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The Effect of Tea Tree Oil *(Melaleuca alternifolia)* on Candida albicans Counts on Heat-Cured Acrylic Resin and 3D-Printed Acrylic Denture Base Materials

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ABSTRACT

Suboptimal cleaning of dentures can increase the overgrowth of Candida albicans, which can cause denture stomatitis. Therefore, it is important to maintain denture hygiene with effective and safe cleaning agents. One natural ingredient that has the potential to be used as a denture cleaner is Tea Tree Oil (Melaleuca alternifolia), which is known to have antifungal activity. This study aims to compare the effectiveness of Tea Tree Oil immersion at concentrations of 0.25%, 0.5%, and 1% against 0.5% sodium hypochlorite in inhibiting the growth of Candida albicans on heat-cured acrylic resin plates and 3D-printed acrylic resin. This study is an experimental laboratory study with a post-test only control group design. The research population consisted of pure culture isolates of Candida albicans ATCC 10231. The research samples consisted of heat-polymerized acrylic resin plates and 3D-printed acrylic resin plates measuring 10×10×2 mm, with 5 samples in each treatment group that were immersed for 8 hours. The number of Candida albicans colonies was counted using the plate count method and calculated with a colony counter. The data were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney test. The results showed that there was a difference in the effectiveness of Tea Tree Oil immersion at various concentrations compared to 0.5% sodium hypochlorite and distilled water against the growth of Candida albicans. Tea Tree Oil concentrations.

Keywords: tea tree oil, candida albicans, acrylic resin, 3D printed, denture cleanser

INTRODUCTION

Teeth play a crucial role in human life, not only in mastication but also in supporting aesthetics and phonetics. Tooth loss can occur due to various factors such as dental caries, periodontal disease, trauma, and the natural aging process. This condition has a significant impact on quality of life, both physiologically and psychosocially. To restore oral function, many individuals choose dentures as a rehabilitative solution. Removable dentures are a common option because they are non-invasive, easy to remove, and adjustable. In the fabrication of removable dentures, the most widely used material is acrylic resin based on polymethyl methacrylate (PMMA). This material is preferred due to its high biocompatibility, favorable physical properties, and relatively low cost [1], [2].

Acrylic resin for denture bases is classified according to its polymerization method, two of which are heat-cured acrylic resin and acrylic resin produced using 3D printing technology. Heat-cured acrylic resin has long been used in clinical practice and is known for its good dimensional stability, high transparency, low toxicity, and ease of repair [1], [3]. However, this material also has several disadvantages, including relatively low mechanical strength, a tendency to fracture, and a high water-absorption capacity that facilitates microbial colonization, particularly by Candida albicans [4].

Advances in manufacturing technology in dentistry have driven the development of 3D-printed acrylic resin as a material for denture bases. This technology enables the fabrication of prostheses with high precision, improved production efficiency, and design flexibility that cannot be achieved using conventional methods. However, despite its promising potential, research on the resistance of 3D-printed acrylic resin to fungal infection remains limited. Several preliminary studies have indicated that the surface characteristics of 3D-printed resin may influence microbial colonization, including Candida albicans. Two

3D-printed resins (NextDent Denture 3D+ and Cosmos Denture) demonstrated higher levels of C. albicans colonization compared to the heat-cured acrylic resin control (Lucitone 550), despite relatively small differences in surface roughness [5]. Consistently, it was found that the 3D-printing fabrication method significantly increased C. albicans adhesion compared with heat-cured methods, whereas milling (CAD-CAM) fabrication resulted in the lowest adhesion [6]. A recent study [7] reported that 3D-printed resin exhibited the highest proportion of biofilm formation after 24 hours of incubation compared with conventional and milled resins.

According to the ISO polymer classification, heat-cured acrylic resin is categorized as a thermoset polymer, which undergoes a permanent chemical transformation during the heating process. In contrast, 3D-printed acrylic resins are generally based on photopolymerization, consisting of reactive polymers that harden upon exposure to ultraviolet light or laser irradiation. These chemical differences may influence the material's surface resistance to microbial accumulation. Several publications have suggested that surface structure and photopolymer characteristics may either enhance or reduce the tendency for fungal biofilm formation.

