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Original Article

THE EFFECT OF ORIGANUM VULGARE GEL ADDITION TO AUTO-POLYMERIZED SILICONE SOFT DENTURE LINER AT VARYING CONCENTRATIONS AND EXPOSURE TIMES ON SURFACE ROUGHNESS AND ANTIFUNGAL ACTIVITY AGAINST *CANDIDA ALBICANS*

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ABSTRACT

Objective: Soft denture liners (SDLs) present certain drawbacks, such as weak bonding with the acrylic resin denture base and increased surface roughness, which may result in porosity. These factors can compromise SDL and the denture base, thereby promoting the colonization of *Candida albicans*.

Methods: A total of 120 samples were divided into four concentration groups (0%, 6.25%, 12.5%, and 25%) and three exposure time groups (7, 14, and 21 days). Each group was tested for antifungal inhibition activity and surface roughness using a universal testing machine. Data were analyzed using one-way ANOVA with Dunnett's T3 post-hoc test to determine the significance of differences among groups ($p<0.05$).

Results: The addition of oregano gel significantly affected the surface roughness between the SDL and the denture base. Higher concentrations and longer exposure times compromised SDL-denture base adhesion, promoting *Candida albicans* colonization. Among the treatment groups, 6.25% oregano gel demonstrated better performance compared to nystatin, maintaining higher bond strength and reducing fungal adhesion.

Conclusion: The addition of 6.25% oregano gel improved the antifungal effect while maintaining adequate surface roughness of the SDL to the denture base, outperforming nystatin under similar conditions.

Keywords: Origanum vulgare, Oregano gel, Auto-polymerized silicone soft denture liner, Silicone soft liner, Surface roughness

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INTRODUCTION

Complete dentures are removable prosthetic appliances designed to replace the entire dentition and associated oral structures of both the maxilla and mandible [1]. These restorations are crucial for restoring masticatory function, aesthetics, speech, and patient confidence. However, despite their benefits, complete dentures have notable drawbacks, such as compromised stability, decreased chewing efficiency, limited retention, and susceptibility to mucosal irritation if not properly maintained [2]. These limitations may significantly reduce the quality of life in denture wearers, particularly in elderly populations.

The prolonged use of complete dentures is associated with further complications, including progressive reduction in denture retention, chronic irritation of the oral mucosa, and increased risk of microbial colonization, especially by *Candida albicans*, the primary pathogen implicated in denture stomatitis [3]. To improve retention and restore intimate contact between the denture base and mucosa, relining procedures are frequently employed. Relining involves applying new base material to the intaglio surface of a denture, enhancing its fit and comfort [4]. Among the materials used for this purpose, soft denture liners (SDLs) have gained popularity due to their viscoelastic properties, which provide a cushioning effect and minimize pressure-induced trauma on the mucosal tissues [5]. SDLs are widely used in full and partial removable dentures to increase patient comfort and adapt to dynamic anatomical changes [6].

Nonetheless, SDLs present several limitations that affect their clinical longevity and performance. These include poor adhesion to the acrylic resin base, increased surface roughness over time, and porosity, all of which create niches for microbial colonization [7]. These surface alterations compromise the durability of SDLs and provide an environment conducive to fungal adherence and biofilm formation, particularly by *Candida albicans* [8]. Various preventive strategies have been proposed to counter these limitations. Surface coatings or sealers can reduce surface roughness and microbial adhesion, although their long-term effectiveness remains questionable. Mechanical cleaning methods, such as brushing, are also used, but they may exacerbate surface degradation and increase roughness [9, 10].

Given the limitations of both mechanical and surface-coating approaches, the incorporation of antifungal agents into SDLs has emerged as a promising alternative to prevent denture stomatitis. Chemical antifungals like nystatin have been effective in inhibiting fungal growth, but their use is limited by side effects such as mucosal irritation, systemic toxicity, drug interactions, and the emergence of resistant fungal strains [11, 12]. In response to these challenges, there has been growing interest in exploring plant-derived antifungal agents, particularly essential oils, as safer and potentially effective alternatives.

