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The antifungal potential of cinnamon oil incorporated into a heat-polymerized soft liner

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Abstract

Objective: This study evaluated the effects of incorporating 1% and 2% cinnamon oil into a soft liner on the growth of *Candida albicans* colonies and the material's hardness.

Methods: Thirty soft liner specimens were prepared for the disk diffusion and thirty for the hardness test. In each test, the specimens were divided into three subgroups based on the concentration of cinnamon oil: A) 0% (control), B) 1%, and C) 2% by weight. Cinnamon oil was added to the monomer of a heat-polymerized soft liner. *C. albicans* was cultured on Mueller-Hinton agar, and the diameters of the inhibition zones around the specimens were measured. The Shore A hardness test was conducted using the Shore durometer. Statistical analysis was performed by the independent t-test, one-way ANOVA, and Tukey post-hoc test (α=0.05).

Results: The control group showed no inhibition zone. The specimens in Group C exhibited a significantly larger inhibition zone (16.72 ± 0.82 mm) than Group B (12.56 ± 0.82 mm; $P < 0.001$). The hardness values differed significantly among groups ($P < 0.001$). Group C demonstrated the highest hardness (48.54 ± 0.95), significantly greater than both Groups B (47.13 ± 0.72; $P < 0.05$) and A (45.23 ± 0.73; $P < 0.05$).

Conclusions: Adding cinnamon oil improved the antifungal activity and hardness of the soft denture liner. Adding 2% cinnamon oil increased the hardness values to the upper limit of clinical acceptability. Therefore, the 1% concentration is recommended to balance antimicrobial efficacy and maintain optimal mechanical properties of the soft liner.

Keywords: Antifungal agent, *Candida albicans*, Cinnamon oil, Denture, Hardness, Soft liners

Introduction

Soft tissue liners are polymer-based materials placed between dentures and oral tissues to alleviate the pressure of masticatory forces on the oral mucosa and improve denture fit (1). These materials must exhibit biocompatibility with oral tissues, retain their shape and color stability, resist abrasion, and form a strong bond with the denture base (2). However, soft liners tend to degrade over time, which increases surface roughness and promotes *Candida albicans* colonization on the denture's mucosal surface (3). Candida-associated denture stomatitis, a common condition among complete denture wearers, is characterized by inflammation of the palatal mucosa and affects nearly 72% of denture users (4, 5). Infections caused by *C. albicans* can lead to severe complications, such as systemic candidiasis, which may spread to internal organs and cause sepsis. Chronic Candida infections can also cause significant skin, oral, or vaginal issues (6).

Maintaining proper denture plaque control is crucial for ensuring oral hygiene in denture wearers and usually involves mechanical and chemical methods. However, soft liners are unsuitable for brushing or other mechanical cleaning techniques, as these methods may damage the material (2). Researchers have explored adding antifungal agents, including plant-based oils and herbal remedies, into soft liner materials to address this issue. For instance, Khanal et al. (7) demonstrated that incorporating henna and turmeric into acrylic denture resin significantly reduced *C. albicans* adherence. Similarly, Al-Jmmal (8) reported that adding coconut oil to heat-cured acrylic resin enhanced its antifungal

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properties, although concentrations above 1% reduced the material's hardness and strength. Ahmed et al. (9) found that Ficus carica and Olea europaea extracts had a notable antimicrobial effect against C. albicans when added to heat-cured soft liners. However, this addition also weakened the bond strength between the liner and the denture base. Current research is focused on identifying herbal additives that can improve antifungal efficacy without compromising the physical properties of soft liners.

There are around 250 recognized species of cinnamon trees worldwide (10). The bark of cinnamon spices is valued for its culinary and medicinal uses. Cinnamon contains critical components, including cinnamaldehyde and trans-cinnamaldehyde, which are responsible for the distinct aroma and various biological activities associated with cinnamon (11). Cinnamon bark also contains procyanidins and catechins (12). These procyanidins have strong antioxidant properties (13). Furthermore, cinnamon has natural solid antimicrobial activities against oral pathogens, making it potentially beneficial in the prevention of caries and periodontal diseases, as well as in the treatment of infected root canals and candidiasis treatment (14).

