ORIGINAL RESEARCH

Surface Modifications and Antifungal Efficacy of Origanum Oil Incorporation in Denture-based Materials: An *In Vitro* Study

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Abstract

Aim: This study aimed to assess if the addition of origanum oil to denture materials could decrease microorganisms counts and biofilm formation without changing their mechanical/surface properties.

Materials and methods: A total of 66 resilient denture liner discs (SoftConfort, Dencril Comércio de Plásticos Ltda, SP, Brazil) were prepared with fixed dimensions of 10 × 3 mm for biofilm assay ($n = 36$) and 12 × 2 mm for sorption-solubility tests ($n = 30$) containing three oil concentrations – 0, 2.5 and 5%, thereby $n = 12$ per each group samples for biofilm assay and $n = 10$ per each group for sorption-solubility test respectively. While the microcosm biofilms of *Streptococcus mutans*, *Candida* species, and total microorganisms formed on denture liner were counted and expressed as colony-forming units per disc surface area, the water sorption (WS) and solubility (SL), was calculated by weighing the samples with an analytical balance at different intervals after storing them in a desiccator and distilled water alternatively. Data was recorded and statistically analyzed.

Results: Surface roughness increased in all groups after biofilm formation ($p < 0.001$), with the most significant change observed in the 2.5% oil group. The tested oil concentrations did not result in sorption changes, but a 5% oil concentration resulted in higher solubility ($p < 0.001$). A reduction of total microorganisms and *S. mutans* was seen after 24 hours for all concentrations ($p < 0.05$). No significant reduction was found for *Candida (C.) albicans* after 24 hours, whilst 2.5% oil concentration presented lower counts of *C. albicans* in comparison to the 5% group after 24 hours ($p < 0.05$).

Conclusions: Incorporating 2.5% origanum essential oil into resilient denture liners seems to reduce microorganisms count in a complex biofilm model. These results need to be confirmed in future studies.

Clinical significance: The addition of natural products like origanum oil into denture-based materials can help manage biofilm onset and development while offering a simple and effective approach for maintaining denture hygiene. This strategy enhances the antimicrobial properties of denture liners without significantly altering their mechanical and surface characteristics, potentially improving patient outcomes.

Keywords: Acrylic resin, Biofilm model, Candidiasis, Denture liner, Origanum oil.

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Introduction

Denture stomatitis is a multifactorial disease involving both local and systemic predisposing factors.[1] *Candida (C.) albicans* is one of the main pathogens responsible for the development of denture stomatitis, but local factors such as continuous use of dentures, inadequate oral hygiene, and poorly manufactured prosthesis may predispose to candidiasis.[2]

Denture base materials present structural and surface characteristics that allow chemical/physical interaction of microbial cells, favoring the adhesion process and further biofilm onset.[3–6] In the presence of irregularities caused by brushing, fissures, and imperfections, a higher concentration and proliferation of these pathogens could be expected.[2] Worse scenarios can be found when denture liners are applied to complete dentures because efficient mechanical cleaning may be difficult and brushing may damage the material surface, while it is known that cleansing agents also act by modifying surface morphology, depending on immersion time and concentration.[7–12]

To overcome this situation, several compounds and drugs have been tested after their incorporation into the formulations of polymethylmethacrylate (PMMA) and silicone-based materials,

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aiming to prevent and control the development of denture stomatitis, with some attempts being made to test the antifungal activity of natural products against *C.* species and especially *C. albicans*.[13–15] Natural products are promising sources of new antibiotics, and their increasing valorization aims to minimize the emergence of resistant microorganisms and discover new drugs. In addition, the possibility that these natural products promote fewer hepatotoxic and nephrotoxic effects as well as fewer allergic reactions and interactions with other drugs should also be considered.[16] *Origanum vulgare* (popularly known as "origanum") is one of these natural products, and it is known for its antimicrobial activity with several applications in the biomedical field.[17,18]

