pathogen that inhabits the human body as a commensal microorganism, and it is considered to be the major cause of fungal infections in humans. Usually, these infections have arisen due to the virulence of *C. albicans*, which presents considerable morphological plasticity as a result of changes in immune response.

The molecular mechanisms of virulence are mostly related to the activation of MAPK (mitogen-activated protein) Kinase signal transduction via. In this sense, cellular responses involved in invasive growth, cell wall formation, osmotic stress adaptation and reproduction occur through intracellular signaling pathways as MKc1, Cek1/2 and HOG1 MAP Kinase.

The activation of MAPK pathway also provides activation of the transcription of Cph1 factor, which is responsible for the filamentous form, considered a virulence factor for the occurrence of systemic infections, and CLA4, responsible for the formation of the germ tube and hyphae. The PKA pathway activation provides the formation of cyclic AMP, which regulates the Efg1 factor, also responsible for the hyphal formation.

In regards to superficial infections, especially those affecting the oropharynx, object of interest in this study, it is known that the mucosa of this region is the most frequent site affected by superficial candidiasis, and colonization by *C. albicans* occurs in 10-50% of healthy individuals. The drug approach to treat this type of candidiasis includes topical and systemic antifungal agents. Miconazole and nystatin have been the drugs of initial treatment. If topical therapy fails to submit results, systemic treatment is initiated, and fluconazole is therefore the most prescribed drug in such cases.

Nevertheless, the number of *Candida* species resistant to the antifungal agents available has been increasing considerably in the last years. Prolonged use of these agents may act as a risk factor for the development of fungal resistance by adaptive mutagenesis. Moreover, there has been a growing population of immunocompromised individuals and an increasingly frequent use of prophylaxis and empirical treatment with antifungals.
Given the above, natural products have been proposed in an attempt to obtain new drugs, since they differ from synthetic products as regards molecular diversity, which is much higher in natural products than in those derived from synthesis processes, that despite of considerable advances, have been still limited. This provides the development of numerous new drugs with diverse therapeutic functions. Within this context, it is highlighted the recognized antifungal activity of the essential oil from C. zeylanicum Blume.

Some studies have proposed the combination of natural products and conventional antimicrobial agents as a way to introduce new formulations in the therapeutic arsenal, capable of tackling multi-resistant microorganisms and preventing or minimizing contact of these microorganisms with synthetic products, thus reducing the risk of selecting new or improved mechanisms of resistance.

In this perspective, this study aimed to evaluate the combined effect between C. zeylanicum essential oil and miconazole against Candida strains.

Methods

Strains

Microbiological tests were performed in the Mycology Laboratory of the Center for Health Sciences, Federal University of Paraíba, which provided strains of C. albicans ATCC 40277, C. tropicalis ATCC 40042 and C. krusei ATCC 40147.

Essential Oil

The EO whose antifungal activity has been under study was obtained from Ferquima Ind. and Comp. Ltd (Vargem Grande Paulista, Sao Paulo, Brazil). Its physical and chemical parameters were described by the supplier, which produced and marketed essential oils on an industrial scale.

Considering the lipid-solubility of the essential oil, an emulsion was prepared by adding TWEEN 80 and sterile distilled water, and that mixture was stirred for five minutes in Vortex apparatus. The essential oil concentration used in the study was determined based on the product’s density (d=1.040g/mL).

Minimum Inhibitory Concentration (MIC)

The MIC determination for the essential oil and for miconazole was performed by the microdilution technique, using 96-well U-bottom microtiter plates (ALAMAR®). Initially, 100μL of Sabouraud Dextrose Broth doubly concentrated were dispensed in the wells. Then, 100μL of the emulsion of C. zeylanicum EO and miconazole were distributed at an initial concentration of 5,000μg/mL and 128μg/mL, respectively. From these concentrations, serial dilutions were conducted by withdrawing an aliquot of 100μL from the most concentrated well and inserting it into the following well. Finally, aliquots of 10μL of inoculum corresponding to the strains under test were dispensed into the wells of each column. In parallel, a yeast viability control was made. Tests were performed in triplicate, and plates were incubated at 35°C for 24-48 hours.

The reading to determine the essential oil MIC on the yeast strains was made through visual method. It was taken into consideration the formation or non-formation of cellular clusters (“button”) at the bottoms of the wells. Thus, MIC was considered as the lowest concentration of the product under test capable of producing visible inhibition on the growth of yeast strains.

In order to confirm the presence of viable microorganisms at non-inhibitory concentrations, 10μL of TTC dye (2,3,5 triphenyl tetrazolium chloride) were inserted into the wells after 24 hours of incubation. The detection of microorganisms viability reflects the activity of dehydrogenase enzymes, which are involved in the fungal respiration process. It makes possible to distinguish the live samples, red-colored, from the dead samples that keep their color.

Synergism assay – Checkerboard method

Combined effect between C. zeylanicum EO and miconazole was determined by the microdilution technique – checkerboard – for derivation of the Fractional Inhibitory Concentration index (FIC index).

