

In vitro susceptibilities of Candida and Aspergillus species to Melaleuca alternafolia (tea tree) oil

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Summary Candida species are an important cause of opportunistic infection in the oral cavity of immunocompromised patients, especially HIV infected patients. Melaleuca oil obtained commercially was investigated since it is known to have broad antifungal properties. The in-vitro susceptibilities of Aspergillus and susceptible and resistant Candida species were performed utilizing serial dilutions in microtiter plates with Sabouraud dextrose agar and the commercial preparation of Melaleuca. As a comparator, in vitro susceptibilities to amphotericin B and fluconazole were also determined using the broth microdilution technique. The results demonstrate that Melaleuca inhibited the Candida species. However, the growth of Aspergillus was not inhibited at the concentrations tested. Thus, preparations containing Melaleuca alternafolia may be a useful alternative for superficial candidal infections. In fact, it may be a useful alternative regimen for advanced HIV-positive patients with oropharyngeal candidiasis refractory to fluconazole. However, controlled clinical studies to evaluate its efficacy are still needed.

Key words Melaleuca alternafolia, Candida, In vitro susceptibility

Susceptibilidad in vitro de Candida y Aspergillus al aceite de Melaleuca alternafolia (Tea Tree)

Resumen La candidiasis oral es una de las causas más importantes de infección en pacientes inmunocomprometidos, especialmente con VIH. Las propiedades antimicóticas del aceite de melaleuca han sido descritas. El aceite de melaleuca, obtenido comercialmente, fue investigado in vitro. Las susceptibilidades in vitro de Candida y Aspergillus fueron estudiadas usando el método de dilución serial y placas de microtítulo en agar de Sabouraud. Como comparación, las susceptibilidades de anfotericina B y fluconazol fueron determinadas simultáneamente usando la técnica del NCCLS. Los resultados demuestran que melaleuca inhibe las especies de Candida que fueron analizadas. Sin embargo, las especies de Aspergillus no fueron inhibidas. En conclusión, las preparaciones que contienen Melaleuca alternafolia posiblemente se podrían usar en infecciones superficiales por Candida. Es también posible que sea un agente alternativo en pacientes con VIH y candidiasis oral resistente a fluconazo o agentes antimicóticos. Se necesitan estudios controlados para establecer su valor clínico.
Oropharyngeal candidiasis develops in 80-95% of patients with AIDS [1,2]. The pathogenesis of this seemingly innocuous disease is very complex. Until now, Candida albicans has accounted for virtually all mucosal candidiasis. Recently, however, other species such as Candida glabrata, Candida parapsilosis, Candida tropicalis and Candida krusei have caused serious symptomatic oropharyngeal candidiasis (OPC), and on occasion it may also be associated with esophageal candidiasis. The oral azole antifungals clotrimazole, ketoconazole, fluconazole and itraconazole are frequently used in patients who are HIV-positive as initial or suppressive therapy for oropharyngeal and esophageal candidiasis. Unfortunately, the incidence of fluconazole-refractory OPC is becoming increasingly more common and frequently may emerge during therapy in advanced HIV-positive patients [3,4]. Many of these patients may suffer from frequent clinical relapses despite high doses of fluconazole and require parenteral amphotericin B. These overwhelming infections frequently impair the quality of life and may result in a reduction of fluid or food intake. In searching for newer and less toxic compounds, we have evaluated the oil of melaleuca. The oil was originally obtained from the leaves of a paperbark tea tree grown in the central coastal region of eastern Australia. Penfold initially discovered the therapeutic value in 1922, when he discovered antibacterial and antifungal properties related to Melaleuca. Several of the active ingredients of the tea tree oil include terpinen-4-ol and alpha-terpineol [5-7]. Several investigators have recently evaluated its in-vitro activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Malassezia furfur, Fusobacterium spp, Bacteroides spp, Prevotella spp, and Calbicans [6-15]. In this study, we evaluate the in-vitro activity of Melaleuca oil against Aspergillus species and known resistant Candida species that have been isolated from either advanced HIV-positive patients suffering from fluconazole and amphotericin B refractory OPC or from immunocompromised patients with disseminated fungal infections.