Mainly responsible for *Origanum vulgare* bioactivity, carvacrol and thymol are monoterpene phenolic compounds that present analgesic, antifungal, and antibacterial effects, showing favorable results against *C. albicans*.[19–23] Thymol acts in the cell membrane structure and the electrostatic surface of the fungus, causing asymmetric stresses in the membrane and increasing its permeability due to the impairment of lipid packaging. As a consequence, the synthesis of enzymes that occurs in cell membranes could be compromised, affecting the formation and viability of hyphae, which are an important virulence factor in the pathogenesis of denture stomatitis.[24,25]

Incorporating natural products into acrylic resin and denture coatings could result in cheaper and easier ways to prevent the onset of biofilm-dependent diseases. Origanum oil has been found to be effective in reducing the adherence of *C. albicans* on denture-base materials, as demonstrated in studies by Srivatstava et al.[26] and Volety et al.[27] It also exhibits superior antifungal activity compared to other herbal extracts, as shown in the study by Eltamimi et al.[28] These findings collectively suggest that origanum oil can be a valuable addition to denture-base materials for its antifungal properties. Although these reports suggest that adding origanum oil to prosthetic materials is valuable, the evaluation of the antimicrobial properties of these formulations has been tested only in studies of dual or single species, and not in complex biofilms such as those found in the oral cavity.[26–28] Furthermore, Brondani et al.[24] revealed that *O. vulgare* essential oil possesses significant inhibitory effects on *C. albicans* mycelial growth by demonstrating significant reductions in the generation of phospholipase enzymes produced by this yeast isolated from patients with prosthetic stomatitis.[24] In addition, the mode of antifungal activity could be related to the intervention of components of the essential oil in enzymatic reactions of cell wall synthesis that affect the morphogenesis and growth of fungi.[29]

Finally, the assessment of changes in the physicochemical properties of denture liners is important as the addition of these natural agents is desired not to modify the material's surface. This study aimed to assess if the addition of origanum oil to denture materials could decrease microorganism counts and biofilm formation without changing their mechanical/surface properties. Thus, the hypothesis tested was that origanum essential oil added to denture liner materials would reduce biofilm formation without modifying the surface characteristics of the material.

Materials and Methods

The present study was designed as a laboratory-based experimental study conducted over one year from January to December 2023 at the Laboratory of Oral Microbiology (LAMICRO). This study was approved by the Local Research and Ethics Committee of the Federal University of Pelotas under Protocol number 0992016.

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Aliquots (100 gm) of *Origanum vulgare* subspecies vulgare, certified botanically, were obtained from Chile (Luar Sul(r) – Indústria e Comércio de Produtos Alimentícios Ltda., Santa Cruz do Sul, RS, Brazil). The collected aliquots were weighed and hydro distilled in a Clevenger type apparatus, for 5 hours. The extracted oil was dried over anhydrous sodium sulfate and concentrated under ultrapure nitrogen (N_2) and stored in dark flasks and kept under refrigeration (4°C) until analysis. The essential oil obtained was then analyzed by gas chromatography and mass spectroscopy (GC/MS) with 1 mL oil injection in Shimadzu QP 2010 system equipped with Rtx-5MS (Restek Corp) capillary columns (30 × 0.25 mm, 0.25 µm film thickness) working with the following temperature scheme: 60–240°C (10 min) at 3°C/min. Injector, transfer line, and ionization chamber temperature were 250, and 200°C, respectively and carrier gas helium (1 mL/min); split ratio 1:20. The components were identified by comparing their retention times and linear retention indices relative to a series of n-alkanes (C8-C20) with those of origanum oil samples. Additionally, computer matching was performed against the Adams Library (2006), NIST (2011), and FFNSC 2.

Specimen Preparation

Thirty-six resilient denture liner discs (SoftConfort, Dencril Comércio de Plásticos Ltda, São Paulo, Brazil) were prepared according to the three oil concentrations – 0, 2.5, and 5% ($n = 12$ for each group). To mimic the conditions of relining, specimens of denture liners were prepared over acrylic resin base discs (10 × 1 mm) using a stainless-steel matrix (10 mm diameter and 3 mm thickness). A uniform surface was ensured by placing glass slides on both sides of the matrix, firmly fixing both ends, and separating the glass slides after curing.

