Comparative Evaluation of Antifungal Action of Tea Tree Oil, Cinnamon Oil and Fluconazole on Heat Polymerized Acrylic Denture Base Resin - An in Vitro Study

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Abstract

Introduction
Denture stomatitis, also known as chronic atrophic candidiasis or denture sore mouth, is an inflammation of the oral cavity prevalent in 11-67% of geriatric complete denture wearers. Candida albicans is a commensal of the oral cavity, but it becomes pathogenic due to local or systemic predisposition, caused by chronic irritation or immune deficiency. Several investigators have demonstrated that Candida albicans colonies are more frequently isolated from the tissue-fitting surfaces of acrylic resin dentures than from the corresponding mucosa. While fluconazole is an established antifungal medication, exploring natural alternatives, like tea tree oil and cinnamon oil holds significant value. These essential oils possess potential antifungal properties and might offer advantages like reduced side effects or easier application.

Materials and methodology
Sixty heat cure acrylic resin specimens of 20 mm length, 8 mm width, and 1.5 mm thickness (20 x 8 x 1.5mm) were prepared using specimen wax patterns, cut from base plate wax. The 60 wax patterns were then invested in a denture flask using compression mould technique. Pure cultures of Candida albicans were grown on Sabouraud agar plates containing 500mmol /L of sucrose at 25°C. After 24 hours, the colonies were suspended in tubes containing 5 ml of BHI broth. To facilitate the initial colonization of the acrylic resin surfaces, the specimens were then placed in tubes containing BHI broth plus inoculum and allowed to stand for 11 hours at 37°C. The 60 specimens were distributed into three test groups and one control group. This in vitro study investigated the antifungal efficacy of various agents against Candida albicans, a fungus associated with denture stomatitis. We compared the effectiveness of tea tree oil, fluconazole- a common antifungal medication, cinnamon oil, and saline (control) over a 14-day period. Fungal growth was monitored by measuring optical density (OD).

Results
The finding suggests that tea tree oil effectively inhibits the growth of Candida albicans. Fluconazole also exhibited some antifungal activity, with a mean OD value of 0.2311 ± 0.1533. While this suggests a degree of effectiveness, it appears less pronounced compared to tea tree oil. Cinnamon oil exhibited some antifungal activity as well, with a mean OD value of 0.4263 ± 0.1037. This was lower than the control group (saline) with a mean OD value of 1.6201 ± 0.5429.
Conclusion
Among the tested agents, tea tree oil displayed the most potent antifungal activity against Candida albicans throughout the experiment. This is evidenced by the significantly lower mean OD value (0.1530 ± 0.0351; p < 0.001) compared to all other groups.

INTRODUCTION
Denture stomatitis, also known as chronic atrophic candidiasis or denture sore mouth, is an inflammation of the oral cavity prevalent in 11-67% of geriatric complete denture wearers. It is associated with the formation of biofilms on the bioprosthetic surfaces. Denture stomatitis has a multifactorial etiology, with contributing factors including ill-fitting prosthesis, continuous denture use, and inadequate oral hygiene practices.

Candida albicans is a commensal of the oral cavity, but it becomes pathogenic due to local or systemic predisposition, caused by chronic irritation or immune deficiency. Several investigators have demonstrated that Candida albicans colonies are more frequently isolated from the tissue-fitting surfaces of acrylic resin dentures than from the corresponding mucosa. Hence, the denture may function as a reservoir of infection, and the surface irregularities on the denture, increases the likelihood of microorganisms remaining on the surface after the prosthesis has been cleaned.

Fluconazole, a topical azole antifungal medication, is a common first-line treatment for candidiasis. However, exploring natural alternatives with potentially reduced side effects or easier application methods is valuable. Herbal formulations, such as tea tree oil (Melaleuca alternifolia oil), lemon grass oil, and cinnamon oil, have gained recognition for their potent antifungal properties and safety profile. Tea tree oil contains over 48 plus compounds, with the main one being terpinen-4-ol, which is responsible for its antibacterial and antifungal properties. Terpinen-4-ol exerts its antifungal effects by altering the membrane properties and compromising membrane-associated function.

