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Effect of Propolis Addition to Auto-Polymerized Silicone Soft Denture Lining Against Inhibition Zone of *Candida Albicans* and Tensile Strength of Heat-Polymerized Acrylic Resin

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Abstract

Soft liners have been used widely to improve comfort in acrylic denture users. Long-term use of soft liners can cause debonding, microporosity, surface roughness, and the growth of *Candida albicans*. To eliminate *Candida*, it is necessary to use an antifungal. One of the natural antifungal ingredients used is propolis. Purpose: This research aimed to determine *Candida albicans* inhibition zone value and soft liners' tensile strength without and with the addition of 76% and 80% propolis and to determine the effect of the addition of 76% and 80% propolis on the inhibition zone and tensile strength. This research was a laboratory experimental research with a post-test-only control group design. Methods: The sample of this research was a heat-polymerized acrylic resin with an auto-polymerized silicone soft liner with total samples of 48, 24 samples each to test the inhibition zone value and tensile strength. The samples were divided into 4 treatment groups, namely without addition, the addition of nystatin, the addition of 76% propolis, and the addition of 80% propolis. Then 24 samples were placed into Petri dishes for *Candida albicans* culture medium and 24 samples were incubated in a solution containing the *Candida albicans* culture for 24 hours. After that, the inhibition zone value and tensile strength test were carried out with the Universal Testing Machine (UTM). The results were recorded and analyzed by SPSS and unpaired T-test. Results: The highest inhibition zone value was found in the group with the addition of 80% propolis, followed by 76% propolis group. The nystatin group and without the addition of propolis didn't show any inhibition zone. The highest tensile strength value was obtained in the 76% propolis group, followed by the nystatin group, 80% propolis, and group without addition. Conclusion: The addition of 76% propolis into the soft liner is the best concentration to inhibit the growth of *Candida albicans* and has the best tensile strength value.

Keywords: Propolis, Soft liner, Heat Polymerized Acrylic Resin, Inhibition zone of Candida Albicans, Tensile Strength

1. Introduction

The use of soft liner or soft denture lining (SDL) to improve comfort for acrylic denture users has been widely used. Soft liners have become dentists' choice due to having many clinical advantages and can improve the adaptation and retention of the denture. In addition, the use of SDL can also improve patients' comfort and quality of life when using removable dentures.[1] Soft liners are soft materials that are often used as a conditioner to unsuitable denture support tissue.[2] SDL materials have viscoelastic properties and the ability to absorb masticatory forces and distribute them evenly over the denture bearing area, thus providing the patient comfort in wearing removable dentures.[3,4] In addition, this material is also easy to manipulate, minimal dimensional changes and water absorption, resistant to

clinical use, easy to clean, does not change color, does not cause pigmentation, biocompatible, tasteless, odorless, good aesthetics, minimal water solubility, 2-3 mm thick, does not irritate tissue, does not colonize bacterial or *Candida*, and have high adhesion to the denture base.[5]

Apart from the advantages provided by SDL, the study reported that microbial colonization, especially *Candida albicans*, was found after the addition of SDL to a heat-polymerized acrylic resin (HPAR) base.[3,5,6] It was reported that fungal and bacterial species could enter the porous space in SDL, where porosity allowed water absorption of water and nutrients diffusion that supports the growth of *candida.*[6] Another problem with SDL is bonding failure between SDL and HPAR, which become unhygienic areas with the growth of fungal and bacterial.[7]

Long-term use of soft liners can cause debonding, microporosity, surface roughness, and the growth of *Candida albicans*.[5,8] Auto-polymerized silicone SDL is a dimethylsiloxane polymer and does not contain plasticizers, so it can maintain its viscoelasticity and softness for a long time. The weakness of this material is the lack or no chemical adhesion to the HPAR base, so the manufacturer provides an adhesive to help in forming a bond between SDL and HPAR.[7] In addition, the elasticity of silicone SDL which lasts longer will cause the growth of *Candida albicans*, disrupting the bonding area of SDL with HPAR. To eliminate *Candida*, it is necessary to use an antifungal in a soft liner. One of the natural antifungal ingredients that can be used is propolis.[10]