For the control group (0% oil), no origanum oil was added, and the materials were prepared according to the manufacturer's recommendation. For experimental groups, 2.5 and 5% of origanum oil were added to the denture liner vehicle. For the 2.5% group, 5.22 gm of powder and 3.9 mL of liquid were formulated with 0.3 mL of oil. For the 5% group, 5.09 g of powder and 3.8 mL of liquid were formulated with 0.6 mL of oil. Materials were prepared according to the manufacturer's recommendation at room temperature (20 ± 1°C and 50 ± 5% relative humidity), under aseptic conditions.

Specimen Handling Procedures

As stated previously, this was a randomized and blinded *in vitro* study. The labeling was performed by assigning a random number that distinguished one test group from another by a member of the laboratory team who did not participate in the evaluations of the tested groups. Samples from each test group were repackaged in identical unmarked containers and coded into a database to have a permanent record of each sample and group tested.

Ultraviolet sterilization of the specimens was performed before biofilm growth. Biofilms were grown for 2 hours (initial biofilm adherence) ($n = 6$) and 24 hours (biofilm development phase)

($n = 6$) on different discs of each oil concentration, totalizing $n = 12$ samples per group using 24-well polystyrene tissue culture plates to allow the microcosm biofilm formation and sequentially evaluate the antimicrobial activity.

Saliva Collection and Biofilm Formation

Stimulated saliva (40 mL) using paraffin film (Parafilm "M"®, American National CanTM, Chicago, IL, USA) was collected from a healthy donor who abstained 24 hours of oral hygiene and had not been under antibiotic therapy for the previous 6 months.[30] To promote the microcosm biofilm formation, the saliva was vortexed and inoculated on the liner denture discs disposed in 24-well plates (1 mL/well) remaining 1 hour at rest (37 °C). After this period, 1.8 mL of Defined Medium with Mucin with 1% sucrose (DMM + s) was added, and the wells were incubated in 5–10% CO_2, < 1% O_2 (Anaerobac - Probac do Brazil Bacteriological Products Ltda, Santa Cecília, SP, Brazil) in anaerobic jars at 37 °C for 2 or 24 hours.[30,31]

Biofilm Activity

At the end of 2 and 24 h of biofilm accumulation, the discs were aseptically removed from the wells and washed by gentle insertion in a new well containing 2 mL of sterilized saline solution.[32] Discs were subsequently added to a falcon tube containing phosphate butter saline (PBS) and sonicated at 30 w with 3 pulses of 10 seconds. The biofilm suspensions were subsequently serially diluted in PBS and 20 µL samples were plated in duplicate on Blood agar, Mitis salivarius agar, and CHROMagar *Candida*. Plates were incubated at 37°C under anaerobic (blood agar and Mitis salivarius agar) or aerobic (CHROMagar) conditions for 24–96 hours. Colony-forming units (CFU) were expressed in colony-forming units per disc surface area.

Surface Roughness

Surface roughness (Ra) of specimens were measured at baseline and after 2 and 24 hours of biofilm formation with a profilometer (SE 1200; Kosaka Laboratory Ltd, Tokyo, Japan) with a 0.01 mm resolution, calibrated with a cut-off value of 0.8 mm, 2.4 mm percussion of measure, and 0.5 mm/s. Three readings were made for each specimen and a mean value was calculated.[31] The mean surface area of specimens was 2.7 ± 0.2 mm^2.