Among herbal antifungals, *Origanum vulgare* (oregano) essential oil has garnered significant attention due to its potent antifungal activity, attributed primarily to its major constituents, carvacrol and thymol [13]. These compounds exhibit broad-spectrum antimicrobial properties, including activity against *Candida* species. In a previous study, Srivastava *et al.* incorporated oregano essential oil at a concentration of 60% into a tissue conditioner and found minimal changes in surface roughness after seven days, suggesting that high concentrations may be effective in maintaining material integrity while exerting antifungal effects [3]. However, using high concentrations of essential oils may raise concerns about cytotoxicity, potential alteration of material properties, and patient acceptance due to strong aroma and taste.

To address these concerns, recent research has shifted toward optimizing the minimal effective dose of oregano essential oil that still maintains antifungal efficacy without compromising the physical properties of SDLs. This approach emphasizes both efficacy and safety. Bhat *et al.* investigated the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of oregano essential oil across a wide range of concentrations (from

50% to 0.024%) [14]. The MIC, defined as the lowest concentration capable of producing an inhibition zone ≥15 mm, was found to be 3.12%, while the MFC, identified as the lowest concentration capable of killing>99% of the fungal population, was established at 6.25% [14, 15]. These thresholds were further supported by the findings of Godil *et al.*, who confirmed 3.12% as a suitable MIC for antifungal screening [16].

Despite the promising results of these *in vitro* studies, relatively few investigations have tested low concentrations of oregano essential oil (e. g., 3.12% and 6.25%) in actual denture liner applications. Most prior studies, such as that by Srivastava *et al.*, have utilized considerably higher concentrations (60%), making it difficult to determine the lowest concentration that remains effective yet safe and stable when incorporated into soft denture materials [3]. Therefore, the novelty of the current study lies in its attempt to optimize the antifungal potential of *Origanum vulgare* essential oil at clinically relevant, lower concentrations. By using 3.12% (MIC) and 6.25% (MFC), this study aims to fill the gap in the literature, particularly in relation to the surface roughness and antifungal activity of SDLs modified with these concentrations over varying exposure durations.

Additionally, this study builds upon and attempts to reconcile conflicting findings in the literature. For instance, while Srivastava *et al.* reported high efficacy at 60%, Bhat *et al.* demonstrated that much lower concentrations may be sufficient to inhibit or eradicate *Candida albicans* [3, 14]. Integrating these insights, alongside recent findings from 2023–2024 on herbal antifungal integration into dental materials, this study aims to clarify the optimal concentration range for oregano oil application in SDLs.

MATERIALS AND METHODS

Ethical approval

This research was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, with approval number: 345/KEPK/USU/2024. All procedures were conducted in accordance with the Declaration of Helsinki.

Sample size and justification

Two types of tests were conducted: surface roughness analysis and antifungal activity assay. The number of samples was determined by referencing similar *in vitro* studies and considering resource feasibility. For the surface roughness test, a sample size of $n = 5$ per group (totaling 40 specimens) was adopted based on comparable studies that detected statistically significant surface changes with similar group sizes [1, 2]. For the antifungal activity assay, each group consisted of $n = 7$ samples (totaling 28), as supported by related antifungal susceptibility testing protocols [3, 4]. Future studies will incorporate formal power calculations to enhance statistical robustness.

Origanum vulgare essential oil specifications

The oregano essential oil used in this study was obtained from Happy Green® (Indonesia). The oil had a declared purity of 98% and was extracted from *Origanum vulgare* via steam distillation. According to the product certificate, the major active compounds were carvacrol (70%) and thymol (15%). The molecular weight of carvacrol is 150.22 g/mol. The concentration of oregano oil used was defined as volume/volume (v/v), not weight/volume (w/v).