A previous study found that depositing 20 wt.% cinnamon-laden nanofibers onto heat-cured poly(methyl methacrylate) (PMMA) surfaces significantly reduced the adhesive and proliferative ability of C. albicans while maintaining the viability of epithelial cells (15). However, no prior research has explored the effects of adding cinnamon to complete denture liners. Therefore, the present study aimed to evaluate the antimicrobial properties and hardness of a soft denture liner incorporated with cinnamon oil. The null hypothesis was that cinnamon does not affect the antimicrobial properties or hardness of the soft liner.

Materials and methods

Specimen preparation

Plastic molds were utilized to prepare the soft liner samples for two distinct tests: the disk diffusion test (a mold with 10 mm in diameter and 3 mm in thickness) and the Shore A hardness test (a mold with 30 mm in diameter and 3 mm in thickness). The impression of the molds was taken using an additional silicone impression-taking material (Zermack, Italy). The impression was then coated with a separating medium (Shanghai New Century Dental Material Co., Ltd, China) and filled with dental stones in the lower flask half. After setting, the impression was coated again with the separating medium, and the upper flask half was applied. After one hour, the flask was opened, the plastic molds were removed, and the mold spaces were created.

Thirty specimens were prepared for the disk diffusion test and another 30 for the Shore A hardness test. Each group was divided into three subgroups based on the concentration of cinnamon oil added: 0% (control group), 1%, and 2% by weight (wt%). For the test groups, 100% pure cinnamon oil (First Botany, USA) was accurately measured using an electronic balance (Kern, Germany) and added to the monomer of a heat-polymerized soft denture liner (Vertex, Netherlands). The mixture was then blended with the powder using a probe sonicator, vibrating at 120 W and 60 KHz for 3 minutes, following the manufacturer's instructions (Figure 1).

Once mixed, the material was transferred to a clean, dry glass beaker, sealed, and left to reach its dough stage for 30 minutes. The mixture was then delivered into the mold cavity and pressed using a polyethylene sheet (Amalgamated Dental T.D., England) to ensure even distribution. The upper flask half was placed on top, and the sealed flask was placed under a hydraulic press at 100 psi for 5 minutes.

Next, clamps (HANUA, Engineering Corp., USA) were used to submerge the flask into a digitally controlled water bath (Memmert, Germany). The water was heated to 70°C for 90 minutes, after which the temperature was elevated to 100°C for 30 minutes, according to the manufacturer's recommendations. Once curing was complete, the flask was removed from

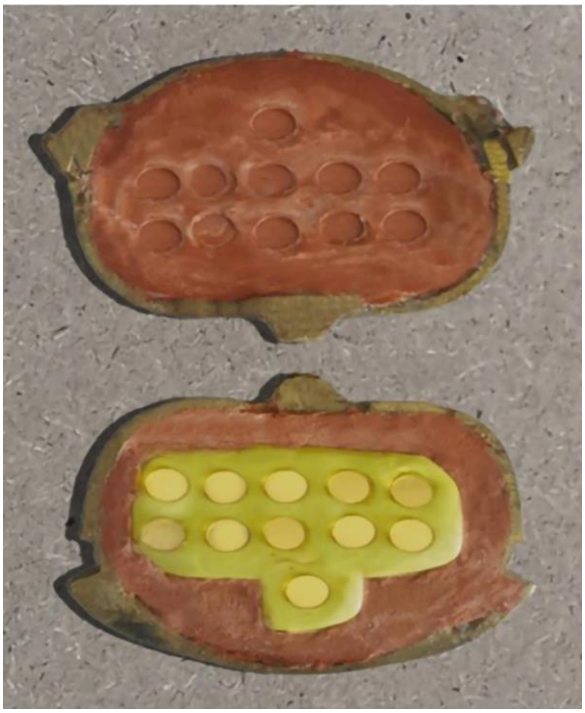

Figure 1. Plastic molds inside a silicone-stone model

the water bath and allowed to cool at room temperature for 30 minutes, followed by 15 minutes under tap water.