Sorption-solubility Tests

For sorption and solubility tests, resilient denture liner and acrylic resin discs ($n = 10$; 15 mm diameter and 2 mm thickness) were prepared for each concentration group. Specimens were prepared as for the microbiological test, except the acrylic resin group (control) that was prepared according to the manufacturer's instructions. All specimens were stored in a desiccator at 37°C with silica gel and weighed daily to verify mass stabilization. The initial dry mass (m1) was recorded once the specimens reached a stable mass, with variations of less than 0.1 mg within any 24-hour period. The specimens were then immersed in distilled water at 37°C for 7 days to measure the saturated mass (m2). Finally, the specimens were returned to the desiccator at 37°C and reweighed until a constant dry mass (m3) was achieved. Specimens were then placed in the desiccator again, at 37°C and reweighed until a constant dry mass (m3) was obtained. The weighing was performed with an analytical balance with 0.01 mg accuracy (AUW220D; Shimadzu). The volume (V) of each specimen was calculated based on the following equation: $V = \pi R^2 h$, where R is the specimen radius. Water sorption (WS) and solubility (SL), given in µg/mm^3, were calculated as follows: WS = (m2–m3)/V; SL = (m1–m3)/V. cm^2.

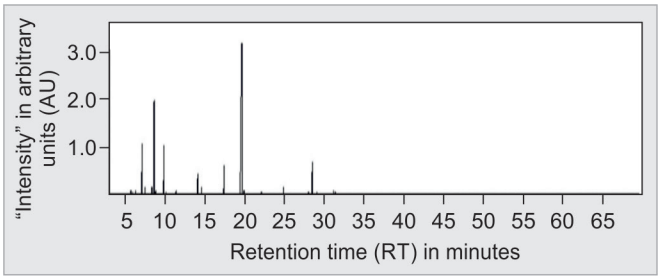

Fig. 1: Chromatogram displaying the retention times of key constituents in origanum oil, with peaks identified for oct-1-en-3-ol at 8.8 minutes, p-cymene at 12.2 minutes, thymol at 20.1 minutes, and β-bisabolene at 24.5 minutes

Statistical Analysis

Statistical analyses were carried out with statistical software (IBM SPSS Statistics v17; IBM Corp) ($\alpha = 0.05$). Water sorption and solubility were analyzed by one-way ANOVA and surface roughness by two-way ANOVA, followed by all pairwise comparisons. For microbiological analyses, data were transformed into rank and analyzed with three-way ANOVA followed by Tukey *post hoc* analysis.

Results

Figure 1 presents the peaks identified through infrared (IR) spectroscopy of origanum oil, with notable compounds including oct-1-en-3-ol, p-cymene, thymol, and β-bisabolene. (Table 1).

An increase in surface roughness was presented in the control group after 2 h ($p = 0.003$) and 24 hours ($p = 0.012$) of biofilm formation (Table 2). Similar results were observed for the 2.5% concentration group: 2 hours ($p < 0.001$) and 24 ours ($p < 0.001$), despite the absence of statistically significant changes in surface roughness in the 5% concentration group. Statistically significant differences were observed among groups after biofilm colonization (24 hours), with the 5% concentration group presenting higher values of surface roughness ($p < 0.001$) than control and 2.5% groups ($p > 0.05$) before and after biofilm colonization.

Sorption and solubility summary measures are presented in Table 3. For the sorption variable, considering the same material with different oil concentrations, no statistically significant differences were detected ($p = 0.347$ for acrylic resin and $p = 0.289$ for denture liner). For solubility, considering the same material with different oil concentrations, both materials presented statistically significant different solubility values ($p < 0.001$), showing a directly proportional increase of the solubility addressed to oil concentration.

The distribution and quantification of total microorganisms are presented in Figure 1.

There was a statistically significant reduction of total microorganisms after 24 hours for both concentrations evaluated ($p < 0.05$). After 24 hours, the 5% group presented a higher count of total microorganisms than the control group ($p < 0.05$) (Fig. 2).

For *Streptococcus mutans* count, there was a reduction after 24 hours in both concentrations evaluated ($p < 0.05$). There were no differences between the oil concentrations for *S. mutans* counts (Fig. 3).

