

Effect of Oregano on Silicone Soft Denture Liner

Ariyani*^{}, Ricca Chairunnisa^{}, Sherley Margan, Sherina Annastasya, Natalia Hotmauli Sianturi

Department of Prosthodontics, Faculty of Dentistry, Universitas Sumatera Utara, Medan, 20155, Indonesia

*Corresponding Author: ariyani@usu.ac.id

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ABSTRACT

Silicone soft denture liner (SDL) is designed to absorb, reduce, and distribute pressure on denture-bearing tissues. The properties include a poor bonding strength to heat-polymerized acrylic resin base and surface roughness which promotes *Candida albicans* adhesion. SDL absorbs disinfectant solution during the immersion of denture in a disinfectant solution, leading to stiffness. Therefore, this study aimed to evaluate the effectiveness of oregano essential oil as an antimicrobial and the impact on the physical and mechanical properties of SDL. Antimicrobial test was conducted on 27 samples (3 groups) using disc diffusion method and measured with a digital caliper, while surface roughness, elastic modulus, and bond strength test was performed on 20 samples (4 groups). Surface roughness was evaluated with a profilometer. Furthermore, elastic modulus and bond strength were assessed using the universal testing machine (UTM). The results of one-way ANOVA test showed significant differences ($p < 0.05$) in all groups of antimicrobial, surface roughness, elastic modulus, and bond strength tests showed significant differences ($p < 0.05$). In conclusion, the addition of oregano essential oil to SDL, enhanced the antimicrobial properties, without compromising its physical and mechanical properties. Oregano extract is expected to be developed as a commercial product.

Keywords: Oregano, Soft Liner, Candida Albicans, Inhibition Zone, Mechanical Properties.

ABSTRAK

Soft Denture Liner (SDL) silikon autopolimerisasi merupakan sebuah bantalan yang memiliki sifat viskoelastik yang dapat menyerap, mengurangi, dan mendistribusikan tekanan ke *denture-bearing tissues*. SDL memiliki kekuatan lekat yang buruk dengan basis resin akrilik polimerisasi panas dan kekasaran permukaan sehingga rentan terhadap perlekatan *Candida albicans*. Perendaman gigi tiruan pada larutan disinfektan dapat menyebabkan penyerapan larutan ke dalam SDL, sehingga SDL menjadi kaku. Penelitian ini bertujuan untuk mengevaluasi efektivitas *essential oil* oregano sebagai bahan antimikroba dan pengaruhnya terhadap sifat fisik dan mekanis dari SDL. Penelitian ini menggunakan 27 sampel (3 kelompok) untuk pengujian antimikroba dan 20 sampel (4 kelompok) untuk pengujian kekasaran permukaan, modulus elastisitas, dan kekuatan lekat. Uji antimikroba dilakukan dengan metode *disc diffusion* menggunakan kaliper digital, uji kekasaran permukaan dilakukan dengan menggunakan profilometer, uji modulus elastisitas dan kekuatan lekat dilakukan dengan menggunakan *universal testing machine* (UTM). Hasil dari penelitian ini dianalisis menggunakan uji one-way ANOVA menunjukkan adanya perbedaan signifikan pada pengujian antimikroba, kekasaran permukaan, modulus elastisitas, dan kekuatan lekat ($p < 0.05$). Kesimpulan penelitian ini, bahwa terdapat pengaruh penambahan *essential oil* oregano pada SDL terhadap sifat antimikroba tanpa memengaruhi sifat fisik, dan mekanisnya. Penulis berharap bahwa ekstrak oregano dapat digunakan sebagai produk komersial.

Kata kunci: Oregano, *Soft Liner*, Candida Albicans, Zona Penghambatan, Sifat Mekanik.



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1. Introduction

Complete dentures are removable dental prostheses designed to replace all teeth and associated structures in the upper and lower jaw [1]. Tooth loss, accompanied by alveolar ridge resorption, can lead to a sharp, painful ridge that causes discomfort when using the denture. This problem can be overcome by relining it with a soft denture liner (SDL) [2].