In modern prosthodontics, various 3D-printing techniques such as stereolithography (SLA), digital light processing (DLP), continuous direct light processing (CDLP), and direct UV printing (DUP) are increasingly utilized [8]. Newer generations of 3D-printed acrylic resins even incorporate hybrid ceramic–resin components, which have been reported to exhibit superior mechanical strength and greater resistance to Candida albicans colonization compared with conventional acrylic resins [9]. Additionally, the high-speed vat photopolymerization (HVP) method enables the production of denture bases with smoother surface quality and shorter processing times [10]. Nonetheless, conclusions regarding the microbiological resistance of these materials remain inconsistent and warrant more targeted comparative research.

Denture hygiene is a key factor in preventing the formation of microbial biofilms. Various cleansing agents have been used, including chemical disinfectants such as sodium hypochlorite. Sodium hypochlorite at a concentration of 0.5% is effective as a disinfecting agent because it can inactivate various microorganisms, including Candida albicans, through mechanisms involving cell wall destruction and protein denaturation. However, long-term use, particularly at high concentrations, may lead to discoloration, surface degradation, and reduced strength of acrylic resin materials [11]. These concerns underscore the need for safer yet still effective disinfectant alternatives, especially for long-term denture wearers.

One alternative that has gained increasing research attention is the use of natural disinfectants, including Tea Tree Oil (TTO), derived from Melaleuca alternifolia. TTO contains active compounds such as terpinen-4-ol, α-terpineol, and 1,8-cineole, which are known for their strong antimicrobial and antifungal properties. Several studies have demonstrated that TTO can inhibit the growth of Candida albicans with effectiveness comparable to, or even greater than, certain chemical disinfectants [12]. In heat-cured acrylic resin, a TTO concentration of 0.25% has been shown to significantly inhibit fungal activity. Nevertheless, information regarding the effectiveness of TTO on 3D-printed acrylic resin remains very limited.

Fungal infections in denture wearers, particularly those caused by Candida albicans, are a major contributor to denture stomatitis, an inflammatory condition of the mucosa beneath the denture base. This condition commonly arises from inadequate denture hygiene, reduced salivary flow, and the ability of C. albicans to form biofilms on acrylic resin surfaces. These biofilms enhance the fungus's adhesive capacity and increase its resistance to antimicrobial agents, thereby slowing the healing process and elevating the risk of recurrence. Studies on acrylic resin surfaces have shown that material porosity and roughness play significant roles in facilitating C. albicans adhesion and proliferation, placing denture wearers whose prosthetic materials exhibit greater micro-retentive properties at higher risk of recurrent infections. These findings underscore the importance of proper denture hygiene and the need to evaluate prosthetic materials with improved resistance to fungal biofilm formation [13], [14].

Several studies have evaluated the incorporation or use of TTO as a disinfecting agent on heat-cured acrylic resins and have shown promising results [15]. However, to date, investigations that directly compare the effectiveness of TTO in inhibiting Candida albicans growth on two different types of denture base materials heat-cured acrylic resin and 3D-printed acrylic resin remain very limited [12], [16]. Moreover, no studies have comprehensively examined the influence of varying TTO concentrations on C. albicans counts or compared biofilm formation between these two resin types.

The limitations of previous studies underscore the need for further investigation. The novelty of the present study lies in the direct comparison of two types of acrylic resins with distinct polymer characteristics thermoset heat-cured resin and photopolymer-based 3D-printed resin as well as the evaluation of the effects of varying concentrations of TTO on the growth of Candida albicans in each material. This approach has not been widely explored and has the potential to generate new recommendations regarding natural disinfectants that are safe for denture base materials.

Therefore, this study aims to analyze the effects of various concentrations of Tea Tree Oil on the number of Candida albicans present on heat-cured acrylic resin and 3D-printed acrylic resin, as well as to compare the effectiveness of these materials in preventing fungal biofilm formation. The findings are expected to contribute scientifically to the development of safer, more effective, and material-friendly denture cleansing methods, and to serve as a basis for clinical recommendations in the prevention of denture stomatitis.