For *Candida* spp. there were no statistically significant changes after 24 hours for both concentrations. However, data revealed

Table 1: Chemical characterization of Origanum oil using infrared (IR) spectroscopy*

TR	*Name*	*%*	*IR c*	*IR L*
4.055	2-E-hexenal	0.08	848	846
5.605	Tricyclene	0.01	924	921
5.690	α-Thujene	0.38	927	924
5.890	α-Pinene	0.30	934	932
6.300	Camphene	0.40	949	946
6.985	Sabinene	0.01	974	969
7.075	Oct-1-en-3-ol	5.39	977	974
7.310	3-Octanone	0.09	986	979
7.460	Myrene	0.64	991	988
7.925	α-Felandrene	0.09	1,006	1,002
8.125	δ-3-carene	0.04	1,012	1,008
8.325	α-terpinene	0.90	1,017	1,014
8.595	p-cymene	11.20	1,025	1,020
8.745	Limonene	0.34	1,029	1,024
8.845	1.8-cineol	0.37	1,031	1,026
9.015	Z-β-ocimene	0.04	1,036	1,032
9.395	E-β-ocimene	0.03	1,047	1,044
9.820	γ-terpinene	6.03	1,058	1,054
10.130	Cis-sabinene hydrate	0.25	1,067	1,065A/1,069F
10.250	n-octanol	0.02	1,070	1,063A/1,076F
10.940	Terpinolene	0.14	1,089	1,086
11.345	Linalol	0.62	1,100	1,095
12.255	cis-p-ment-2-en-1-ol	0.03	1,122	1,118
12.980	trans-pinocarveol	0.07	1,140	1,135
13.220	Camphor	0.14	1,145	1,141
14.080	Borneol	3.20	1,166	1,165
14.570	Terpinen-4-ol	1.01	1,178	1,174
14.675	Dec-1-en-3-ol	0.12	1,180	1,177
14.885	p-cimen-8-ol	0.25	1,185	1,179
15.150	α-terpineol	0.16	1,192	1,186
15.745	n-decanal	0.03	1,206	1,201
17.225	Cuminic aldehyde	0.03	1,240	1,238
17.400	Carvacrol methyl ether	4.19	1,245	1,241A/1,239F
19.635	Thymol	52.87	1,297	1,289
19.905	Carvacrol	0.66	1,303	1,298
22.095	Thymol acetate	0.38	1,355	1,349
23.055	α-Ilangene	0.03	1,377	1,373
23.440	β-bourbonene	0.08	1,387	1,387
24.870	E-caryophyllene	1.02	1,421	1,417
25.270	β-copaene	0.04	1,431	1,430
25.525	α-trans-bergamotene	0.02	1,437	1,432
25.665	Aromadendrene	0.16	1,441	1,439
26.255	α-humulene	0.03	1,455	1,452
26.375	E-β-farnesene	0.01	1,458	1,454
26.555	allo-aromadendrene	0.03	1,462	1,458
27.195	γ-muurolene	0.12	1,478	1,478
27.375	Germacrene D	0.23	1,483	1,484
27.950	Viridiflorene	0.19	1,497	1,486
28.005	Bicyclogermacrene	0.32	1,498	1,50A0/1,497F
28.140	α-muurolene	0.05	1,501	1,500A/1,497F
28.490	β-bisabolene	5.19	1,510	1,505
28.695	γ-cadinene	0.09	1,516	1,513
29.070	δ-cadinene	0.27	1,525	1,522
29.810	E-α-Bbisabolene	0.02	1,544	1,540
31.160	Espatulenol	0.49	1,579	1,577
31.380	Caryophyllene oxide	0.36	1,585	1,586
32.340	β-atlantol	0.04	1,610	1,608
	Total identified	99.30		

*Table 1 shows the chemical characterization of Origanum vulgare essential oil based on infrared (IR) spectroscopy. The table includes retention times (TR), compound names, the percentage of each identified compound (%), and the corresponding IR absorption values (IR c and IR L). IR c represents the calculated IR values observed from the experimental data, while IR L refers to the literature IR values

that the 2.5% concentration after 24 hours presented a statistically lower amount of *Candida* spp. than the 5% group at the same time ($p < 0.05$) (Fig. 4).