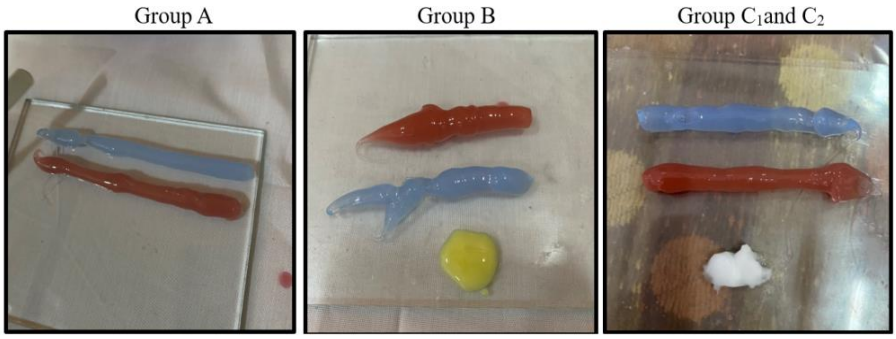

Fig. 1: Base and catalyst were mixing according to the manufacturer's instructions. For groups B, C_1 and C_2, before mixing, 10% antifungal agents were added and then mixed with the base and catalyst

Antifungal activity assay

Twenty-eight disc-shaped SDL specimens (6 mm diameter × 1 mm thickness) were prepared and divided into four groups (n = 7/group):

Group A: SDL without antifungal agent

Group B: SDL+10% nystatin

Group C: SDL+3.12% *Origanum vulgare* gel

Group C3: SDL+6.25% *Origanum vulgare* gel

The antifungal testing used the disc diffusion method with Candida albicans ATCC®10231™ on Sabouraud Dextrose Agar (SDA). Fungal suspensions were adjusted to 1×10^6 colony-forming units per milliliter (CFU/ml) (McFarland 0.5 standard). Sample discs were placed on the inoculated SDA surface and incubated at 37 °C for 24 h. Inhibition zones were measured in three directions and averaged.

Candida albicans culture and inhibition zone test

The antifungal activity was evaluated using the disc diffusion method. Candida albicans ATCC®10231™ was cultured on Sabouraud Dextrose Agar (SDA) and incubated at 37 °C for 24 h. The suspension was adjusted to a 0.5 McFarland standard (~1×10^6 colony-forming units per milliliter (CFU/ml)). A sterile cotton swab was used to evenly inoculate the SDA plate.

Sterile SDL discs were placed onto the inoculated agar and incubated at 37 °C for 24 h. The inhibition zones were measured in millimeters using a digital caliper on day 7 and day 14. The mean of three measurements was calculated.

While *Candida albicans* ATCC®10231™ is a reference strain for reproducibility, it does not encompass clinical variability or resistance profiles seen in patient-derived isolates, which is a limitation of this study [6, 7].

Determination of MIC and MFC

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *O. vulgare* oil were pre-determined using concentrations ranging from 0.024% to 50% (v/v). MIC was defined as the lowest concentration that produced a clear inhibition zone>15 mm, which was identified at 3.12%, consistent with literature [5, 8]. MFC was determined via total plate count, defined as the lowest concentration resulting in>99% reduction in colony formation, achieved at 6.25% [9, 10].