After this, the flasks were opened and left to cool for another 20 minutes. Finally, the soft liner specimens were removed and polished with fine-grit silicone polishing burs (Vertex) and fine-grit sandpaper. Then, samples were autoclaved (Tuttnauer, USA) and stored in sterile containers until further testing.

Antifungal test

C. albicans samples were obtained from six male volunteers with denture stomatitis using sterile cotton swabs. The identification of Candida species primarily relies on the characteristic features of the colonies. *C. albicans* colonies on sabouraud dextrose agar exhibit a convex, smooth, and creamy morphology. For the antifungal testing, Mueller-Hinton agar (MHA) was prepared by dissolving 38 grams of the medium (HiMedia Laboratories, India) in 1000 mL of distilled water and heating it for ten minutes, following the manufacturer's instructions. The medium was sterilized by autoclaving at 121°C and 15 psi for 15 minutes. After autoclaving, the solution was allowed to cool at room temperature. Then, the solution was poured into sterile petri dishes on a flat surface to ensure consistent depth. The final pH of the medium was 7.3 ± 0.1 at 25°C, and the plates were stored in an incubator (Memmert, Germany) at a temperature between 2°C and 8°C, as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The Kirby-Bauer technique was employed to evaluate the antifungal properties of the soft liner specimens. Two isolated *C. albicans* colonies from the incubated culture were placed into a test tube containing 4 mL of normal saline. This resulted in a suspension with a turbidity of approximately 1.5×10^8 CFU/mL. The fungal suspension was then evenly spread over the surface of the Mueller-Hinton agar by streaking with a sterile cotton swab. After ten minutes, the specimens were carefully placed onto the agar surface using sterile forceps and pressed firmly to ensure proper contact. The plates were then incubated upside down at 37°C for 18-24 hours. After the incubation period, the diameter of the inhibition zones around the specimen discs was measured using a caliper to assess the antifungal efficacy of the soft liner samples.

Shore A hardness

The Shore A hardness test was performed by applying the Shore A durometer instrument (Ezitown, China) to the surface of the soft liner (Figure 1). Measurements were taken from five points on each sample: one in the center and four in the peripheral locations. The durometer was allowed a 5-second penetration duration for each reading to ensure consistent and accurate results.

Table 1. Mean ± standard deviation of inhibition zone (mm) in the study groups

Group	Mean
Group (B)	12.56 ± 0.82
Group (C)	16.72 ± 0.82
P-value	0<0.001*

*Values less than 0.05 indicate a significant difference among the groups based on the independent sample t-test.

Statistical analysis

Statistical analysis was done using SPSS version 26 (IBM Inc., NY, USA). The independent samples t-test, one-way ANOVA, and Tukey post-hoc tests were employed to analyze the results from the disk diffusion test and the Shore A hardness test. A p-value of less than 0.05 was considered statistically significant.

Results

In the disk diffusion test, Group A exhibited no inhibition zone. As shown in Table 1, the results from the independent-sample t-test demonstrated that the inhibition zone diameter in Group C (16.72 ± 0.82 mm) was significantly larger than that in Group B (12.56 ± 0.82 mm; P < 0.001).

The results of the Shore A hardness test are presented in Table 2. According to the ANOVA test, there was a statistically significant difference in hardness values among the groups (P < 0.001). Pairwise comparisons using the Tukey post-hoc test revealed that the hardness of Group C (48.54 ± 0.95) was significantly higher than that of the other groups (P < 0.05). Additionally, Group B (47.13 ± 0.72) demonstrated significantly higher hardness compared to Group A (45.23 ± 0.73; *P* < 0.05).

Discussion

In this study, incorporating cinnamon oil into heat-cured soft denture liners at 1% and 2% concentrations

Table 2. Mean ± standard deviation of shore A hardness values in the study groups

Group	Mean
Group (A)	45.23 ± 0.73 [a]
Group (B)	47.13 ± 0.72 [b]
Group (C)	48.54 ± 0.95 [c]
P-value	0<0.001*

*Values less than 0.05 indicate a significant difference among the groups based on ANOVA.
Different lowercase letters represent significant differences between the groups according to the Tukey post-hoc test (P < 0.05).

caused enhanced antifungal activity against C. albicans in the disk diffusion test. The Shore A hardness test revealed that adding cinnamon oil increased the hardness of the soft liner. Therefore, the null hypothesis was rejected.