**MATERIALS AND METHODS**

**Fungal strains.** The organisms included clinical specimens recovered from patients with candidemia, OPC, esophageal candidiasis, or asymptomatic colonization. The distribution of species included 50 C. albicans isolates, 21 C. glabrata isolates, 10 C. tropicalis isolates, seven C. parapsilosis isolates and five isolates each of C. krusei, Candida lusitaniae, Candida kefyr, and Candida guilliermondii. The quality control strains included C. albicans ATCC 90028, C. parapsilosis ATCC 90018, and C. glabrata ATCC 9030. In addition, five isolates of Aspergillus fumigatus and five isolates of Aspergillus nidulans were also evaluated.

**In-vitro susceptibility analysis.** Amphotericin B and fluconazole were obtained from their respective manufacturers. Oil of melaleuca T36-C7 (Tea-tree oil) was obtained from Melaleuca Inc., Idaho Falls, Idaho, U.S.A. This formulation contains 36% Terpinen-4-ol and less than 10% 1,8 cineole as determined by gas liquid chromatography. The MICs of all of the antifungal agents for all of the isolates were determined in accordance with the National Committee for Clinical Laboratory Standards M27-A by a microdilution method [16]. A standard inoculum of Candida was diluted to a final concentration of 1x10^6 to 5x10^6 CFU/well in microtiter plates. As previously published by other authors, the Aspergillus inoculum was prepared by suspending Aspergillus conidia in buffered-saline. The conidia were counted by a hemocytometer and then diluted to a concentration of 10^6 conidia/ml [17]. Controls were grown on drug-free and one drug containing media.

*Candida* and *Aspergillus* species were tested against doubling dilutions of the oil of melaleuca [range, 2%-0.03 % (v/v)] as previously published by Hammer *et al.* [11,12] prepared in RPMI in a 96-well microtiter plate. Tween 80 (Sigma, St Louis, Mo.) was added at a final concentration of 0.001% (v/v).

The MICs for amphotericin B and melaleuca were defined as the lowest concentration that inhibited 100% of the visible growth. The MICs of fluconazole were defined as the lowest concentration that inhibited 80% of visible growth when compared with the growth control. The data are reported as the concentrations of each antifungal agent necessary to inhibit 50% (MIC50) and 90% (MIC90) of the isolates evaluated. All assays were done in duplicate to verify the results. Since there are no definitive MIC breakpoints that separate resistant from susceptible strains, we used an MIC of ≥ 16 mg/ml to define fluconazole resistance.

The MFC (mean fungicidal concentration) was determined by subculturing 0.1 ml from the first microtiter well demonstrating complete growth inhibition and from all wells with no visible growth onto Sabouraud dextrose agar plates that were incubated at 30°C for 72 hours. Afterwards, colonies were counted, and the MFC was defined as the lowest concentration at which 99% of the initial inoculum was killed.

**RESULTS**

Melaleuca oil had the lowest MIC50 and the lowest ranges against *C. albicans*, *C. parapsilosis* and *C. kefyr* with a range of 0.06 - 0.25 % (Table 1). The most susceptible of all of the *Candida* species is still *C. albicans*, with an MIC range of 0.06 – 0.25 %, an MIC50 of 0.12%, an MIC90 of 0.25%, and an MIC50 of 0.50% (Table 1). The *C. albicans* strains included 10 isolates for which the MIC50 of fluconazole was 32 µg/ml and the MIC90 was 64 µg/ml. The MIC50 of melaleuca for *C. albicans* isolates for which fluconazole MICs were ≥ 16 µg/ml or ≤ 8 µg/ml were the same (0.12 %) (Table 2). The second most susceptible group of yeast isolates includes the five *C. lusitaniae* and five *C. guilliermondii* that have an MIC50 of 0.25% to melaleuca, with a similar MIC range of 0.12 – 0.25%. *C. krusei* and *C. tropicalis* Table 1. In-vitro susceptibilities of Candida albicans, non-albicans Candida species and Aspergillus species to Melaleuca oil using broth microdilution assays.