Propolis is a natural dark-colored resin material, a material that is collected by bees from shoots and plant exudates, mix it with wax and use in nest making and hive adaptation, especially to fill gaps in beehives. Propolis has been used in traditional medicine since ancient times.[10] The effectiveness of propolis in dentistry has been proven and it has anti-inflammatory, antibacterial, antifungal, hemostatic properties and positive reactions to superficial tissue reorganization.[14]

Research by Valerio et al, 2016, stated that propolis exhibits an antifungal effect against *Candida albicans*, able to prevent the formation of biofilms, and eliminate mature biofilms of *Candida albicans*. The presence of *Candida albicans* biofilms can reduce the tensile strength of SDL with HPAR.[3] The addition of 100% propolis concentration of 10% v/v on soft liner showed a greater inhibition zone against *Candida albicans* compared to tetracycline as a control for up to 60 days, which confirms the antifungal activity of propolis against *Candida albicans*.[3] Research by Leonilda et al, 2015, which added a 100% concentration of propolis of 75 mg to 4 g of SDL showed that the physical properties of SDL (in the form of cohesive adhesive tensile strength) did not change compared to the addition of 150 mg and 300 mg.[4] Wibowo's study (2017) tested propolis activity with concentrations of 72%, 73%, 74%, 75%, 76%, and 77% against *Candida albicans*. The results showed that the minimum inhibitory concentration of propolis was 76%.[13] Hartini's research (2017) tested the antifungal activity of propolis extract with a concentration of 20%, 40%, 60%, and 80% against *Candida albicans* found that 80% propolis had a greater diameter of the inhibition zone. 80% and to determine the effect of 76% and 80% addition of propolis on the value of the inhibition zone and adhesion strength.[14] Based on previous research, we would like to determine the inhibition zone value of *Candida albicans* and tensile strength of the soft liner without and with the addition of 76% and 80% propolis.

II. Material and Method

This research is a laboratory experimental research with a post-test-only control group design. This research used HPAR (Meliodent, Heraeus Kulzer, Germany) and auto-polymerized silicone SDL (Mollosil plus Detax GmbH, Ettlingen, Germany), with four treatment groups: without addition, with the addition of nystatin, 76% propolis, and 80% propolis, with total sample 48 samples. Samples divided into 24 samples each to study the inhibition zone value and tensile strength, with the number of samples for each treatment group was 6 samples.

Sample Making

The sample for the inhibition zone test was a HPAR base in the form of a disc with 6 mm in diameter and 1.5 mm thickness (Figure 1) on which above that was placed an auto-polymerized silicone SDL. This sample aimed to facilitate the diffusion disc test by placing the sample in agar media to assess the inhibition zone diameter of each treatment group. Samples were made by mixing base and catalyst of auto-polymerized silicone SDL without and with the addition of propolis and the nystatin. Before the polymerization process is complete, the soft liner is dropped on the HPAR disc sample to examine the candida inhibition zone on agar media, as based on previous research.[6]

Figure 1 Master Cast and Wax disc to Determine the Inhibition Zone

For the tensile test, the samples were two bases of heat-polymerized acrylic resin in a rectangular shape with a size of 83x10x3mm. The sample size was made based on the instructions for making samples of the HPAR material, which made it easier to make based on previous research.5 Autopolymerized silicone SDL with a size of 3x10x3 mm was placed between the two HPAR bases. To obtained samples, samples were planted in a cuvette and hold with a UTM clamp then 2 main models of brass are needed, namely the master cast I (measuring 83x10x3 mm) to get the mold (Figure 2) and master cast II (measuring 3x10x3 mm) is placed in the center of the mold that has been obtained as a spacer to get space for the auto-polymerized silicone SDL material when filling HPAR (Figure 3).

