Research Article

Effect of tea tree oil on candida adherence and surface roughness of heat cure acrylic resin

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Abstract: Background: Denture cleansing was an important step that could prevent the spread of infection and improve a patient's health, the durability of the dentures, and the overall quality of life; therefore, it was necessary to choose a suitable cleanser that, in addition to being effective, did not have an unfavorable effect on the qualities of the denture base resin itself when used for an extended period. For this purpose, this study aimed to evaluate the effect of tea tree oil (TTO) on *Candida albicans* adhesion and the surface roughness property of poly(methyl methacrylate) denture material after immersion in TTO. Methods: A total of 55 heat-cured acrylic resin specimens were used for *C. albicans* adherence and surface roughness tests. They were distributed into groups (0.25%, 0.5%, 0.75%, and 1%) of TTO, distilled water (DW), and 2% chlorhexidine digluconate, totaling five specimens for each group. The specimens were immersed following their group for 10 minutes. Surface roughness was determined by a profilometer, and *C. albicans* adherence was determined by measuring optical density with a spectrophotometer. For comparisons between groups for surface roughness and disinfection tests, one-way ANOVA was performed on the SPSS program, considering $\alpha = 0.05$. Results: TTO had a statistically significant effect on *C. albicans* adhesion to heat-cured acrylic resin ($P < 0.05$) compared with negative control. Meanwhile, no statistically significant difference was found between 0.75% and 1% TTO concentrations ($P > 0.05$), whereas the surface roughness test showed a statistically non-significant difference between TTO concentrations and DW ($P > 0.05$). Conclusions: Immersion of acrylic resin in TTO was effective in decreasing *C. albicans* adhesion to it, and the greatest decrease was obtained by 1% TTO. The surface roughness test showed a non-significant difference in acrylic roughness after immersion in TTO.

Keywords: Tea tree oil; acrylic resin; candida albicans; surface roughness

Introduction

The most popular material for manufacturing denture resin was polymethyl methacrylate (PMMA), which had good aesthetics, a low absorption rate of water, and low toxicity. However, this material was porous, so food could adhere and microorganisms could flourish, potentially causing oral disease [1]. *Candida albicans* was one of the microorganisms that was frequently detected in denture bases [2].

Dental prostheses were classified as semi-critical devices and must undergo high-level disinfection or sterilization in accordance with the Centers for Disease Control and Prevention (CDC) criteria [3]. Dentures could be sterilized mechanically, chemically, or by a mix of both. Using a chemical solution to disinfect was beneficial for those who wear dentures [4]. The disinfectant must have virucidal, fungicidal, and bactericidal properties. The U.S. Food and Drug Administration (FDA) had recommended using high-level disinfectants and those that had received FDA approval for cleaning up reusable dental and medical equipment. They included different mixtures and formulations, such as 2.5% glutaraldehyde, 3% hydrogen peroxide, 5.25% sodium hypochlorite, and 2%–4% chlorhexidine gluconate (CHX) [5]. CHX had broad-spectrum antimicrobial activity [6]. CHX molecules interacted with negatively charged cell walls through their positive charges. Due to this interaction, the membrane, the cytoplasmic composition, and the enzyme inhibition could be permanently lost [7]. Therefore, CHX was a widely used and accessible disinfectant, but it had a bad taste, a bad smell, and a bleaching effect [8]. Its mouthwashes have been rarely linked to delayed hypersensitivity responses [9]. Although these compounds were

frequently employed to eliminate biofilm, they affected the PMMA surface by making it rougher and altering the color of the material [10]. As a result, research on possible natural denture cleansers has been conducted [11].

Aside from their economic value, the main advantages of employing natural plant extracts were safety, biocompatibility, and no adverse effects [12].

Melaleuca alternifolia, an Australian native plant from which tea tree oil (TTO) was derived, could be steam-distilled to produce oil from its leaves. TTO was made up of various substances, mostly monoterpene and sesquiterpene hydrocarbons and their alcohols. Several studies showed that TTO had antiseptic, antibacterial [13], anti-inflammatory, and antifungal characteristics, particularly those that had anti-*Candida* properties [14].

Moreover, phytoconstituents were chemical substances found in natural agents that had a specific target effect for treating and preventing biofilm-related disorders [15]. A few of these compounds were α-terpineol, which came from *M. alternifolia* [16]. These phytoconstituents displayed antifungal activity against *C. albicans* [17].

A wide variety of cosmetic products, such as moisturizers, body lotions, conditioners, shampoos, mouthwashes, soaps, and hand washes, included TTO in conventional amounts as part of their compositions [18].