<table>
<thead>
<tr>
<th>Organisms (No. tested)</th>
<th>MIC (% vol/vol)</th>
<th>MFC (%vol/vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td><em>C. albicans</em> (50)</td>
<td>0.06 – 0.25</td>
<td>0.12</td>
</tr>
<tr>
<td><em>C. glabrata</em> (21)</td>
<td>0.25 – 0.50</td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. tropicalis</em> (10)</td>
<td>0.12 – 0.50</td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> (7)</td>
<td>0.06 – 0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. kefyr</em> (5)</td>
<td>0.06 – 0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. krusei</em> (5)</td>
<td>0.12 – 0.50</td>
<td>0.5</td>
</tr>
<tr>
<td><em>C. lusitaniae</em> (5)</td>
<td>0.12 – 0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. guilliermondii</em> (5)</td>
<td>0.12 – 0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em> (5)</td>
<td>NI &gt; 2.0</td>
<td>-</td>
</tr>
<tr>
<td><em>Aspergillus nidulans</em> (5)</td>
<td>NI &gt; 2.0</td>
<td>-</td>
</tr>
</tbody>
</table>
| NI = non-inhibitory
have very similar MIC\textsubscript{50} of 0.5 and 0.25\%, respectively, and an MIC range of 0.12 – 0.5\% for both species. The least susceptible of the Candida species to melaleuca were the C. glabrata, with an MIC range of 0.25 – 0.50 \%, an MIC\textsubscript{50} of 0.25\%, and an MIC\textsubscript{90} of 0.50\% (Table 1). As was the case with C. albicans, the MIC\textsubscript{50} of melaleuca for the strains of C. glabrata for which fluconazole MICs were ≥ 16 \micro g/ml or ≤ 8 \micro g/ml were similar at 0.25\% and 0.12\% for both groups, respectively (Table 2).

Unlike the activity detected against the Candida species; the melaleuca oil had essentially no effect against any of the Aspergillus isolates we tested.

**DISCUSSION**

The results of this study confirm the excellent in\textit{vivo} efficacy of the tea tree oil Melaleuca, against the more common Candida species. Melaleuca oil (tea tree oil) is an old over the counter remedy with that possesses potent in-vitro antifungal activity against a broad spectrum of Candida species.

Melaleuca demonstrates the lowest MICs and is the most active against C. albicans, C. kefyr, and C. parapsilosis, with similar MIC\textsubscript{50} and narrow MIC ranges. Melaleuca also has similar activity against C. lusitaniae, C. guilliermondii and C. tropicalis. On the other hand, melaleuca demonstrates less activity against C. glabrata although still within the efficacy range, and not much higher than the MICs for the very susceptible strains of Candida. Moreover, the MIC and MFC results indicate that melaleuca is fungicidal for all of the Candida species evaluated, including those Candida species that were fluconazole resistant.

In addition to the broad anti-candidal activity of melaleuca oil, the most exciting observation was the remarkably good activity it demonstrated against the strains of C. albicans and C. glabrata for which fluconazole MICs were high. These putatively resistant strains were collected from patients with clinical failure to respond to high dose fluconazole (1.2 - 1.5 \text{g/day}). Essentially the same melaleuca concentration was demonstrated for both the putatively fluconazole-susceptible and fluconazole-resistant strains of C. albicans and C. glabrata. Moreover, melaleuca also demonstrated good activity with low MICs against several Candida species for which the MICs of fluconazole were high.

Unfortunately, melaleuca oil demonstrated poor in\textit{vivo} activity against the two filamentous fungi, A. fumigatus and A. nidulans.

In summary, the oil of melaleuca demonstrates great potential as a novel antifungal compound with potent in-vitro fungicidal activity against C. albicans, C. glabrata, C. tropicalis and C. parapsilosis, the four most commonly isolated species causing disseminated and mucocutaneous candidiasis in the United States [1,3]. Melaleuca compounds may be a valuable addition to the management of bacterial and fungal infections in the future [13,15,18,19]. In addition, because of its excellent in\textit{vivo} activity against azole-resistant strains of C. albicans, C. glabrata, and C. krusei including the multi-azole resistant strains which have been recovered from AIDS patients, melaleuca should be particularly useful for the management of these clinically resistant candidal infections.

Recently, we published a small prospective study evaluating a melaleuca based oral solution in patients with AIDS and fluconazole-refractory oropharyngeal candidiasis [20]. At the 4-week evaluation, eight of the 13 patients enrolled showed a significant response. Additionally, seven out of 12 patients also demonstrated a significant mycological response rate with a decrease in the colony counts of Candida species recovered during follow-up.

In addition, comparison of our data with previously published data by Hammer et al. and Concha et al. demonstrate very similar in\textit{vivo} susceptibility results [10,11]. Unfortunately, as previously stated by Hammer et al., it is difficult to compare data from different investigators since the chemical composition of the oils may be different, as well as the methodology of the studies [11].

We feel that the results of the in-vitro assays and the small clinical study are extremely promising and that further large, comparative prospective clinical studies are warranted to determine the efficacy of the melaleuca compounds for multi-drug resistant thrush. Especially in the population of HIV-positive patients with advanced disease who suffer from repeated episodes of mucosal candidiasis.