Figure 2 Master Cast I for Tensile Test

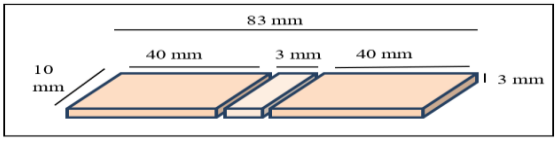
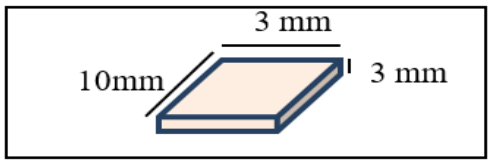


Figure 3 Master Cast II for Tensile Test



Treatment Group

Treatment groups in this study were divided into eight groups with the number of samples each group was six.

a. HPAR Disc with auto-polymerized silicone SDL

- Without propolis addition as negative control (Group A)
- With nystation addition as positive control (Group B)
- With 76% propolis addition (Group C)
- With 80% propolis addition (Group D)

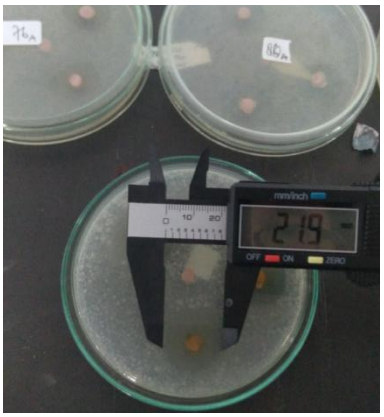
b. HPAR Base with auto-polymerized silicone SDL

- Without propolis addition as negative control (Group A1)
- With nystation addition as positive control (Group B1)
- With 76% propolis addition (Group C1)
- With 80% propolis addition (Group D1)

Sample Testing

Candida albicans inhibition zone values testing were carried out with agar disc diffusion method (Potato Dextrose Agar MERCK German KGA) by making culture on the medium and incubated them at 37°C for 24 hours and measuring the inhibition zone of *Candida albicans* growth with digital calipers in Microbiology Laboratory, Faculty of Pharmacy, University of Sumatera Utara. Each measurement is repeated and the test for each sample is repeated 3 times (Figure 4).[3,6]

Figure 4 *Candida Albicans* Inhibition Zone Testing



Tensile strength samples testing was carried out by tensile test with Universal Testing Machine (UTM Tensilometer, AND, RTF-1350, Japan) in Mechanical Engineering Laboratory, University of Sumatera Utara. The samples were first immersed in a solution containing *Candida albicans,* then tested with a crosshead speed of 5 mm/minute until a crack occurred. The value that appears when the first crack occurs is recorded. Tensile strength is calculated in Newton, KgF/mm², or Mpa. The clinically acceptable standard tensile strength value was 0.45 MPa. The calculation of tensile strength is done with the formula: Bond strength = Maximum load (KgF) / Cross-sectional area (mm²) (MPa or KgF/mm²). The cross-sectional area of the sample is 10x10xmm². Disconnection or detachment of the auto-polymerized silicone SDL attachment from the HPAR base surface was observed by one operator. The detachment can be in the form of adhesive failure, cohesive failure, or mixed failure which can be observed visually (Figure 5).

Figure 5 Tensile Strength Testing with UTM Tensilometer, AND, RTF – 1350, Japan

Data Analysis

Data obtained were analyzed with univariate analysis to determine the mean and standard deviation value of each group. To determine the effect of propolis addition to the auto-polymerized silicone SDL on the *Candida albicans* inhibition zone and tensile strength of heat-polymerized acrylic resin, a one-way ANOVA test was performed.

III. Results

Table 1. Mean Value of *Candida Albicans* Inhibition Zone on Auto-Polymerized Silicone SDL Without and with the Addition of 76% and 80% Propolis.

Sample Number	Inhibition Zone			
	A (Without Propolis)	**B (Nystatin)**	**C (76% Propolis)**	**D (80% Propolis)**
1	0	0	22.10	22.40
2	0	0	22.00	22.70
3	0	0	22.30**	22.30*
4	0	0	22.20	24.20**
5	0	0	21.90	23.60
6	0	0	21.70*	24.20**
X ± SD (mm)	0	0	22.03 ± 0.21	23.23

Note: *: Lowest value
**: Highest value

Table 2. Mean Value of Tensile Strength Value of Auto-Polymerized Silicone SDL and HPAR without and with the Addition of 76% and 80% Propolis.