In this study, the integration of origanum oil into denture materials significantly reduced microbial counts and biofilm formation, with changes, compared to the control group, in solubility from 2.5% concentration and in surface roughness at 5% concentration at baseline and after 24 hours of biofilm formation.

Discussion

This study aimed to evaluate if the addition of a natural compound would result in a decrease of microorganisms by using a microcosms biofilm model, which is the closest to the oral environment *in vitro* model available. Although an antimicrobial property is desirable, the new material should maintain its original properties to be of clinical use. The assessment of these properties is important, considering, for example, that a high surface roughness could result in more biofilm accumulation and an increased potential to develop biofilm-based diseases.[31,32]

The results presented by this study are new and promising, as a desirable antimicrobial effect was achieved by incorporating a natural compound into resin-based materials. Previous preclinical studies have demonstrated the pharmacological properties of origanum oil as antimicrobial, antitumor, antioxidant, antimutagenic, insecticidal, hepatoprotective as well as an anti-inflammatory and anti-obesity agent.[17,33] Its nano technological applications as a promising pharmaceutical product have also been studied. Nevertheless, Origanum has been reported to cause some side effects such as angioedema, perioral dermatitis, allergic reactions, inhibition of platelet aggregation, hypoglycemia and miscarriage. However, conclusive evidence is still needed to decide on its clinical applications in human diseases.[18]

On the other hand, the extensive use of oregano in traditional medicine, as well as its pharmacological activities, lacks conclusive studies in animal models. Although the active constituents of the plant are already known, detailed studies on the bioactivities of isolated active constituents are still lacking. Furthermore, structure-activity studies on the isolated compounds can also reveal insights into the molecular mechanism of action of their bioactive fractions and/or compounds as health promoters as well as disease modifiers.[18]

It is already known that origanum essential oil presents a recognized antifungal activity shown in *Candida* biofilms,

Table 2: Surface roughness (Ra-µm) differences before and after biofilm growth in denture liners

	Origanum oil concentration			
Biofilm exposure	*Control (0%)*	*2.5%*	*5%*	*p-value***
Baseline	2.34 ± 0.28A[a]	2.22 ± 0.29A[a]	4.38 ± 0.90C[b]	<0.001
2 hours	2.88 ± 0.53B	3.04 ± 0.37B	4.81 ± 0.81C	
24 hours	2.98 ± 0.65B[a]	3.17 ± 0.63B[a]	4.30 ± 0.94C[b]	<0.001
p-value*	<0.05	<0.001	>0.05	

Values are mean ± standard deviation. Different uppercase letters in columns show Ra differences between baseline and biofilm exposure times. Different lowercase letters in rows show Ra differences between oil concentrations. *Paired sample *t*-Test. **ANOVA and Tukey *post hoc* tests

Table 3: Comparative analysis of sorption and solubility variables between different materials and oil concentrations

	Concentration			
	Control (0%)	*2.5%*	*5%*	
Variables	*Med (1ºQ; 3ºQ)*	*Med (1ºQ; 3ºQ)*	*Med (1ºQ; 3ºQ)*	*p-value***
Sorption				
Acrylic resin	16.5 (14.5; 17.9)[a]	16.5 (15.2; 17.9)[a]	14.6 (14.1; 16.7)[a]	0.347
Denture liner	18.3 (17.1; 21.0)[a]	20.2 (17.1; 23.8)[a]	18.0 (16.8; 20.2)[a]	0.289
p-value*	0.150	0.012	0.008	
Solubility				
Acrylic resin	0.1 (–1.8; 0.7)[a]	33.6 (31.4; 34.6)[b]	38.7 (37.9; 39.9)[c]	<0.001
Denture liner	6.3 (5.0; 21.0)[a]	15.4 (14.3; 16.3)[b]	20.9 (19.4; 22.9)[c]	<0.001
p-value*	<0.001	<0.001	<0.001	