Surface imperfections made it more likely that microbes persisted on the denture surface even after the prosthesis had been cleaned; hence, the roughness of acrylic resin surfaces was a crucial feature [19]. The present study aimed to evaluate the antifungal effect of TTO and its effect on the surface roughness property of heat-cured acrylic resin after immersion in TTO.

Methods

Specimen preparation

A total of 55 heat-cured acrylic (SR Triplex Hot, Ivoclar Vivadent, Liechtenstein) specimens were prepared in accordance with the manufacturer's recommendation. A master mold with a size of 10 × 10 × 2.3 mm [20] was used for *C. albicans* testing (30 specimens), and for surface roughness testing, a master mold with a size of 30 × 15 × 2.5 mm (length, width, and thickness, respectively) was used (25 specimens) [21]. The excess material was removed from all acrylic specimens (aside from those being prepared for surface roughness testing) by using a prosthetic engine equipped with stone and acrylic burs, which were continuously cooled by water to prevent overheating that could cause specimen distortion. Polishing was carried out using a rouge that was placed in a dental lathe machine and spun at 1500 rpm with continuous water cooling to make the surface glossy [22].

Specimen grouping

A total of 55 specimens were fabricated and divided into two sets.

1. *C. albicans* adhesion test: A total of 30 specimens were subdivided into six groups, with five specimens in each group.

Group 1: immersed in 0.25% TTO,

Group 2: immersed in 0.5% TTO,

Group 3: immersed in 0.75% TTO,

Group 4: immersed in 1% TTO,

Group 5: immersed in distilled water (DW, negative control), and

Group 6: immersed in 2% CHX (positive control for antifungal test).

2. Surface roughness test: A total of 25 specimens were subdivided into five groups, with five specimens in each group.

Group 1: immersed in 0.25% TTO,

Group 2: immersed in 0.5% TTO,

Group 3: immersed in 0.75% TTO,

Group 4: immersed in 1% TTO, and

Group 5: immersed in DW (negative control).

Preparation of TTO solution

Pure tea tree essential oil (Now Foods; Bloomingdale, IL 60108, USA): TTO solution was prepared by mixing it (in accordance with the specimen groups) with 1% Tween 80 as an emulsifying agent with the use of a magnetic stirrer and DW.

C. albicans adhesion test

All specimens were sterilized using an autoclave at 121 °C and 15 psi for 15 minutes. Each specimen was individually infected with 10 ml sterile tryptic soy broth (TSB), which was saturated with *C. albicans*, and incubated for 24 hours at 37 °C under aerobic conditions. The tubes' turbidity was adjusted on day 2 to match that of McFarland tube No. 5, which corresponds to 10^7 organisms/ml [23]. After the specimens were washed with saline, they were submerged in denture cleaner solution [24] in accordance with the specimen groups for 10 minutes. The specimens were then washed and dyed with crystal violet. Next, they were gently rinsed with sterile DW and immersed in 3 ml of 96% ethanol for 3 minutes to remove the adhered C. albicans. The adhered *C. albicans* was examined using a spectrophotometer set to 0.5 at 540 nm [25].

Surface roughness test

The stylus-type electronic roughness tester's contact surface roughness (Ra) measurement instrument (profilometer, VTSYIQI, China) was used to measure the specimen's surface roughness with precision (0.001). The specimens were immersed in accordance with their group for 10 minutes and then washed, dried, and tested.

When the stylus was permitted to touch the sample's initial area while it was on a steady, stiff surface, the reading on the digital scale appeared on its own [26]. The stylus had a diamond tip that moved physically in five access points and was 2.5 mm long while still retaining contact with the surface and giving an average reading.

Statistical analysis

Individual surface roughness readings and readings for *C. albicans* adhesion were computed and summarized. The mean and standard deviation of each group were calculated using SPSS (IBM SPSS

version 20). Comparisons of the results between each of the groups were performed using one-way ANOVA and Tukey HSD. The level of significance was set at $P < 0.05$.

Results

The results of the *C. albicans* adhesion test for each group are reported in Table 1.

Group 5 was found to have the highest mean value (0.132) after examining the stained specimens from each group, followed by the experimental groups and group 4, which exhibited the lowest mean value (0.0024).

Table 1: Descriptive statistics for candida adherence test

Groups	N	Mean	SD	SE	Minimum	Maximum
Group 5	5	0.13200	0.008367	0.003742	0.120	0.140
Group 1	5	0.11600	0.011402	0.005099	0.100	0.130
Group 2	5	0.02040	0.004278	0.001913	0.017	0.026
Group 3	5	0.00660	0.001673	0.000748	0.005	0.009
Group 4	5	0.00240	0.000894	0.000400	0.001	0.003
Group 6	5	0.00260	0.001673	0.000748	0.001	0.005

One-way ANOVA test produced a highly significant result when comparing between groups($P < 0.05$) Table 2.