Auto-polymerized silicone SDL has elastic properties, serving as a shock absorber or cushion to absorb, reduce, and distribute the pressure exerted on the base to denture-bearing tissues [3,4]. However, the limitations include poor bond strength to the heat-polymerized acrylic resin base and surface roughness that promotes the adhesion of *Candida albicans*, a common cause of denture stomatitis. The prevalence of denture stomatitis among complete denture users ranges from 30-50%. Therefore, the selection of methods to prevent the growth of *Candida albicans* is very necessary [5,6].

Immersion in disinfectant solution is a commonly used method for prevention. For instance, sodium hypochlorite is effective in eliminating microorganisms. Immersion can reduce bond strength, increase water absorption, increase surface roughness, and decrease the elastic properties of SDL over time [7,8]. An alternative method to mitigate the limitation of immersion is the incorporation of antimicrobial agents, either synthetic or natural. Overuse of synthetic antifungal agents, such as nystatin, may lead to side effects and resistance with prolonged application. Meanwhile, natural antifungal ingredients, such as oregano (*Origanum vulgare*) essential oil, present fewer side effects when incorporated into SDL [9].

Oregano essential oil has been studied as an antifungal agent in tissue conditioners, effectively reducing fungal colonization [10]. Its application for 1, 3, and 7 days showed effective natural antifungal properties without adversely affecting the physical and mechanical properties of tissue conditioners. Based on the aforementioned background, oregano (*Origanum vulgare*) essential oil has been incorporated into SDL as a preventive antimicrobial agent [3,11,12].

2. Materials and Methods

The ethical clearance of this study was approved by the Ethics Committee of Health Study, University of Sumatera Utara, with number 510/KEPK/USU/2022. This study was a laboratory experiment with a post-test-only control group design conducted between June and October 2022.

For the antimicrobial test, 27 samples of auto-polymerized silicone SDL (Mollosil Detax GmbH, Ettlingen, Germany) with a 6 mm diameter and 1 mm thickness were used. The samples were divided into three groups, namely SDL without antifungal agent addition (group A), SDL with 10% nystatin (Taisho Mycostatin® Nystatin Oral Suspension, Japan) (group B), and SDL with 3.125% (based on pilot study) gel-based oregano essential oil (Happy Green, Indonesia) (group C). As for the measurement of surface roughness, elastic modulus, and bond strength, 20 samples were used. The samples were divided into four groups, namely SDL without an antifungal agent (group A), SDL with immersion in sodium hypochlorite solution (Group B), SDL with 10% nystatin (Taisho Mycostatin® Nystatin Oral Suspension, Japan) (Group C), and SDL with 3.125% gel-based oregano essential oil (Group D).

The procedure began with a chemical analysis of oregano essential oil using gas chromatography-mass spectrophotometry (GC-MS). Subsequently, the sample was prepared by mixing the base and catalyst (1:1) for 30 seconds until homogeneous according to the group distribution.

Antimicrobial testing was performed using Mueller Hinton Agar plates streaked with *Candida albicans* suspension. Sterile samples were then placed on the agar plates and incubated at 37°C for 24 hours.

The inhibition zones of *Candida albicans* were measured using a digital caliper and replicated three times, as shown in Figure 1

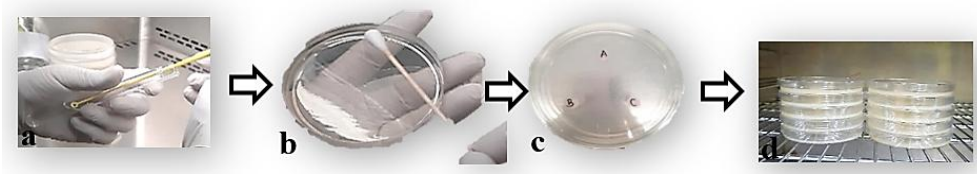


Figure 1. The procedure of antimicrobial test: (a) *Candida albicans* ATCC® 10231™ were grown in sterile aquadest (b) *Candida albicans* suspension was streak using sterile cotton swab (c) Sterile samples were placed on an agar plate (d) Incubated at 37°C for 24 hours.