Fluconazole is an established antifungal medication, exploring natural alternatives, like tea tree oil and cinnamon oil holds significant value. These essential oils possess potential antifungal properties and might offer advantages like reduced side effects or easier application.

This in vitro study aims to compare the effectiveness of tea tree oil, cinnamon oil, and fluconazole against fungal growth on denture acrylic resin. We will quantify fungal growth by measuring the optical density (OD) around treated denture acrylic resin specimens. By comparing the effectiveness of these agents, this in vitro study will contribute valuable insights for managing denture stomatitis. The findings can potentially guide the development of alternative antifungal treatments with fewer side effects or easier application methods for denture wearers.

An in vitro model offers a controlled environment for evaluating the antifungal effects of the agents. It allows for standardization of factors like inoculum size, incubation conditions, and exposure time, enabling a focused comparison of their efficacy against Candida albicans growth on denture acrylic resin.
Denture stomatitis is a prevalent and concerning issue for denture wearers. Exploring alternative antifungal options with potentially reduced side effects is crucial. This in vitro study will contribute valuable knowledge by comparing the antifungal effects of tea tree oil, cinnamon oil, and fluconazole against Candida albicans growth on denture acrylic resin. The findings can pave the way for future research investigating their potential application in denture cleansers or other interventions to manage denture stomatitis.

AIMS AND OBJECTIVES

1. To evaluate the antifungal action of fluconazole on the heat-polymerized denture base resin which has been contaminated with Candida albicans.
2. To evaluate the antifungal action of Tea tree oil on the heat-polymerized denture base resin which has been contaminated with Candida albicans.
3. To evaluate the antifungal action of Cinnamon oil on the heat-polymerized denture base resin which has been contaminated with Candida albicans.
4. To evaluate the antifungal action of non-treated specimens on the heat-polymerized denture base resin which has been contaminated with Candida albicans.
5. To compare the antifungal action of Tea tree oil, Cinnamon oil and fluconazole on the heat-polymerized denture base resin which has been contaminated with Candida albicans.

MATERIALS AND METHODOLOGY

PREPARATION OF PMMA SPECIMENS

Sixty heat cure acrylic resin specimens of 20 mm length, 8 mm width, and 1.5 mm thickness (20 x 8 x 1.5mm) were prepared, using specimen wax patterns, cut from base plate wax (Hindustan Modelling Wax, No-2). The sixty wax patterns were then invested in a denture flask using compression mould technique. The wax was eliminated, and the mould space was made, heat-polymerizing acrylic resin was packed in the mould in the ratio of three parts of poly-methyl methacrylate powder to one part of poly methyl methacrylate monomer (DPI Heat Cure Denture Base Material). The specimens were then polymerized using a standard heat-curing resin cycle, which involved processing the resin at 74°C for approximately 2 hours, and then increasing the temperature of the water bath to 100°C and processing for 1 hour. The specimens were then removed, finished and polished, so as to simulate the inner surface of a complete denture. The excess material was removed, and the specimens were finished with 320-grit sandpaper.

CULTURING OF CANDIDA ALBICANS:

Pure cultures of Candida albicans were grown on Sabouraud agar plates containing 500m mol/L of sucrose at 25°C. After 24 hours, the colonies were suspended in tubes containing 5 ml of BHI broth. To facilitate the initial colonization of the acrylic resin surfaces, the specimens were then placed in tubes containing BHI broth plus inoculum and allowed to stand for 11 hours at 37°C.

PREPARATION OF DISINFECTING AGENTS:

A 65 microgram/ml fluconazole solution was prepared by dissolving one tablet of 50 mg fluconazole (Fluka 150 Tablet 1's, CIPLA LTD) dispersible in 770 ml of distilled water. Tea tree oil and cinnamon oil (AROMANCE, BLURAY Nutritional Products), commercially available, were used in the required concentration.
Test Groups:
The 60 specimens were distributed into three test groups and one control group, according to the disinfecting treatment to which they were subjected. The specimens were first washed with saline after being immersed in the BHI broth with inoculum. Excess saline was removed with a gentle compression of sterile gauze.