Table 2: ANOVA test of candida adherence test

	Sum of Squares	Df	Mean Square	F	P-value
Between Groups	0.091	5	0.018	488.266	0.000*
Within Groups	0.001	24	0.000		
Total	0.092	29			

*=Significant at p<0.05, ^=not significant at p>0.05

Table 3 revealed that groups 3, groups 4, and groups 6 had non-significant differences ($P > 0.05$), whereas other groups had significant differences ($P < 0.05$).

Table 3: Multiple comparisons of candida adherence between groups using Tukey HSD

(I) Groups	(J)Groups	Mean Difference (I-J)	SE	P-value
Group 5	**Group 1**	0.016000	0.003870	0.004*
	Group 2	0.111600	0.003870	0.000*
	Group 3	0.125400	0.003870	0.000*
	Group 4	0.129600	0.003870	0.000*
	Group 6	0.129400	0.003870	0.000*
Group 1	**Group 2**	0.095600	0.003870	0.000*
	Group 3	0.109400	0.003870	0.000*
	Group 4	0.113600	0.003870	0.000*
	Group 6	0.113400	0.003870	0.000*
Group 2	**Group 3**	0.013800	0.003870	0.017*
	Group 4	0.018000	0.003870	0.001*
	Group 6	0.017800	0.003870	0.001*
Group 3	**Group 4**	0.004200	0.003870	0.882^
	Group 6	0.004000	0.003870	0.902^
Group 4	**Group 6**	-0.000200-	0.003870	1.000^
Group 6	**Group 4**	0.000200	0.003870	1.000^

*=Significant at p<0.05, ^=not significant at p>0.05

Regarding surface roughness, the mean of group 5, shown in Table 4, was lower than that of the other groups, indicating that the experimental groups' roughness increased.

Table 4: Descriptive statistics for surface roughness test

Groups	N	Mean	SD	SE	Minimum	Maximum
Group 5	5	1.28340	0.101923	0.045581	1.131	1.407
Group 1	5	1.32520	0.092834	0.041517	1.182	1.425
Group 2	5	1.33220	0.070404	0.031486	1.239	1.424
Group 3	5	1.35080	0.129405	0.057872	1.162	1.465
Group 5	5	1.38040	0.105876	0.047349	1.237	1.506

Table 5: ANOVA test for surface roughness test

	Sum of Squares	Df	Mean Square	F	P-value
Between Groups	0.025	4	0.006	0.611	0.660^
Within Groups	0.208	20	0.010		
Total	0.233	24			

^=Not significant at p>0.05, *= significant at p<0.05

A one-way ANOVA table of roughness data revealed a non-significant difference among the groups (P > 0.05).

Discussion:

Elderly and immunocompromised individuals were more prone to developing denture stomatitis, which mostly results from an imbalance of the microflora caused by local variables, such as wearing dentures [27]. The goal of treatment for denture stomatitis was to eliminate the pathogens that cause denture contamination [28].

Several mechanical and chemical methods are available today to disinfect dentures [29]. Chemical cleaning involved submerging dentures in solutions that had antibacterial, antifungal, and solvent properties. These solutions could be used on their own or in combination with mechanical or ultrasonic cleaning [30]. However, obtaining a secure sterilization or disinfection procedure without impairing the mechanical and physical properties of the resins used in denture bases was crucial [29].

Considering the majority of plants had therapeutic qualities, such as antimicrobials, making them a potential alternative for disinfectant materials [30], TTO has been used in clinical studies to treat a range of fungal infections, including oral candidiasis [31].

Tween 80 was mixed with TTO as an emulsifying agent to obtain microemulsions that enhance oil's activity. Hence, it did not have any antimicrobial properties. It might be used to create a dilutable U-type microemulsion, and surfactants could be used to increase membrane fluidity and improve cell permeability. Even after a 1000-fold dilution, such microemulsions might maintain their shape. The antimicrobial effect of dilutable microemulsions, which were structure-dependent, remains to be proven [32].