Surface roughness measurements were conducted using a profilometer (Mitutoyo Surfest SJ-210), with a measuring range of 4.00 to 10.0 µm and an accuracy of 0.001 µm. The sample was placed on a flat surface, and the stylus was perpendicular to the sample surface while the measurement was taken in 3 replications, as shown in Figure 2.

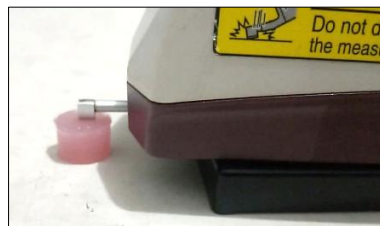


Figure 2. Measurement of surface roughness

The elastic modulus was measured through a stress-relaxation test using the Universal Testing Machine (UTM). The procedure included placing the sample on the two grips of the machine. The crosshead speed used to obtain the elastic modulus for each sample was 20 inches/minute, as shown in Figure 3.



Figure 3. Measurement of elastic modulus and bond strength

Univariate Analysis was used to determine each group's mean and standard deviation. Furthermore, a one-way ANOVA test was adopted to determine the effect of oregano essential oil addition.

3. Results

The GC-MS test showed some active chemical composition of oregano essential oil (Happy Green, Indonesia), including carvacrol (70.84%), p-cymene (6.82%), gamma-terpinene (5.33%), thymol (3.28%),

beta-caryophyllene (2.99%), beta-myrcene (1.44%), alpha-terpinene (1.33%), linalool (1.14%), alpha-thuyene (1.11%), alpha-pinene (0.89%), terpinene-4-ol (0.72%), and cis-beta-ocimene (0.03%).

The results in Figure 1 showed that Group A had no inhibition zone, which indicated the absence of antimicrobial activity. Group B had a lower inhibition zone of 12.54 ± 0.35 mm compared to 14.52 ± 0.57 mm of Group C.

A normality test was performed with the Saphiro-Wilk test ($n < 50$), and the results showed a normal distribution ($p > 0.05$). Subsequently, the one-way ANOVA test presented a significant effect of adding oregano essential oil to SDL on the inhibition zone of *Candida albicans* ($p < 0.001$), as detailed in Table 1.

Graphic 1. The average value and standard deviation of the inhibition zone

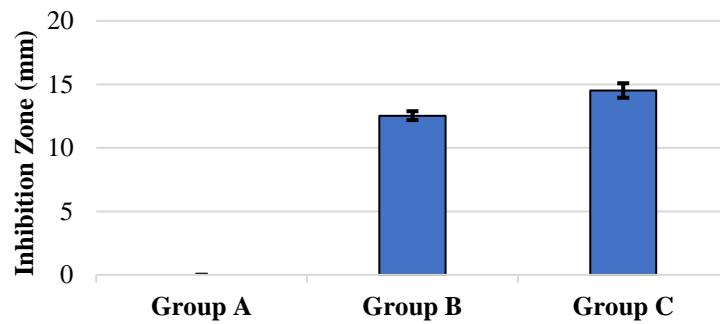


Table 1. Effect of oregano essential oil to SDL on the *Candida albicans* inhibition zone

| Group | n | Inhibition Zone (mm) | P-Value |
|-------|---|------------------------|-------------|
| | | Average value \pm SD | |
| A | 9 | 0 | $< 0.001^*$ |
| B | 9 | 12.54 ± 0.35 | |
| C | 9 | 14.52 ± 0.57 | |

Figure 2 showed that the mean and standard deviation of surface roughness in group A was 0.18 ± 0.01 μm . In groups B and C, the values were 0.06 ± 0.02 μm and 0.13 ± 0.04 μm , respectively. Meanwhile, in group D, the mean and standard deviation of surface roughness was 0.05 ± 0.01 μm . The one-way ANOVA test showed that the effect of the addition of oregano essential oil to the SDL on surface roughness was significant with $p\text{-value} = 0.001$ ($p < 0.05$), as presented in Table 2.