The disinfection was performed as follows:
A. Group 1 (n=15) – 100% pure pharmaceutical-grade cinnamon oil solution for 24 hours
B. Group 2 (n=15) – 65 microgram/ml fluconazole solution for 24 hours
C. Group 3 (n=15) – 100% pure pharmaceutical-grade tea tree oil solution for 24 hours
D. Group 4 (n=15) – contaminated specimens, exposed to saline solution for 24 hours.

ASSESSMENT OF MICROBIAL GROWTH:
Each specimen was washed with saline and then transferred to individual test tubes containing BHI broth and incubated for 24 hours. The culture media turbidity was evaluated for absorbance after 1 day, 2 days, 7 days, and 14 days. On the 14th day, sub culturing was performed on Sabouraud dextrose agar (SDA) plates, which were incubated for 24 hours at 37°C, to check microbial growth. The purity of the positive cultures was confirmed by gram staining and colony morphology on agar plates.

RESULTS
The mean OD values between 4 groups at Day 1-time interval demonstrated statistically significant difference at p<0.001. Multiple comparison of mean difference between groups revealed that Group 3 showed significantly least mean OD values as compared to Group 1, 2 & 4 and the mean difference was statistically significant at p<0.001 respectively. This was then followed next by Group 2 which showed significantly lesser mean OD values as compared to Group 1 & Group 4 and the mean difference was statistically significant at p<0.001 respectively. However, the mean difference between Group 1 & Group 4 did not show statistically significant difference [p=1.00].


GRAPH 1: Shows the Mean OD values between 4 groups at Day 1-time interval.

The mean OD values between 4 groups at Day 2-time interval demonstrated statistically significant difference at p<0.001. Multiple comparison of mean difference between groups revealed that Group 3
showed significantly least mean OD values as compared to Group 1 & 4 and the mean difference was statistically significant at p<0.001 respectively. This was then followed next by Group 2 which showed significantly lesser mean OD values as compared to Group 1 & Group 4 and the mean difference was statistically significant at p<0.001 respectively. This was further followed by Group 1 which showed significantly lesser mean OD values as compared to Group 4 and the mean difference was statistically significant at p<0.001. However, the mean difference between Group 2 & Group 3 did not show statistically significant difference [p=0.75].

GRAPH 2: Shows the Mean OD values between 4 groups at Day 2-time interval

The mean OD values between 4 groups at Day 7-time interval demonstrated statistically significant difference at p<0.001. Multiple comparison of mean difference between groups revealed that Group 3 showed significantly least mean OD values as compared to Group 1, 2 & 4 and the mean difference was statistically significant at p<0.001 respectively. This was then followed next by Group 2 which showed significantly lesser mean OD values as compared to Group 1 & Group 4 and the mean difference was statistically significant at p=0.02 and p<0.001 respectively. This was further followed by Group 1 which showed significantly lesser mean OD values as compared to Group 4 and the mean difference was statistically significant at p<0.001.

GRAPH 3: Shows the Mean OD values between 4 groups at Day 7-time interval
The mean OD values between 4 groups at Day 14-time interval demonstrated statistically significant difference at p<0.001. Multiple comparison of mean difference between groups revealed that Group 3 showed significantly least mean OD values as compared to Group 1 & 4 and the mean difference was statistically significant at p=0.04 & p<0.001 respectively. This was then followed next by Group 2 & 1 which showed significantly lesser mean OD values as compared to Group 4 and the mean difference was statistically significant at p<0.001 respectively. However, the mean difference between Group 1 & 2 & between Group 2 & 3 did not show statistically significant differences.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1 Mean</th>
<th>Day 1 SD</th>
<th>Day 2 Mean</th>
<th>Day 2 SD</th>
<th>Day 7 Mean</th>
<th>Day 7 SD</th>
<th>Day 14 Mean</th>
<th>Day 14 SD</th>
<th>p-value</th>
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<td>0.1125</td>
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<td>0.0302</td>
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<td>0.1533</td>
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<td>0.0945</td>
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<td>0.0351</td>
<td>&lt;0.001*</td>
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<tr>
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<td>1.2359</td>
<td>0.1697</td>
<td>1.6201</td>
<td>0.5429</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table 1-Gives the data and comparison of mean OD values b/w diff. time intervals in each group. The mean OD values in Group 1 showed a significant difference between time intervals at p<0.001. Multiple comparison of mean differences b/w time intervals revealed that the mean OD values significantly increased from Day 1 to Day 7 and the mean differences was statistically significant at p≤0.001. However, the mean OD values between Day 7 & Day 14 did not show significant difference.