The antifungal effect observed in this study is likely attributed to cinnamaldehyde, the primary component of cinnamon bark oil. It makes up 60–80% of the oil and is well-known for its antifungal properties (16). Cinnamaldehyde's primary mechanism of action is disrupting fungal cell walls and compromising membrane integrity. It inhibits ergosterol formation, an essential component of fungal cell membranes, thus disrupting membrane permeability and causing leakage of cellular contents (17). This disruption further causes osmotic imbalance, ultimately resulting in cell death. Additionally, eugenol, which constitutes 5-10% of cinnamon bark oil, supports its antifungal properties (18, 19). Eugenol can penetrate fungal cell membranes, causing leakage of internal constituents, such as potassium ions, which are vital for cellular equilibrium (20). Moreover, eugenol interferes with fungal metabolic processes by inhibiting enzyme functions (21).

Although cinnamaldehyde and eugenol are the main antimicrobial components, other compounds like benzyl benzoate, linalool, and beta-caryophyllene exhibit additional antimicrobial effects. Linalool alters membrane permeability even at low concentrations, while beta-caryophyllene reduces localized inflammation caused by fungi (22). Benzyl benzoate enhances antifungal activity by compromising the integrity of the fungal membrane. Furthermore, polyphenolic compounds such as procyanidins and catechins in cinnamon bark add an extra layer of antifungal defense by inducing oxidative damage in fungi, further preventing fungal growth (23).

The present findings corroborate the results of several studies that proved the antimicrobial potential of cinnamon oil in denture materials. For instance, cinnamon-laden nanofibers on PMMA surfaces reduced C. albicans adhesion while maintaining epithelial cell viability (15). Additionally, an in vitro study has identified cinnamon and citronella oils as effective agents against Candida biofilm formation, making them suitable for daily denture cleansing (24). A clinical trial has also demonstrated that cinnamon essential oil is as effective as nystatin in treating Candida infections (25).

In the present study, the Shore A hardness of the Vertex soft liner without additives was 45.23 ± 0.73. Białożyt-Bujak et al. (26) reported a higher hardness of 47.4. However, they measured hardness after 24 hours, whereas the present study evaluated it shortly after setting. After incorporating 1% and 2% cinnamon oil, the hardness increased to 47.13 ± 0.72 and 48.54 ± 0.95, respectively. This increase can be attributed to the reactive aldehyde groups in cinnamaldehyde, which may link with the polymer chains of the soft liner material, enhancing the matrix's bridging density (19). Moreover, catechins and procyanidins strengthen the polymer matrix by filling its gaps (20). Cinnamon oil induces a shrinkage in the matrix of soft liner polymer, reducing the material's free volume and increasing its stiffness (21). The oil also promotes a phase change in the polymeric structure, contributing to a more crystalline structure (22, 23).

Clinically acceptable Shore A hardness values for soft liners typically range between 13 and 49 units within 24 hours (27). While all groups in this study fell within this acceptable range, the group containing 2% cinnamon oil approached the upper limit. Therefore, a 1% concentration may be more appropriate for maintaining antimicrobial properties without excessively increasing hardness.

Despite these promising results, there are limitations to consider. Some patients may experience allergic reactions to cinnamon oil, potentially restricting its clinical use. Additionally, the hardness values should be evaluated over an extended period, as the hardness of soft liners tends to increase over time (26). Future studies should assess the effects of adding cinnamon oil with different concentrations to other soft liner brands.

Conclusions

Incorporating 1% and 2% cinnamon oil into a soft denture liner enhanced its antimicrobial properties against *C. albicans*. However, increasing the oil concentration also raised the material's hardness. Since adding the 2% concentration reached the clinically acceptable hardness values limit, a 1% concentration appears to be the better option for balancing antimicrobial efficacy with optimal mechanical properties.

Conflict of Interest

None

Authors' contributions

ASA, RSA, and LNA contributed to the study design, data gathering, and manuscript preparation.

Ethical approval

Not applicable.

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