RESEARCH METHODS

This study is laboratory-based experimental research using a post-test only control group design to evaluate the effects of Tea Tree Oil (TTO) on the number of Candida albicans present on two types of denture base materials: heat-cured acrylic resin and 3D-printed acrylic resin. The study was conducted at the Dental Testing Laboratory, Faculty of Dentistry, Universitas Sumatera Utara (USU), and the Microbiology Laboratory, Faculty of Medicine, USU, during September–October 2025. Samples were fabricated following ISO 18416:2015 standards and ADA Specification No. 12, with dimensions of 10 × 10 × 2 mm, consisting of heat-cured acrylic resin and 3D-printed acrylic resin specimens. A total of eight treatment groups were used, including TTO at concentrations of 0.25%, 0.5%, and 1%, as well as a positive control of 0.5% NaOCl for both material types, with five specimens in each group (totaling 40 samples).

The heat-cured acrylic resin samples were produced using a conventional curing process, whereas the 3D-printed resin samples were fabricated using a digital design (.STL) and DLP/SLA technology with DENTCA Denture Base material. Both types of samples underwent sterilization, followed by immersion in artificial saliva for 24 hours, and were subsequently contaminated in a suspension of Candida albicans ATCC 10231 adjusted to the McFarland standard of 1.5×10^8 CFU/mL. After 24 hours of incubation at 37°C, the samples were immersed in the treatment solutions for 15 minutes.

The fungal cell count was determined using dilution and inoculation methods on SDA media. Following vortexing in physiological saline, the suspension was diluted to 10^{-2} and inoculated onto SDA plates, then incubated for 24 hours at 37°C to quantify the resulting colonies. Statistical analysis was performed using SPSS version 24. The Shapiro–Wilk and Levene tests were used to assess data normality and homogeneity. Differences among groups were analyzed using the Kruskal–Wallis test, followed by the Mann–Whitney test when significant differences were identified.

RESULT AND DISCUSSION

The analysis revealed differences in the response of Candida albicans to Tea Tree Oil (TTO) treatment across the two denture base materials, namely heat-cured acrylic resin and 3D-printed acrylic resin. Variations in TTO concentration produced differing colony counts for each material, and the 0.5% NaOCl positive control was used as a benchmark to confirm antifungal effectiveness. The colony count data and statistical test results are presented to demonstrate significant differences among treatments and to compare the effectiveness of TTO between the two resin types.

Table 1. Mean Colony Count of Candida albicans

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Material Groups		NaOCl 0.5	k 1	k 0.5	k 0.25
3D	Mean	9.80	621.40	746.60	1004.40
	N	5	5	5	5
	Std. Deviation	2.588	26.340	26.111	79.714
RAPP	Mean	1.00	9.40	58.60	200.20
	N	5	5	5	5
	Std. Deviation	1.225	3.847	13.686	31.933

The results showed that the mean colony counts of Candida albicans in the 3D-printed resin groups treated with Tea Tree Oil (TTO) at concentrations of 1%, 0.5%, and 0.25% were 621.40 ± 26.340, 746.60 ± 26.111, and 1004.40 ± 79.714, respectively. In the heat-cured acrylic resin groups, the mean colony counts for TTO concentrations of 1%, 0.5%, and 0.25% were 9.40 ± 3.847, 58.60 ± 13.686, and 200.20 ± 31.933, respectively. Meanwhile, in the 0.5% sodium hypochlorite control group, the colony count was 9.80 ± 2.588 for the 3D-printed resin and 1.00 ± 1.225 for the heat-cured resin. These differences in mean values indicate varying levels of effectiveness among the materials in inhibiting the growth of Candida albicans. Subsequently, an effectiveness test was performed to evaluate the antifungal activity of Tea Tree Oil as a denture cleanser against Candida albicans on heat-cured acrylic resin plates.