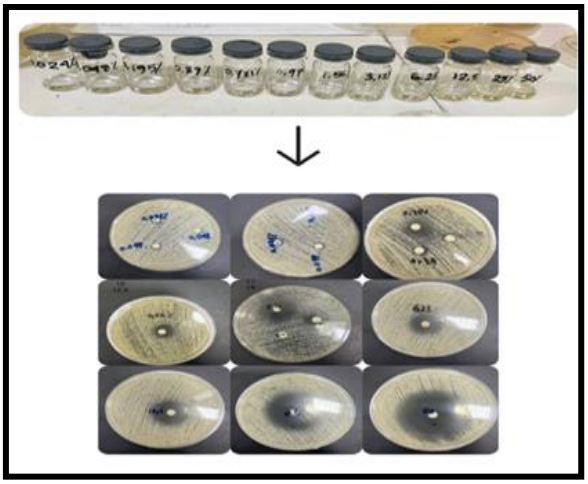

Fig. 2: Minimum inhibition concentration test

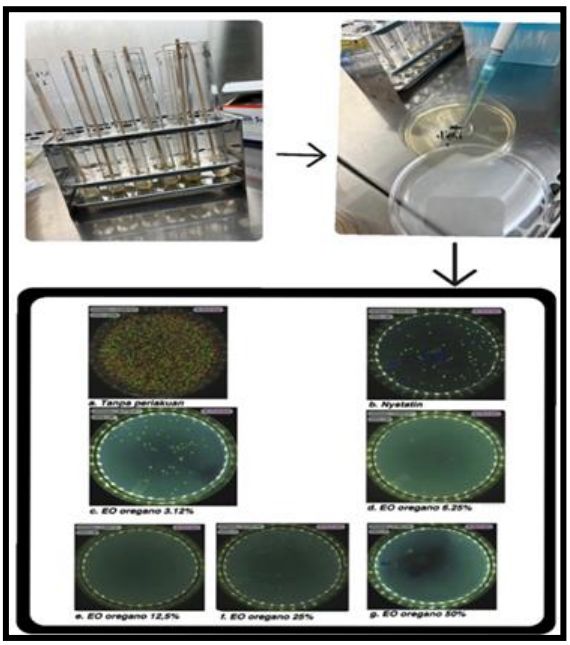

Fig. 3: Minimum fungicidal concentration test

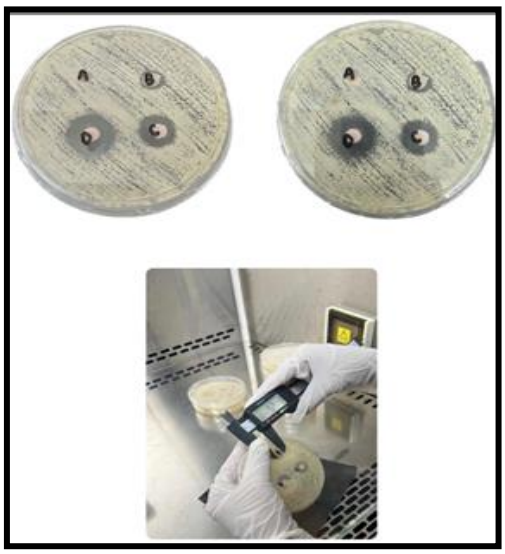

Fig. 4: Procedure of *Candida albicans* inhibition zone

Surface roughness sample preparation

Forty specimens of auto-polymerized silicone soft denture liner (SDL) (Mollosil, Detax GmbH, Germany) were prepared in cylindrical molds (15 mm diameter × 10 mm thickness). The samples were allocated into eight groups (n = 5/group):

-A1: SDL without antifungal agent, tested at 7 d

-A2: SDL without antifungal agent, tested at 14 d

-B1: SDL+10% nystatin (Taisho Mycostatin®, Japan), tested at 7 d

-B2: SDL+10% nystatin, tested at 14 d

-C1: SDL+3.12% *Origanum vulgare* oil gel, tested at 7 d

-C2: SDL+3.12% *Origanum vulgare* oil gel, tested at 14 d

-C3: SDL+6.25% *Origanum vulgare* oil gel, tested at 7 d

-C4: SDL+6.25% *Origanum vulgare* oil gel, tested at 14 d

Antifungal agents were mixed evenly into the base material prior to the addition of the catalyst. The mixture was poured into molds and polymerized under pressure using a hydraulic press. The surfaces were trimmed with a scalpel and coated with varnish to prevent contamination.

Surface roughness testing

Specimens were immersed in distilled water at 37 °C for 7 and 14 d. After incubation, they were rinsed and air-dried. Surface roughness was measured using a Mitutoyo Surftest SJ-210 Profilometer (Mitutoyo Corp., Japan). Measurements were taken in triplicate per sample across a 2-mm path, and the mean average roughness (Ra) value (µm) was recorded.