Combined effect was calculated as the ratio of MIC0.5 of miconazole / MIC0.5 of EO, MIC0.5 MIC0.5 / MIC0.5 MIC0.5 and MIC0.5 MIC0.5 / MIC0.5 MIC0.5. This index was interpreted as follows: synergism (<0.5), additive (0.5-1.0), indifference (1 and <4) or antagonism (>4.0).

Results and Discussion

As seen in Table 1, C. zeylanicum EO and miconazole when assessed alone presented, respectively, MIC of 31.25μg/mL and 32μg/mL on strains of C. albicans, C. tropicalis and C. krusei. These findings confirm the data presented by other studies. The antimicrobial activity of the EO from C. zeylanicum may be related to the action of trans-cinnamaldehyde, an important compound found in large amounts in this EO composition.
the antifungal effect of the combination between products tested alone. Produced by the association, when compared with the both strains, thereby indicating indifference of the effect values. Then, it was observed a FIC value of 1.1248 for μg/mL) and unaltered miconazole MIC (32μg/mL) va-

The combined use of medicinal plants and/or their products and byproducts with the concomitant use of conventional drugs may act by inhibiting, enhancing the therapeutic effects of drugs or otherwise by interfering with the expected response24. Oliveira et al.25 emphasize that such combined use on special occasions may put the patient at risk, since it might trigger acquisition of resistance by microorganisms or might initiate mechanisms of irritation or other adverse effects. On the other hand, with respect to strains of *C. tropicalis* and *C. krusei*, there was a decrease in the EO MIC (39 μg/mL) and unaltered miconazole MIC (32μg/mL) values. Then, it was observed a FIC value of 1.1248 for both strains, thereby indicating indifference of the effect produced by the association, when compared with the products tested alone.

In the literature, no study has been found evaluating the antifungal effect of the combination between *C. zeylanicum* EO and miconazole against *Candida* spp. The findings of the present investigation warrant the completion of other studies to further investigate the association of *C. zeylanicum* with other conventional agents used in the medical and dental fields.

According to Cuenca-Estrella26, the combined antifungal compounds can promote greater effectiveness of each drug, thus allowing the use of lower doses of each product. The checkerboard method and the microbial death curve have been often used in the in vitro evaluation of combined antimicrobials activity25. This information was pointed out by Odds22, who reaffirmed the viability of the checkerboard test in the study of interactive effects between molecules.

**Conclusions**

The findings of this study indicated that the combination between *C. zeylanicum* essential oil and miconazole was not found to be an advantageous possibility for in vitro growth inhibition of *Candida* spp., since antagonist or indifferent effects were verified when compared with the potential of these products alone. Nevertheless, the combination of this essential oil with other standard antifungals should be considered in further trials in order to diminish the use/dose of synthetic agents due to their adverse effects.

**Table 1. Minimum Inhibitory Concentration (MIC) of *C. zeylanicum* essential oil and miconazole on *Candida* strains**

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC (μg/mL)</th>
<th>C. zeylanicum</th>
<th>Miconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> ATCC 40277</td>
<td>312.5</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td><em>C. tropicalis</em> ATCC 40042</td>
<td>312.5</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td><em>C. krusei</em> ATCC 40147</td>
<td>312.5</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Fractional Inhibitory Concentration Index (FIC) and MIC (μg/mL) after combination between *C. zeylanicum* essential oil and miconazole against strains of *C. albicans* ATCC 40277, *C. tropicalis* ATCC 40042 and *C. krusei* ATCC 40147**

<table>
<thead>
<tr>
<th>Strains</th>
<th>C. albicans ATCC40277</th>
<th>C. tropicalis ATCC40042</th>
<th>C. krusei ATCC40146</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. zeylanicum EO MIC</td>
<td>39 μg/mL</td>
<td>39 μg/mL</td>
<td>39 μg/mL</td>
</tr>
<tr>
<td>Miconazole</td>
<td>128 μg/mL</td>
<td>32 μg/mL</td>
<td>32 μg/mL</td>
</tr>
<tr>
<td>FIC</td>
<td>4.1248</td>
<td>1.1248</td>
<td>1.1248</td>
</tr>
<tr>
<td>Effect</td>
<td>Antagonism</td>
<td>Indifference</td>
<td>Indifference</td>
</tr>
</tbody>
</table>

The findings of the present investigation warrant the completion of other studies to further investigate the association of *C. zeylanicum* with other conventional agents used in the medical and dental fields.

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**References**


Corresponding author:
Dr. Ricardo Dias de Castro
Universidade Federal da Paraíba – Campus I – Cid. Universitária
Centro de Ciências da Saúde
Dep. Odontologia Clínica e Social
João Pessoa - PB, CEP 58059-900
Brazil
E-mail: irlan.almeida@gmail.com, ricardodiasdecastro@yahoo.com.br

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