Table 2. Effectiveness of Tea Tree Oil Immersion as a Denture Cleanser Against the Growth of Candida albicans on Heat-Cured Acrylic Resin Plates

Group	Mean	Std. Deviation
TTO 0,25%	200.20	31.933
TTO 0,5%	58.60	13.686
TTO 1%	9.40	3.847
NaOCL 0,5%	1.00	1.225

The table demonstrates the effectiveness of various concentrations of Tea Tree Oil (TTO) on the colony count of Candida albicans grown on heat-cured acrylic resin. The data show that higher TTO concentrations correspond to lower colony counts. TTO at 0.25% produced the highest number of colonies (mean 200.20 CFU), indicating the lowest inhibitory effect. At a concentration of 0.5%, the colony count decreased markedly to 58.60 CFU, while TTO at 1% exhibited the strongest antifungal activity with a mean of only 9.40 CFU. As a comparison, 0.5% NaOCl, used as the positive control, demonstrated the highest level of effectiveness with the lowest colony count (mean 1.00 CFU). These findings indicate that although TTO is capable of reducing Candida albicans growth particularly at the 1% concentration its effectiveness remains lower than that of NaOCl. The observed reduction pattern confirms that the antifungal response of TTO is concentration-dependent. Subsequently, an effectiveness test of 0.25% Tea Tree Oil immersion as a denture cleanser against Candida albicans on 3D-printed resin plates was conducted. The results are presented in Table 3 below.

Table 3. Effectiveness of Tea Tree Oil Immersion as a Denture Cleanser Against the Growth of Candida albicans on 3D-Printed Acrylic Resin Plates

Group	Mean	Std. Deviation
TTO 0,25%	1004.40	79.714
TTO 0,5%	746.60	26.111
TTO 1%	621.40	26.340
NaOCL 0,5%	9.80	2.588

The table illustrates the number of Candida albicans colonies after immersion of 3D-printed resin plates in various concentrations of Tea Tree Oil (TTO). The results show that all TTO treatment groups produced relatively high colony counts. The 0.25% TTO concentration yielded the highest colony count (mean 1004.40 CFU), followed by 0.5% TTO (746.60 CFU) and 1% TTO (621.40 CFU). Although an increase in concentration corresponded with a reduction in colony numbers, the antifungal effectiveness of TTO on 3D-printed material remained limited and did not demonstrate strong inhibitory activity.

As a comparison, the 0.5% NaOCl positive control showed the highest level of effectiveness, with a mean colony count of only 9.80 CFU. This substantial difference indicates that although TTO possesses inhibitory potential, it is far less effective than NaOCl in reducing Candida albicans growth on 3D-printed resin surfaces. These findings also highlight that antifungal response is strongly influenced by the type of denture base material. Subsequently, a Kruskal–Wallis statistical test was conducted to assess the differences in TTO effectiveness between 3D-printed resin and heat-cured acrylic resin. The results are presented in the following table.

Table 4. Kruskal–Wallis Test Results on the Effectiveness of Tea Tree Oil Extract Against the Growth of Candida albicans on 3D-Printed Resin and Heat-Cured Acrylic Resin

Resin Types	Groups	$\bar{x} \pm$ SD	p value
3D Printed Resin	Tea tree oil extract 0.5%	746.60±26.111	0.000
Heat-cured Acrylic Resin	Tea tree oil extract 0.5%	58.60±13.686	
3D Printed Resin	TTO extract 0.25%	1004.40±79.714	0.009
Heat-cured Acrylic Resin	TTO extract 0.25%	200.20±31.933	
3D Printed Resin	Tea tree oil extract 1%	621.40±26.340	0.000
Heat-cured Acrylic Resin	Tea tree oil extract 1%	9.40±3.847	
3D Printed Resin	Sodium hypochlorite 0.5%	9.80±2.588	0.001
Heat-cured Acrylic Resin	Sodium hypochlorite 0.5%	1.00±1.225	

This table shows the comparative results of the effectiveness of Tea Tree Oil (TTO) extract at various concentrations against the growth of Candida albicans on two types of denture base materials, namely 3D-printed resin and heat-cured acrylic resin. At a TTO concentration of 0.5%, there was a highly significant difference between the two resin types (p = 0.000), where the 3D-printed resin exhibited a much higher colony count (746.60 CFU) compared to the heat-cured resin (58.60 CFU), indicating a weaker antifungal response on the surface of the 3D-printed resin. A significant difference was also observed at TTO 0.25% (p = 0.009), where the 3D-printed resin again showed a considerably higher colony count (1004.40 CFU) compared to the heat-cured resin (200.20 CFU). At a TTO concentration of 1%, a similar pattern emerged; the 3D-printed resin produced 621.40 CFU, while the heat-cured resin produced only 9.40 CFU, with p = 0.000 indicating a highly significant difference.