Graphic 2. The average value and standard deviation of surface roughness

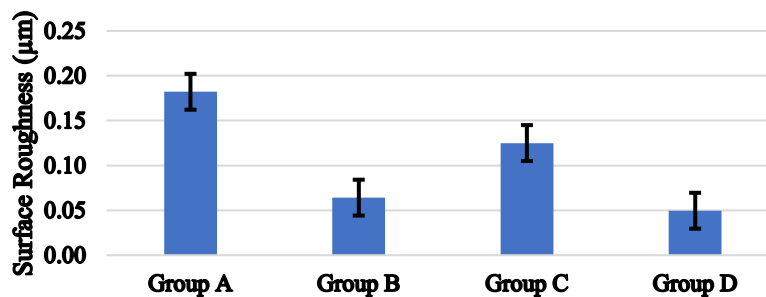


Table 2. Effect of oregano essential oil on the surface roughness of SDL

| Group | n | Surface Roughness (µm) | P-Value |
|-------|---|------------------------|---------|
| | | Average value ± SD | |
| A | 5 | 0.18 ± 0.01 | 0.001* |
| B | 5 | 0.06 ± 0.02 | |
| C | 5 | 0.13 ± 0.04 | |
| D | 5 | 0.05 ± 0.01 | |

Figure 3 shows the mean and standard deviation of the elastic modulus for each group. In group A, the mean elastic modulus was 0.29 ± 0.01 MPa. In groups B and C, it was 0.27 ± 0.01 MPa and 0.23 ± 0.02 MPa, respectively. The lowest mean elastic modulus and standard deviation values were observed in group D, with 0.2 ± 0.03 MPa. The one-way ANOVA test showed that the addition of oregano essential oil to the SDL at one day of use had a significant effect with p-value = 0.001 ($p < 0.05$), as presented in Table 3.

Graphic 3. The average value and standard deviation of elastic modulus

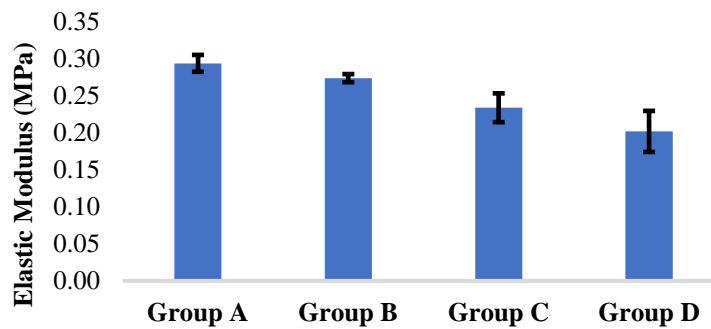


Table 3. Effect of oregano essential oil on the elastic modulus of SDL

| Group | n | Elastic Modulus (MPa) | P-Value |
|-------|---|-----------------------|---------|
| | | Average value ± SD | |
| A | 5 | 0.29 ± 0.01 | 0.001* |
| B | 5 | 0.27 ± 0.01 | |
| C | 5 | 0.23 ± 0.02 | |
| D | 5 | 0.2 ± 0.03 | |

Based on Figure 4, the mean value and standard deviation of the adhesive strength in group A were 0.72 ± 0.04 MPa. In groups B and C, the values were 0.84 ± 0.02 MPa and 0.8 ± 0.02 MPa, respectively. The mean and standard deviation values obtained in group D were 0.99 ± 0.05 MPa, as shown in Table 4.