Fig. 5: Surface roughness are measurement with profilometer

Statistical analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Normality was tested using the Shapiro–Wilk test. One-way ANOVA followed by post-hoc Dunnett's T3 correction was used to assess differences between groups. A p-value<0.05 was considered statistically significant. Adjustments for multiple comparisons were made to control for Type I error across all post-hoc analyses.

RESULTS

The research data were processed using SPSS version 21 for statistical analysis. Prior to conducting One-Way ANOVA, data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was verified with Levene's test. Both tests confirmed that the data met the assumptions for ANOVA. The statistical analysis included univariate tests to evaluate surface roughness and *Candida albicans* inhibition zones, One-Way ANOVA to examine the effect of adding *Origanum vulgare* essential oil gel at different concentrations, and Dunnett's T3 post-hoc test with adjustments for multiple comparisons to identify significant differences between groups. The rationale for selecting specific post-hoc comparisons, particularly between Group B2 (nystatin at 14 d) and Groups C2 and C4 (oregano oil at 3.12% and 6.25% for 14 d), was based on matching usage duration to assess relevant clinical differences. Effect sizes (partial eta squared, η^2) were also reported alongside p-values to indicate the practical significance of the findings.

Surface roughness measurements after 7 d of application showed mean values of 0.16±0.09 µm for the control group without antifungal agent (A1), 0.13±0.11 µm for the 10% nystatin group (B1), and 0.06±0.03 µm and 0.06±0.007 µm for the oregano oil groups at 3.12% (C1) and 6.25% (C2), respectively. After 14 d, the values were 0.33±0.07 µm (A2), 0.69±0.21 µm (B2), 0.12±0.02 µm (C3), and 0.08±0.01 µm (C4). One-Way ANOVA revealed that neither nystatin nor oregano oil gel at 3.12% and 6.25% concentrations produced statistically significant differences in surface roughness after 7 d ($p = 0.080$; $\eta^2 = 0.13$; $p>0.05$). However, a significant effect was observed after 14 d of gel application ($p = 0.001$; $\eta^2 = 0.47$; $p<0.05$). Post-hoc Dunnett's T3 analysis indicated statistically significant differences between the nystatin 14 d group (B2) and both oregano oil groups at 3.12% (C2) and 6.25% (C4) with p-values of 0.016, 0.012, and 0.006, respectively ($p<0.05$). These results suggest that *Origanum vulgare* essential oil gel at these concentrations significantly reduces surface roughness of the soft denture liner compared to 10% nystatin after 14 d of use. The choice of comparisons was justified by the matching exposure durations, ensuring clinically relevant interpretation of the differences observed.

Inhibition zone of *Candida albicans* activity

The univariate test results for the inhibition zone of *Candida albicans*, based on the 7 d usage duration, showed the following mean and standard deviation: group A1 (0 mm), group B1 (9.5±0.44 mm), group C1 (13.6±0.85 mm), and group D1 (15.67±0.55 mm). For the 14 d usage duration, the results were: group A2 (0 mm), group B2 (9.06±0.31 mm), group C2 (11.54±0.94 mm), and group D4 (15.23±0.62 mm). Group a (control) showed no inhibition zone, indicating no antifungal activity.

As shown in table 2, the inclusion of *Origanum vulgare* essential oil at 3.12% and 6.25% markedly impacted the inhibition zone of *Candida albicans* on the silicone-based soft liner. Statistical

evaluation revealed that there were substantial differences in inhibition zones between groups treated with the essential oil and the control group, both after 7 and 14 d of use. Post-hoc analyses using Dunnett's T3 test for the 7 d usage duration showed significant differences between all groups: A1 vs. B1 ($p<0.001$), A1 vs. C1 ($p<0.001$), and B1 vs. C1 ($p<0.001$). Similarly, for the 14 d usage duration, the differences were significant for all groups: A2 vs. B2 ($p<0.001$), A2 vs. C2 ($p<0.001$), and B2 vs. C2 ($p = 0.001$).

