INVITRO ACTIVITIES OF MELALEUCA ALTERNIFOLIA(TEA TREE OIL) AGAINST VARIOUS ORAL CANDIDA SPECIES - A PILOT STUDY

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**Context:**
Denture stomatitis is an inflammatory reaction occurring in denture wearers and oral yeasts like Candida species were predominantly associated with this condition. This in vitro study intends to investigate the inhibitory effect of natural alternatives like Tea Tree oil (Melaleuca Alternifolia) on growth of different Candida species.

**Aims:** The aim of the current pilot study was to investigate the in-vitro activities of Melaleuca Alternifolia against various oral Candida species.

**Settings and Design:** Standard strains of five species of Candida in liophilized form were used to determine the MIC of Melaleuca Alternifolia with incubation period of 48hrs.

**Methods and Material:**
Microbiological tests were used to perform this study. A total of five oral Candida isolates (C.albicans, C.dubliniansis, C.galbrata, C.Krusei and C.tropicalis) in liophilized form were used and revived in Sabourad’s dextrose broth. Fifty tubes each having 100 µl of BHI (Brain Heart Infusion) broth were used. The concentrations of the test solutions were achieved by serial dilution method. After incubation period, by visual inspection of the tubes, the MIC values were determined. We have compared the MIC values of test solution Melaleuca Alternifolia with 0.2% fluconazole.

**Results:** The results showed that 30% Melaleuca Alternifolia exhibited antifungal activities against Candida species which were comparable to the antifungal activity of 0.2% fluconazole.

**Conclusions:** The results signify that tea tree oil has a comparable/much better anti-fungal effect than the control (0.2% fluconazole).

**Key-words:** Candida species, Denture stomatitis, Fluconazole, Melaleuca Alternifolia.

**Introduction:**
Denture stomatitis is an inflammatory reaction, occurring mostly in the palatal surface of maxilla, in denture wearing patients either partial or complete. Denture stomatitis has been strongly associated with poor hygiene and continuous denture wearing, which
facilitates denture plaque formation in which Candida albicans can be regularly isolated, suggesting a pathogenic association between bacteria and fungi.

Various antifungal agents have been proposed for the treatment of denture stomatitis but because of numerous side effects, recurrence and resistance these have been less popular. Thus, new therapeutic strategies like use of natural products can play an important role in the treatment. Among natural products, essential oils are emerging as promising therapeutic tools for oral infection.

**MATERIALS AND METHODS:**

The experiment was carried out in the Department of Prosthodontics and crown and bridge & Department of Microbiology at Maratha Mandal’s Nathajirao G. Halsekar Institute of Dental Sciences and Research center, Belgaum-590010.

Five standard strains of oral candida isolates (C.albicans, C.dubliniansis, C.galbrata, C.Krusei and C.tropicalis) in liophilised form were used and revived in Sabouraud’s dextrose broth (Fig.1, 2).

![Fig 1](image1), ![Fig 2](image2), ![Fig 3](image3)

Fifty tubes, each having 100 µl of BHI (Brain Heart Infusion) broth were used to which 100µl stock solution was added in the first MIC tube. After mixing well, 100µl solution from this tube was transferred to the second tube. This process was continued till the 10th tube. From the 10th tube which was the last tube 100µl of the final solution was discarded.

The concentrations of the test solutions achieved by this serial dilution method were as following - 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2 and 1 mcg/ml (Fig.3). Now 100µl standard isolated strains of different species of Candida (C.albicans,
C. dubliniensis, C. galbrata, C. Krusei, C. tropicalis) were added to each of the 10 such prepared MIC tubes with varying concentrations such that the final volume per tube was 200µl. These tubes were then incubated at 37°C for 24-48 hours. After incubation period, by visual inspection of the tubes, the MIC values of different candida species against control and test solutions were determined.

**Results:**

The comparisons showed that for Candida albicans the MIC value for both control and test was 4, where as for other four candida species MIC values showed wide variations, which were tabulated in (table 1 and 2).