Graphic 4. The average value and standard deviation of bond strength

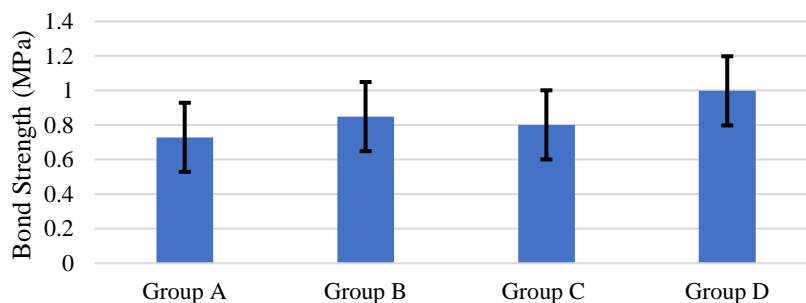


Table 4. Effect of oregano essential oil on the bond strength of SDL

| Group | n | Bond Strength (MPa) | P-Value |
|-------|---|------------------------|---------|
| | | Average value \pm SD | |
| A | 5 | 0.72 \pm 0.04 | 0.001* |
| B | 5 | 0.84 \pm 0.02 | |
| C | 5 | 0.8 \pm 0.02 | |
| D | 5 | 0.99 \pm 0.05 | |

4. Discussion

Oregano contains antifungal compounds that are highly effective against pathogenic microorganisms, making it a safe alternative to modern medicine for treating infectious diseases [13]. The herb also has anti-candidal properties, particularly against oral isolates of *Candida* associated with denture stomatitis [12]. The GC-MS test in this study identified carvacrol as the major component. Baj T et al. (2020) reported that carvacrol was one of the most important bioactive compounds for antifungal activity. The effectiveness of antifungal activity can change due to the synergistic and antagonistic effects of some components in essential oil. Several studies have reported a synergistic activity of carvacrol and thymol, with p-cymene further enhancing the antimicrobial effect [14]. These components primarily inhibit germination and filament formation, which are essential for tissue invasion [3,10,12].

The addition of materials to the polymer chain can affect the physical and mechanical properties of SDL, such as surface roughness and hardness, which directly influence its elastic properties. The adherence of microorganisms on denture base material, as the first stage for colonization and development of pathologies such as denture stomatitis, is affected by surface roughness [15]. Compared to another group, the value of the surface roughness in this study was lower. It experienced a significant effect due to the addition of *Origanum vulgare* essential oil. Auto-polymerized SDL had filler that can absorb the water molecules after polymerization and cross-linking. Due to the hydrophobic nature of carvacrol and thymol, the essential oil infiltrates the micropores of the SDL, preventing further water absorption by the filler [16–19].

The addition of oregano essential oil is observed to improve the elastic properties of SDL. This could be attributed to the plasticizing effect which makes the material softer or more pliable [20]. In addition, the primary compounds of the oil, namely carvacrol and thymol are hydrophobic [19]. These compounds allow the penetration of oil component into the micropores of the SDL, preventing the absorption of more water [18]. The enhanced softness and elasticity of SDL provide a significant advantage and are essential for cushioning mucosal tissue and maintaining shape during use. In addition, good elastic properties help distribute the masticatory load evenly to the mucosal tissue and absorb energy from the denture base. The results showed that auto-polymerized silicone SDL with added oregano essential oil had a higher value than the entire group. According to Huddar et al and Yonikogtlu et al, an increase in the bond strength of the soft liner was obtained from leaching out the plasticizer [21]. Based on this study, the best concentration for each characteristic is 3.125% gel-based oregano essential oil.

5. Conclusion

In conclusion, oregano essential oil as a natural antimicrobial agent, inhibited the growth of *Candida albicans*. Furthermore, it had a higher inhibition zone value compared to when nystatin was added. The lowest surface roughness and elastic modulus values of all groups, was produced by oregano essential oil. This implied that the oil did not destroy the properties of auto-polymerized silicone SDL. However, the value of the bond strength with the addition of oregano essential oil was higher compared to the other groups.

6. Acknowledgment

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7. Conflict of Interest

The authors declare that there are no conflicts of interest to disclose concerning this study.

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