Table 1: Effect of the addition of 3.12% *Origanum vulgare* essential oil gel on the zone of inhibition *CFU = colony-forming units; SD = standard deviation of *Candida albicans* on a silicone soft denture liner based on usage duration (7 and 14 d)

Usage duration	Group	Average candida inhibition zone±SD (mm)	p-Value
7 d	A1	0	<0.001*
	B1	9.5±0.44	
	C1	13.6±0.85	
	C3	15.67±0.55	
14 d	A2	0	<0.001*
	B2	9.06±0.31	
	C2	11.54±0.94	
	C4	15.23±0.62	

Post-hoc dunnett's T3 test, Group A1 vs. B1: $p<0.001$*, Group A1 vs. C1: $p<0.001$*, Group B1 vs. C1: $p<0.001$*, Group A2 vs. B2: $p<0.001$*, Group A2 vs. C2: $p<0.001$*, Group B2 vs. C2: $p = 0.001$*, *Significant at $p<0.05$.

Table 2: Effect of the addition of 6.25% *Origanum vulgare* essential oil gel on the zone of inhibition, *CFU = colony-forming units; SD = standard deviation of *Candida albicans* on a silicone soft denture liner based on usage duration (7 and 14 d)

Usage duration	Group	Average candida inhibition Zone±SD (mm)	p-Value
7 d	A1	0	<0.001*
	B1	9.5±0.44	
	C3	15.67±0.55	
14 d	A2	0	<0.001*
	B2	9.06±0.31	
	C4	15.23±0.62	

Post-hoc dunnett's T3 test**:** Group A1 vs. B1: $p<0.001$*, Group A1 vs. C3: $p<0.001$*, Group B1 vs. C4: $p<0.001$*, Group A2 vs. B2: $p<0.001$*, Group A2 vs. C4: $p<0.001$*, Group B2 vs. C4: $p = 0.001$*, *Significant at $p<0.05$.

Table 3: Effect of the addition of nystatin and *Origanum vulgare* essential oil gel (3.12% and 6.25%) based on usage duration, *Ra = average roughness; SD = standard deviation (7 and 14 d) on surface roughness of auto-polymerized silicone soft denture liner

Usage duration	Group	Average surface roughness±SD (µm)	p-Value
7 d	A1	0.16±0.09	0.080
	B1	0.13±0.11	
	C1	0.06±0.03	
	C2	0.06±0.007	
14 d	A2	0.33±0.07	<0.001*
	B2	0.69±0.21	
	C2	0.12±0.02 µm	
	C4	0.08±0.01 µm	

Post-hoc Dunnett's T3 Test: Group B2 vs. C2: $p = 0.016$*, Group B2 vs. C4: $p = 0.012$*, Group C2 vs. C4: $p = 0.006$*, **Significant at $p<0.05$.*

DISCUSSION

Soft denture liners (SDLs) are commonly formulated with fillers such as nanoscale amorphous fumed silica, which have a high affinity for water molecules. The polymerization process of these liners involves crosslinking, during which two parallel reactions occur: the dissolution of water-soluble substances from the liner into the surrounding medium, and the absorption of water molecules into the polymer matrix itself. The dynamic equilibrium between these two processes is critical for preserving the dimensional stability and surface integrity of the material. Particularly in silicone-based SDLs, water penetration is a key factor contributing to surface roughness, as absorbed water can swell the matrix and increase porosity.

In this study, all samples were immersed in distilled water and maintained at a consistent temperature to mimic intraoral conditions. The untreated control group (Group A) consistently exhibited the highest surface roughness values at both 7 and 14 d. This finding can be explained by the lack of any antifungal or modifying agents, allowing free water absorption that increased microporosity and consequently surface irregularities. This correlates well with existing literature that reports increased surface roughness due to unimpeded water uptake in silicone soft liners [17, 18].