These findings are consistent with the antifungal mechanism of TTO reported by [17], who stated that "Tea Tree Oil 5% showed strong antifungal activity against Candida species with inhibition effects comparable to nystatin," supporting the evidence that TTO possesses potent antifungal properties that can reduce fungal colony formation on oral surfaces. This strengthens the interpretation that increasing TTO concentration improves its antifungal potential, although its effectiveness can still vary depending on the physical characteristics of the denture base material.

The positive control using 0.5% NaOCl also demonstrated a significant difference (p = 0.001), although both resin types yielded very low colony counts. The 3D-printed resin showed 9.80 CFU, while the heat-cured resin produced only 1.00 CFU. Overall, these findings confirm that heat-cured acrylic resin exhibits a better Candida albicans elimination response compared to 3D-printed resin across all treatment groups. Further testing was conducted to evaluate the effectiveness of Tea Tree Oil (TTO) extract at various concentrations in inhibiting Candida albicans growth on two denture base materials, namely heat-cured acrylic resin plates and 3D-printed resin. In addition, 0.5% sodium hypochlorite was used as a positive control to compare the antifungal inhibitory ability between TTO and a conventional cleaning agent. The results of colony counts observed after treatment are summarized in the following table.

Table 5. Comparison of the Effectiveness of TTO and NaOCl Against the Growth of Candida albicans on Heat-Cured and 3D-Printed Resin

Material Type	Group	$\bar{x} \pm SD$	*p value*
3D Printed Resin Plate	Sodium Hypochlorite 0.5%	9.80±2.588	0.000
	TTO Extract 1%	621.40±26.340	
	TTO Extract 0.5%	746.60±26.111	
	TTO Extract 0.25%	1004.40±79.714	
Heat-cured Acrylic Resin Plate	Sodium Hypochlorite 0.5%	1.00±1.225	0.000
	TTO Extract 1%	9.40±3.847	
	TTO Extract 0.5%	58.60±13.686	
	TTO Extract 0.25%	200.20±31.933	

The table shows that 0.5% sodium hypochlorite demonstrated the highest effectiveness in suppressing Candida albicans growth on both resin types, with very low average colony counts on both the 3D-printed resin (9.80 CFU) and the heat-cured resin (1.00 CFU). In the TTO treatment group, antifungal efficacy increased with increasing concentration. However, the 3D-printed resin consistently produced significantly higher colony counts at all TTO concentrations, for example, 1004.40 CFU at 0.25% TTO and 621.40 CFU at 1% TTO. In contrast, the heat-cured resin showed a significantly better response, with significant decreases at both 1% TTO (9.40 CFU) and 0.5% TTO (58.60 CFU). A p-value of 0.000 confirms that the difference between the two materials for all treatments is highly significant. Overall, this table shows that heat-cured acrylic resin has better resistance to Candida albicans colonization than 3D-printed resin and that TTO has antifungal potential. However, its effectiveness is still below that of 0.5% sodium hypochlorite.

This study evaluated the effectiveness of soaking in tea tree oil (TTO) at concentrations of 0.25%, 0.5%, and 1% and 0.5% sodium hypochlorite (NaOCl) in inhibiting the growth of Candida albicans on two types of denture base resins, namely heat-cured resin and 3D-printed resin. The results showed that heat-cured samples had a lower number of colonies than 3D-printed resin samples. This finding is in line with recent research, which stated that 3D-printed resin has higher porosity, greater water absorption properties, and higher surface energy, thereby increasing biofilm retention and Candida adhesion [18]. The rougher surface and internal porosity of digital materials make it easier for microorganisms to adhere and grow, in contrast to heat-cured resin, which has a more stable polymer density.

Sodium hypochlorite 0.5% showed the highest inhibitory power against Candida albicans on both types of resin with an average number of colonies (9.80±2.588 and 1.00±1.225 CFU/ml. This is in line with evidence that NaOCl works through strong oxidation of active chlorine components (HOCl) entering microbial cells, causing protein aggregation, membrane disruption, lipid and DNA damage, and triggering oxidative stress, which ultimately leads to rapid fungal cell lysis [19]. In addition, NaOCl is known to alter intracellular osmotic pressure, thereby accelerating fungal death. However, several studies warn that long-term use of NaOCl can affect the surface roughness and color stability of acrylic resin [20]. This is important to consider in daily clinical use, especially in patients who wear long-term dentures.