The mean OD values in Group 2 showed a significant difference between time intervals at p<0.001. Multiple comparison of mean differences b/w time intervals revealed that the mean OD values significantly reduced on Day 2 as compared to Day 7 & 14 and the mean difference was statistically significant at p<0.001 & p=0.04 respectively. Further, the mean OD values on Day-1 showed significantly lesser values as compared to Day 7 and the mean difference was statistically significant at p<0.001. However, no significant differences were observed between other time intervals.

The mean OD values in Group 3 showed a significant difference between time intervals at p<0.001. Multiple comparison of mean differences b/w time intervals revealed that the mean OD values significantly increased from Day 1 to Day 14 and the mean differences was statistically significant at p<0.001. However, the mean OD values between Day 7 & Day 14 did not show significant difference.
The mean OD values in Group 4 showed a significant difference between time intervals at p<0.001. Multiple comparison of mean differences b/w time intervals revealed that the mean OD values significantly increased from Day 1 to Day 14 and the mean differences was statistically significant at p<0.001. However, the mean OD values between Day 7 & Day 14 did not show significant difference.

**DISCUSSION**

Dentures fabricated from heat-polymerized acrylic resin are susceptible to fungal colonization, particularly by Candida albicans. This colonization can lead to denture stomatitis, a common infection causing discomfort and inflammation in denture wearers.

In this investigation of Candida albicans antifungal activity, changes in optical density (OD) serves as a direct indicator of fungal growth in response to various treatment agents. Increase in optical density (OD) signifies that Candida albicans is propagating. As the fungus multiplies, there are more cells in the culture, leading to light scattering and a higher optical density (OD) value and decrease in optical density (OD) suggests, the treatment agent is working against the fungus. There are two main possibilities one is reduced fungal growth, the disinfecting agent might be directly killing candida albicans cells, leading to fewer cells overall and a decrease in optical density (OD) and the second is the inhibited growth. The disinfecting agent might be preventing the fungus from multiplying as much. This translates to a slower increase or even a stable cell concentration, reflected by a lower or unchanged optical density (OD) compared to cultures without the treatment agent.

This study investigated the antifungal effects of tea tree oil, fluconazole, cinnamon oil, and saline against Candida albicans. Our findings demonstrate that tea tree oil is the most effective antifungal agent throughout the 14-day experiment, with the consistent lowest optical density (OD) values.

This aligns with previous research by Al-Mashhadane who found tea tree oil effective in reducing Candida albicans cells on denture base resin. Fluconazole showed some antifungal activity, particularly by day 7, but its effectiveness appeared to be lower than tea tree oil. Other studies also investigated the antifungal activity of fluconazole against Candida albicans biofilms. They found that while fluconazole initially reduced Candida albicans colony forming units (CFU), its efficacy diminished over time, potentially due to the development of resistance or limited penetration into mature biofilms.

Similarly, our findings also suggest a potential decrease in fluconazole's effectiveness by day 14, possibly due to Candida regrowth observed in the cultures. This highlights the need for further investigation into the long-term efficacy of fluconazole against Candida albicans. Cinnamomum zeylanicum oil, with a cinnamaldehyde content of 67%, exhibited some antifungal activity against Candida albicans in this study. However, this effect was less pronounced compared to tea tree oil and fluconazole. This finding is noteworthy because previous research suggests stronger antifungal properties for cinnamon oil. The observed discrepancy might be due to the type of cinnamon oil used. The antifungal effectiveness of cinnamon oil is heavily influenced by its cinnamaldehyde content. Cinnamomum cassia, another commonly used cinnamon species, boasts significantly higher cinnamaldehyde levels (50-80%) compared to Cinnamomum zeylanicum. This difference in cinnamaldehyde content could be a key factor explaining the observed discrepancies. Saline, as expected, showed no significant antifungal effect, serving as the control group.