The addition of antifungal agents, specifically nystatin and *Origanum vulgare* essential oil gel at concentrations of 3.12% and 6.25%, significantly influenced surface roughness. At 14 d, the nystatin-treated group (B2) showed the highest surface roughness among the treated groups. This increase is likely due to the gradual release of nystatin's active nanoparticles, which can create microscopic voids and pores as antifungal agents diffuse out from the matrix. The reactivity of these nanoparticles has been documented in earlier studies as a factor that increases surface roughness by physically altering the material surface over time [19, 20].

Conversely, the group treated with 6.25% *Origanum vulgare* essential oil gel exhibited the lowest surface roughness (C4). The hydrophobic nature of key bioactive compounds such as carvacrol and thymol is thought to reduce water absorption by filling microvoids within the polymer matrix, thereby stabilizing the surface texture and preventing excessive swelling. This observation aligns with Abdallah *et al.* (2021), who demonstrated that natural additives with hydrophobic properties, like curcumin extract, reduce

water penetration and improve surface smoothness in acrylic-based soft liners [21].

The present findings underscore the importance of the chemical and physical interactions between the polymer matrix and incorporated antifungal agents. Surface roughness is not solely dependent on water absorption but is also influenced by how additives interact with the matrix to modify its mechanical and physicochemical characteristics [22]. The smooth surface maintained by the essential oil gel suggests better clinical durability and patient comfort due to lower plaque accumulation risk and irritation.

Regarding the antifungal activity, the presence of *Candida albicans* on denture surfaces poses a significant risk for oral fungal infections, especially in immunocompromised individuals. Incorporating antifungal agents into SDLs aims to mitigate this risk by preventing microbial colonization. In the control groups without antifungal additives (Group A), no inhibition zones were observed against *Candida albicans*, confirming the absence of intrinsic antifungal activity.

Both nystatin and *Origanum vulgare* essential oil gel showed antifungal efficacy, but the essential oil, particularly at 6.25%, demonstrated a larger and more sustained inhibition zone. The phenolic compounds carvacrol and thymol exert their antimicrobial effects by disrupting fungal cell membranes and inhibiting enzymatic activities critical for fungal viability [23, 24]. Nystatin, by contrast, targets ergosterol in fungal membranes to form pores that lead to cell death; however, its limited solubility and slower release kinetics from the soft liner may reduce long-term effectiveness [25].

The larger inhibition zones seen with *Origanum vulgare* essential oil gel suggest a more consistent release of active antifungal compounds compared to nystatin. This result concurs with studies showing that higher concentrations of natural herbal extracts improve antifungal activity, although the effect may decline over extended periods due to gradual compound depletion [26]. The superior antifungal performance combined with the preservation of surface smoothness highlights *Origanum vulgare* essential oil as a promising natural alternative for SDL incorporation.

While nystatin remains a gold standard antifungal agent, its influence on the mechanical and physical properties of the liner such as increasing surface roughness could compromise liner longevity and patient comfort [27].

CONCLUSION

The study demonstrated that the addition of 6.25% Origanum vulgare essential oil gel to auto-polymerized silicone soft denture liners significantly reduced surface roughness and exhibited stronger antifungal activity against Candida albicans compared to lower concentrations (3.12%) and nystatin. This higher concentration of oregano oil showed superior efficacy in maintaining the material's surface integrity while effectively inhibiting fungal growth over a 14 d period. Clinically, these findings suggest that incorporating 6.25% *Origanum vulgare* essential oil into soft denture liners could potentially reduce the incidence of denture stomatitis by minimizing fungal colonization and improving material performance. Further long-term studies are recommended to confirm the durability and safety of this natural antifungal agent in prosthodontic applications.

AUTHORS CONTRIBUTIONS

All authors contributed significantly to the conception, research, data preparation, data analysis, and interpretation. They were also involved in preparation and revision of the article. All authors agreed to submit their work to the current journal and agreed to take responsibility for all aspects of the research.

CONFLICT OF INTERESTS

The authors affirm that they have no conflicts of interest related to this research.

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