Tea tree oil exhibits significant antifungal activity, particularly at 1% concentration, which exhibits the highest colony reduction compared to lower concentrations with mean values of 621.40±26.340 and 9.40±3.847 on 3D printed resin and heat-cured acrylic resin, respectively. The mechanism of action of TTO, specifically its active component terpinen-4-ol, inhibits membrane ergosterol biosynthesis and increases fungal cell membrane leakage [12], [21]. The concentration-dependent effectiveness of TTO is also supported by other studies that note that increasing the concentration of essential oils is directly proportional to their fungistatic ability [22]. Thus, the findings of this study are consistent with current scientific evidence that TTO is a natural antifungal agent with potential clinical use, although still lower than NaOCl.

The statistical test results also showed a significant difference in disinfectant effectiveness between the two resin types. Heat-cured resin showed a better antifungal response to all concentrations of TTO and NaOCl, while the 3D-printed resin showed a relatively smaller decrease in colonies. This difference confirms the findings [18] and [23] that the resin microstructure is a significant determinant of disinfection success. Therefore, the choice of disinfectant should take into account the type of resin used by the patient.

Although NaOCl is the most effective agent, the use of TTO as a natural alternative still has clinical value. Several recent studies have noted that plant extract-based disinfectants exhibit better biocompatibility and do not cause significant discoloration or roughness of the base resin [24]. These findings confirm that TTO, especially at high concentrations such as 1%, can be considered as an alternative denture cleaning agent, especially for patients who are sensitive to strong chemicals or wish to avoid the abrasive effects of NaOCl.

Overall, this study demonstrates that both the type of resin and the type of disinfectant significantly influence the ability to inhibit Candida albicans. The Kruskal–Wallis analysis revealed significant differences among treatments, indicating that TTO concentration and the properties of the resin material directly affect its fungistatic capacity. The combination of antifungal effectiveness, material safety, and long-term impact on the physical properties of the resin should be considered when selecting an optimal denture cleanser.

One of the limitations of this study is the use of the spread plate method for counting Candida albicans colonies, which may introduce inaccuracies because this technique heavily depends on the consistency of inoculum spreading. Variations in spreading pressure, agar thickness, and cell distribution on the media surface can result in uneven or closely clustered colonies, thereby increasing the risk of errors in manual enumeration. To mitigate this potential bias, the study employed a digital colony counter (Interscience Scan® 300), which is capable of automatically detecting colonies through digital imaging. The use of this instrument has been shown to enhance accuracy and precision while reducing human error in colony counting [25].

Another limitation to consider is that the 3D-printed resin samples in this study did not undergo finishing or polishing. This was done to comply with the manufacturer's instructions and the standard operating procedures (SOP) for 3D-printed materials, as some manufacturers state that certain 3D-printed resins can be used immediately after curing without additional finishing steps. However, this decision may have influenced the study outcomes because the absence of a polishing step results in 3D-printed resin surfaces that are rougher and more porous compared to heat-cured resin. Scientifically, rougher surface textures have been shown to enhance Candida albicans adhesion and colonization [18], [23]. Therefore, the lack of polishing may have contributed to the higher colony counts observed in the 3D-printed samples in this study, which should be considered when interpreting results and comparing resin groups. Consequently, the sample preparation procedure for 3D-printed resin in this study represents a research limitation that can serve as a point of evaluation and consideration for future studies.

CONCLUSION

Based on the study results, Tea Tree Oil (TTO) was proven to inhibit the growth of Candida albicans on both heat-cured and 3D-printed acrylic resins, with effectiveness increasing alongside concentration and reaching optimal results at 1%. The antifungal performance of TTO was more pronounced on heat-cured resin compared to 3D-printed resin, likely influenced by the latter's rougher and more porous surface due to the absence of a polishing process. Nevertheless, compared to 0.5% sodium hypochlorite, which exhibited the highest inhibitory effect against C. albicans on both materials, TTO remains a promising alternative because it is safer for denture base materials and does not cause discoloration or surface damage.

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