In this study, acrylic disks submerged in TTO for 10 minutes had a statistically significant decrease (P < 0.05) in *C. albicans* adhesion compared with group 5. Moreover, groups 3 and 4 had a non-significant difference with group 6. These results may be because TTO's chemical composition interferes with *C. albicans* adhesion to the acrylic resin surface, and their effect increases with increased concentration. However, terpinen-4-ol and α-terpineol were shown to be the main compounds responsible for the antifungal and antibacterial effects [33]. In addition, the components linalool and limonene combined with α-pinene could interact with microbial activity, which was similar to the result of Sikkema et al. (1995) [34]. Consequently, TTO damages fungal strains' cell walls and cytoplasmic membranes, leading them to leak cytoplasmic materials. Given that the lipophilicity of essential oils allowed them to cross the cytoplasmic membrane, Furthermore, TTO and terpinen-4-ol might harm fungal organelle membranes by penetrating them. Finally, these irreversible TTO-mediated alterations result in cell death and exhibit antifungal properties against *C. albicans* strains. This finding was in agreement with that of Li et al. (2016), who studied the dynamics and mechanism of the antimicrobial activity of TTO against bacteria

and fungi at different concentrations and times [35], and Krishnaveni et al. (2021), who discussed the immersion of denture soft liner in pure TTO [36].

The roughness result showed a statistically non-significant difference in surface roughness ($P > 0.05$) in all groups compared to group 5. These results agreed with those of Abed (2022), who found a non-significant difference in acrylic roughness after immersion in electrolyzed water [37]. Heidrich et al. (2018) obtained the same results, that was, rosemary and castor oils and propolis extract had no damaging effect on the surface roughness of pink acrylic resin until the fourth month [38]. Similarly, Salman and Saleem (2011) demonstrated that when the heat acrylic resin was immersed in different denture cleaner solutions, the resin's surface was not affected [39]. Therefore, TTO did not cause major deterioration and was considered safe to be used as a natural antifungal denture cleanser.

Conclusion

Heat-cured acrylic resin immersed in TTO might decrease *C. albicans* adhesion to it, and the greatest decrease was obtained by 1% TTO. The results of the acrylic surface roughness test showed a non-significant difference following immersion in TTO.

Conflict of interest

The authors have no conflicts of interest to declare.

Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all aspects of this work.

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Informed consent

Informed consent was obtained from all individuals or their guardians included in this study.

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تأثير زيت شجرة الشاي على التصاق المبيضات وخشونة السطح لراتنج الأكريليك المعالج بالحراره

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المستخلص:

الخلفية: كان تنظيف طقم الأسنان خطوة مهمة يمكن أن تمنع انتشار العدوى وتحسن صحة المريض، ومتانة طقم الأسنان، الجودة الشاملة للحياة؛ لذلك، كان من الضروري اختيار منظف مناسب، بالإضافة إلى فعاليته، لم يكن له تأثير سلبي على خصائص راتنج قاعدة طقم الأسنان نفسه عند استخدامه لفترة طويلة. ولهذا الغرض هدفت هذه الدراسة إلى تقييم تأثير زيت شجرة الشاي على التصاق المبيضات البيضاء وخشونة السطح لمادة أطقم الأسنان المتعددة (ميثيل ميثاكريلات) بعد الغمر فيها. الطريقة: تم إجراء خمسة وخمسين عينة من راتنج الأكريليك المعالج بالحرارة لاختبار التصاق المبيضات وخشونة السطح وتوزيعها على مجموعات (0.25٪ ، 0.5٪ ، 0.75٪ ، 1٪) من زيت شجرة الشاي والماء المقطر و 2٪ كلورهيكسيدين digluconate ، مجموع خمس عينات لكل مجموعة. تم غمر العينات وفقًا لمجموعتهم لمدة 10 دقائق. حدد مقياس البروفايل خشونة السطح ، بينما تم تحديد مدى التزام المبيضات عن طريق قياس الكثافة الضوئية باستخدام مقياس الطيف الضوئي. قارن برنامج SPSS مجموعات خشونة السطح واختبارات التطهير باستخدام ANOVA أحادي الاتجاه و Tukey HSD ، مع مراعاة $\alpha = 0.05$. النتائج: بناءً على نتائج الدراسة ، قلل زيت شجرة الشاي ذو دلالة إحصائية من التصاق المبيضات براتنج الأكريليك المعالج بالحرارة ($P < 0.05$) مقارنة بمجموعة التحكم السلبية ، بينما لم يكن هناك فرق ذو دلالة إحصائية بين التراكيز (0.75٪ و 1٪) زيت شجرة الشاي والمجموعة الضابطة الإيجابية ($P < 0.05$). كشف اختبار خشونة السطح عن وجود فرق غير معنوي إحصائياً عند مقارنة تركيزات زيت شجرة الشاي مع الماء المقطر ($P < 0.05$). الاستنتاجات: راتنجات الأكريليك المعالجة بالحرارة المغمورة في زيت شجرة الشاي قد تقلل من التصاق المبيضات البيضاء بها ، مع 1٪ زيت شجرة الشاي الذي يوفر أكبر قدر من التخفيض. في المقابل ، أظهرت خشونة سطح الأكريليك اختلافًا غير معنوي بعد الغمر في زيت شجرة الشاي