Med, median; 1ºQ, first quartile; 3rd Q, third quartile; Different lowercase letters mean statistically significant differences between groups. *Mann–Whitney test. **Kruskal–Wallis followed by Dunn's test

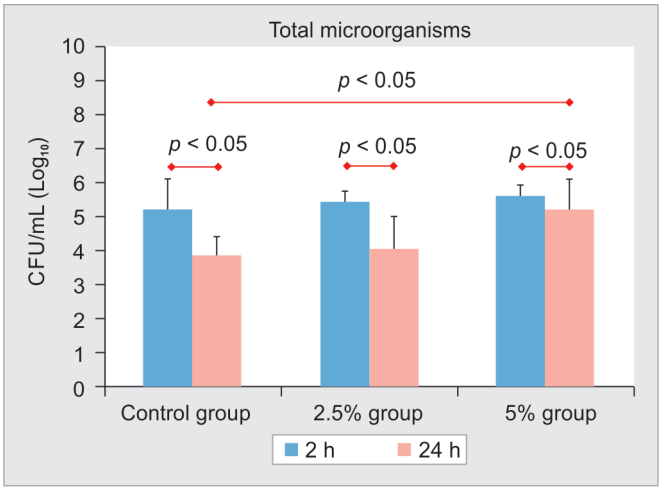

Fig. 2: Total microorganisms count in CFU/mL (Log_{10}) among groups and times tested

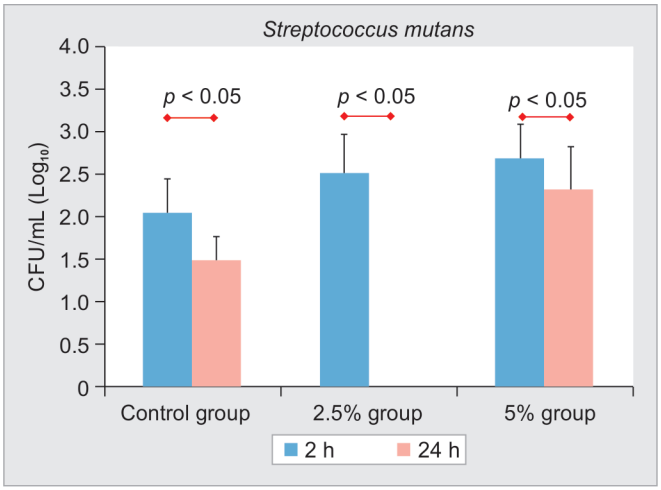

Fig. 3: *Streptococcus mutans* distribution in CFU/mL (Log_{10}) among groups and times tested

especially due to several compounds such as carvacrol and thymol.[23] Due to their hydrophobic character, these phenolic compounds act by dissolving the hydrophobic part of the bacteria membrane, increasing the membrane permeability, and causing a loss of the structure in the phospholipid bilayer. Hence, these two terpenes cause structural and functional damage to the cell membrane.[34] Moreover, thymol represented more than half of the oil composition used in this study, which could explain the presence of an antimicrobial effect. These two are well-known phenolic compounds and present analgesic, antifungal, and antibacterial effects, also showing to be effective against *Candida*.[22,23,34] Even with increasing improvement, resilient materials present physical-biological problems that compromise their clinical use for long periods. Due to the high absorption rate of oral fluids, these materials are affected by the loss of dimensional stability, poor adaptation of the denture, discoloration, and rupture between materials.[26,27] The loss of water, plasticizer, and ethanol leads the resilient materials to increase their hardness and consequently makes the material more porous, facilitating biofilm formation and maintenance over the surface.[35] Although only one relining material was tested, and this is a limitation of this study, it was clear that the addition of a