**Table 1:** Comparison of MIC values of Test solutions on Five different Candida species

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Test solutions</th>
<th>500</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.25</th>
<th>16</th>
<th>08</th>
<th>04</th>
<th>02</th>
<th>01</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Candida albicans</strong></td>
<td>30% melaleuca alternifolia</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>0.2% Fluconazole</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><strong>Candida dubliniensis</strong></td>
<td>30% melaleuca alternifolia</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>0.2% Fluconazole</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><strong>Candida galbrata</strong></td>
<td>30% melaleuca alternifolia</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>0.2% Fluconazole</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><strong>Candida krusei</strong></td>
<td>30% melaleuca alternifolia</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>0.2% Fluconazole</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td><strong>Candida tropicalis</strong></td>
<td>30% melaleuca alternifolia</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>0.2% Fluconazole</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

**Table 1:** Comparison of MIC values of Test solutions on Five different Candida species
Table 2: Comparison of MIC values of 30% melaleuca alternifolia with 0.2% fluconazole (in mcg/ml)

<table>
<thead>
<tr>
<th>Candida species</th>
<th>30% Melaleuca alternifolia</th>
<th>0.2% Fluconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Candida dubliniansis</td>
<td>31.25</td>
<td>125</td>
</tr>
<tr>
<td>Candida galbrata</td>
<td>16</td>
<td>62.5</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>31.25</td>
<td>62.5</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>4</td>
<td>62.5</td>
</tr>
</tbody>
</table>

In the graphical representation we can appreciate that the quantity of 30% Melaleuca alternifolia used to inhibit growth of Candida isolates was less compared to the quantity of 0.2% fluconazole (fig 4).

Discussion:

Candida species are considered important opportunistic pathogens due to the increasing frequency of infections they cause in the compromised patient groups and those on cancer chemotherapy, broad spectrum antibiotics. Of the many pathogenic Candida species, C.albicans, C.galbrata, C.tropicalis and C.krusei are the most commonly found in the oral cavity. They frequently inhabit as commensals predominantly within the biofilms, which are spatially organized heterogeneous communities of fungal cells encased in the matrix of extra-cellular polymeric substances (EPS). Candida biofilms can also develop on surfaces of prosthesis and medical devices, and exhibit resistance to both anti-fungal and host defences compared
with their free-living planktonic counter parts. Melaleuca alternifolia mainly alters the permeability of candida cell, it also inhibits respiration in a dose dependent manner. Earlier studies have also shown that it inhibits formation of germ tubes or mycelial conversion in candida.\(^6\)

In our study we compared the anti-microbial activity of 30% melaleuca alternifolia (tea tree oil) and 0.2% fluconazole against five different candidal strains out of which the tea tree oil showed significant inhibition of various candidal strains at lower concentrations when compared to flucanazole.

Other authors have also observed the antifungal and fungicidal effects of \(\alpha\)–terpineol and terpinen-4-ol. Mondallo et al (2006) reported that terpinen-4-ol (main component of malaleuca alternifolia –tee tree oil) was fungstatic (MIC\(_{90}\) of 0.06% ) and fungicidal (MFC\(_{90}\) of 0.125%) against fluconazole susceptible and resistant candidal isolates. These authors suggested that this compound could be a mediator of the in vivo activity of tea tree oil in a rat model of vulvovaginal candidiasis.

(Mondello F, De Bernardis F, Girolamo A, Cassone A, Salvatore G: Invivo activity of terpenin-4-ol. The main bioactive component of melaleuca alternifolia cheel (tea tree) oil against azole-susceptible and resistant human pathogenic candida species. BMC Infect Dis 2006, 6:158.)

Our study demonstrates anti microbial activity in vitro only. However since tea tree oil is known to have immune modulating activity(Cox SG, MannCM, MarkhamJL, BellHC, GustafsonJE, WarmingtonJR, WyllieSG:the mode of antimicrobial action of essential oil of melaleuca alternifolia (tea tree oil). J ApplMicrobiol 2000,88:170-175.

Its effectiveness clinically could be much better and in vivo studies would probably demonstrates better control of infections due to synergistic actions many active substances are present in tea tree oil and these individually contribute to bioactivity observed invitro some roles of individual constituents are known whereas some still unknown.

To conclude tea tree oil with its multipotential constituents may play an important role as an adjunct in the treatment of infectious and inflammatory diseases with candidal etioly. Since our sample size is less and in vitro results cannot be
extrapolated in vivo, further investigation is needed by launching in vivo clinical trials.

**CONCLUSION:**

There is an increasing trend of resistance shown by various Candida species. So there is an increasing demand to introduce natural materials. Tea tree oil with its proven antifungal activity can be an alternative to these antifungal agents. These in vitro results cannot be extrapolated in vivo and so further research is needed by launching in vivo clinical trials to assess whether any adverse effects exists or not.

**REFERENCES:**

4. Jean Barbeau, PhD, Jacynthe Se´guin, ART, b et el. Reassessing the presence of Candida albicans in denture-related stomatitis.