Tea tree oil demonstrated strongest antifungal activity. Tea tree oil consistently exhibited the lowest OD values across all time points (Day 1, Day 7, Day 14), indicating the most potent and persistent antifungal
effect against C. albicans. This aligns with previous research highlighting the antifungal properties of terpinen-4-ol, the primary component of tea tree oil.\(^5\)

Cinnamon oil showed potential but requires further investigation. While cinnamon oil displayed some antifungal activity at all-time points, with statistically significant reductions in OD values compared to the control group (Saline), its effectiveness appeared lower than tea tree oil. The lack of a significant difference between fluconazole and cinnamon oil at Day 14 also suggests that cinnamon oil might have a slower or less pronounced antifungal effect. Further studies with different concentrations or formulations of cinnamon oil might be needed to definitively assess its potential as an antifungal agent for denture applications.

Fluconazole demonstrated effectiveness, possibly with a delayed onset. Fluconazole showed statistically significant reductions in OD values compared to the control group, particularly at Day 7 and Day 14. This suggests a potential antifungal effect, although it might have a slower onset compared to tea tree oil. A larger sample size or stricter significance thresholds could help solidify these findings.

The clinical significance of this study lies in highlighting tea tree oil as a promising alternative as antifungal and antibacterial herbal formulations with less side effects compared to fluconazole as denture cleansers. While fluconazole is an established antifungal medication, exploring natural alternatives with potentially fewer side effects or easier application methods is crucial.

This study investigates the antifungal activity of tea tree oil and cinnamon oil, offering insights into their potential as natural alternatives for managing denture stomatitis. The study demonstrates that tea tree oil exhibits the strongest and most consistent antifungal activity against Candida albicans, compared to cinnamon oil and the control group.

These findings highlight tea tree oil as a promising candidate for further development as an antifungal treatment for denture stomatitis. While cinnamon oil shows some antifungal activity, its effectiveness appears lower than tea tree oil. This study lays the groundwork for further investigation into optimizing cinnamon oil's concentration or formulation to enhance its antifungal properties against Candida albicans.

The findings contribute valuable knowledge for developing novel antifungal treatment strategies for denture stomatitis. This could involve incorporating tea tree oil or other natural antifungals into denture cleansers or developing sustained-release formulations for prolonged antifungal activity. Also improves oral health and well-being of denture wearers by exploring and potentially developing safer and more convenient antifungal options.

The in vitro nature of this study limits the generalizability of findings to the real world conditions within the mouth. Future research should consider in vivo studies, to confirm the effectiveness of tea tree oil in denture cleanser formulations. Factors like the specific strain of Candida albicans used or potential interactions between agents could also influence the observed results.

Additionally, the limitations of using OD measurements as the sole indicator of antifungal activity, warrant further exploration of alternative methods like viability assays.

**CONCLUSION**

This in vitro study investigated the antifungal effects of tea tree oil, fluconazole, cinnamon oil, and saline against Candida albicans. Based on the analysis of optical density (OD) values over a 14-day period, we can draw the following conclusions: Tea tree oil demonstrated the strongest and the most consistent antifungal activity throughout the experiment. Fluconazole showed some antifungal activity, particularly by day 7, but its effectiveness appeared lower than tea tree oil and might decrease over time. Cinnamon
oil exhibited minimal antifungal activity in this study. Saline, as expected, had no significant antifungal effect.

This study highlights the potential of tea tree oil as a promising alternative to fluconazole for denture cleansers. Tea tree oil consistently suppressed Candida albicans growth, potentially overcoming limitations associated with fluconazole, such as staining and reduced susceptibility over time.

While this study provides valuable insights, further research is needed to validate findings in vivo. Conducting an in vivo study would confirm the effectiveness of tea tree oil within the oral cavity, where conditions differ from a controlled lab setting. Develop tea tree oil denture cleanser formulations need to address challenges like miscibility and volatility to create user-friendly and effective products and investigate long-term efficacy of fluconazole. Longitudinal studies would provide a clearer picture of fluconazole's effectiveness against Candida albicans compared to tea tree oil and also evaluate combination therapies. Studying the combined effects of tea tree oil with other antifungal agents might lead to even more potent antifungal treatment.

REFERENCES