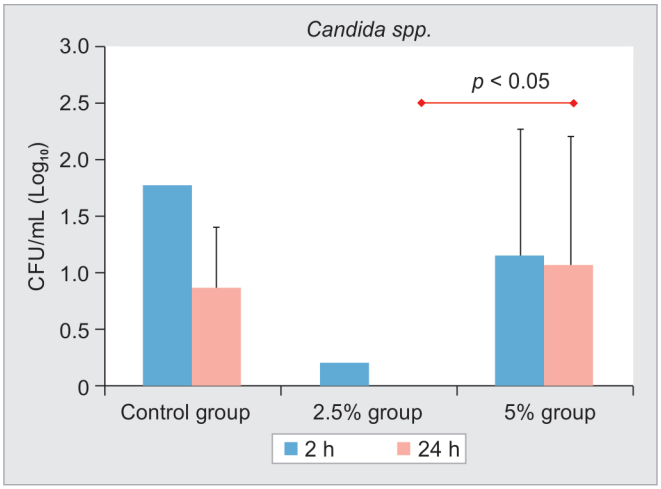

Fig. 4: *Candida* spp. distribution in CFU/mL (Log_{10}) among groups and times tested

5% concentration is not feasible for denture liners, considering that there was a huge increase in surface roughness even before microbial colonization. In contrast, adding 2.5% oil resulted in characteristics similar to the control group and still presenting the desirable antimicrobial activity, making it a possible choice for the addition of essential oil at this concentration.

The results showed that while the addition of origanum oil reduced microorganism counts, particularly *S. mutans*, it also increased surface roughness and solubility of the materials, albeit only at the higher concentration. These changes suggest a partial alteration of the material properties, likely due to chemical interactions or mechanical stress during incorporation. On the other hand, further studies are needed to confirm these findings and optimize the use of origanum oil in denture materials for antimicrobial purposes.

Considering the antimicrobial characteristics, although *Candida* counts were not affected by the origanum oil addition, total microorganisms and *S. mutans* were, and this fact is probably more important than trying to decrease one specific microorganism, as almost all biofilm-based diseases are polymicrobial in nature. This is the reason why single species biofilm tests, although important, may not stand for mouth-like conditions. Single-species biofilm tests, while valuable, may not fully capture the complexity of oral biofilms. Biofilm-based diseases often arise from polymicrobial interactions. Multi-species biofilm models, such as the microcosm model, better simulate the intricate microbial dynamics and environmental conditions found in the mouth.[30,31] These models are indispensable for developing effective antimicrobial treatments tailored to combat the diverse microbial communities responsible for biofilm-related diseases.

It is known that *C. albicans* may benefit from other bacteria by using the extracellular matrix as a shield, while bacteria present in the biofilm modulate host response, upregulating cytokines when co-cultured with fungi.[36] Also, some bacteria have synergisms with *C. albicans*, making environmental conditions adequate for its maintenance. A clinical study performed by Valentini et al. demonstrated these microbial interactions, showing that the decrease of *S. mutans* is beneficial and impaired biofilm formation rate.[37] The choice of those microorganisms is because *S. mutans* is regularly found in denture plaque, while *C. albicans* and other species are the main microorganisms responsible for denture stomatitis. Still, it is known that *S. mutans* increases the growth of *Candida* spp., presenting mutual growth stimulation and co-aggregation with each other, which may enhance the adhesion process.[38] In addition, *S. mutans* affects virulence parameters of *Candida* and this has to be taken into account in studies dealing with the prevention and treatment of denture stomatitis.[39]

Furthermore, this is the first study to gather the antimicrobial effect together with the possible changes of the material with origanum oil added to the acrylic resin and resilient denture liners assessed at different times of biofilm formation. However, the hypothesis of the present study was partially accepted, since the addition of origanum oil reduced the biofilm formation, but changed the properties of tested materials only in terms of surface roughness and solubility.

Conclusion

Within the limitations of this study and based on the results obtained, it is concluded that 2.5% origanum oil concentration showed effective antimicrobial properties against *Candida* spp. and *S. mutans* in a complex biofilm model without significant changes in the resilient denture lining material. Therefore, adding origanum oil into resilient denture liners could be a good alternative as an antimicrobial substance with simple steps and low costs for implementation.

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