Sample Number	Tensile Strength			
	A1 (Without Propolis)	**B1 (Nystatin)**	**C1 (76% Propolis)**	**D1 (80% Propolis)**
1	0.17	0.35	0.52	0.24
2	0.13*	0.33*	0.49	0.27**
3	0.18**	0.37	0.58**	0.22*
4	0.13*	0.37	0.48*	0.22*
5	0.18**	0.38**	0.53	0.25
6	0.18**	0.34	0.49	0.25
X ± SD (mm)	0.16 ± 0.02	0.36 ± 0.01	0.51 ± 0.03	0.24 ± 0.01

Note: *: Lowest value
**: Highest value

Graphic 2. Mean Value of Tensile Strength Value of Auto-Polymerized silicone SDL and HPAR without and with the addition of 76% and 80% Propolis.

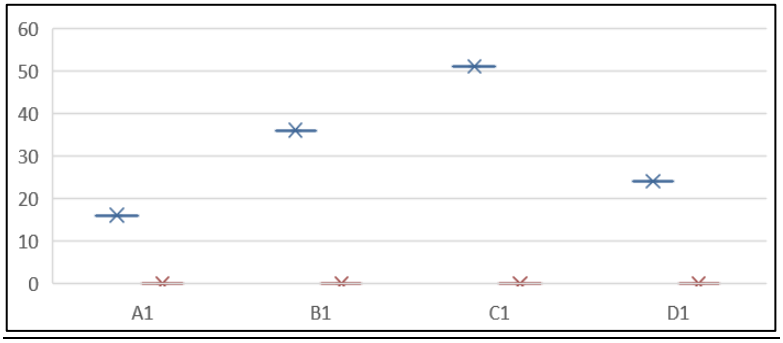


Table 3. The Effect of Propolis Addition to Auto-Polymerized Silicone SDL Against *Candida Albicans* Inhibition Zone

Group	**Inhibition Zone**		**p**
	N	Mean ± SD	
A (without addition)	6	0	0.001
B (nystatin)	6	0	
C (76% propolis)	6	22.03 ± 0.21	
D (80% propolis)	6	23.23 ± 0.87	

Note: *significant ($p < 0.05$).

Table 4. The Effect of Propolis Addition to Auto-Polymerized Silicone SDL against Tensile Strength of SDL with HPAR

Group	**Tensile Strength**		**p**
	N	**Mean ± SD**	
A1 (without addition)	6	0.16 ± 0.02	0.001
B1 (nystatin)	6	036 ± 0.01	
C1 (76% propolis)	6	0.51 ± 0.03	
D1 (80% propolis)	6	0.24 001	

IV Discussion

In this study, the group without the propolis addition was used as a negative control because various studies showed that auto-polymerized silicone SDL did not have an inhibition zone against *Candida albicans*. After all, it did not contain antifungal properties. For group A (without propolis addition), the mean value of the inhibition zone was 0 mm (Table 1), which means that there it didn't have the potential to inhibit the growth of *Candida albicans*. This result is the same as the research of Pachava (2014) which obtained the inhibition zone value of *Candida albicans* 0 mm in the acrylic and silicone soft liner groups.[15] This could be because SDL does not have an active component that is antifungal. Auto-polymerized silicone SDL (Mollosil Plus) in this study has a liner chemical composition, consisting of polydimethylsiloxane, filler, pigment, and platinum catalyst, and primary composition of 60-100% ethyl-acetate.[16,17] SDL alone cannot inhibit the growth of fungal and long-term use can cause denture stomatitis so that it is necessary to add an antifungal agent to SDL to prevent the formation of biofilms.[18]

Auto-polymerized silicone SDL group added with nystatin was used as a positive control because nystatin is the most commonly used clinically in the oral cavity and various studies showed a clinically good positive inhibition zone. In group B (with the addition of nystatin), the mean value of the inhibition zone was 0 mm (Table 1). In this study, the addition of nystatin in the soft liner could not inhibit the growth of *Candida albicans*. This result is different from the results of Krishnamurthy (2016), which found that the inhibition zone of nystatin against *Candida albicans* was 30.37 ± 3.33 mm using a factory-made antimicrobial sensitivity test disc (Himedia Laboratories Pvt., Ltd., India).[6] Results of this study is also different from Bueno (2015), where the addition of antifungal drugs (nystatin, miconazole,

ketoconazole, itraconazole), was induced in disc-shaped SDL sample.

In group C (with the addition of 76% propolis), the mean value was 22.03 ± 0.21 mm, while for group D (with the addition of 80% propolis) the mean value was 23.23 ± 0.87 mm (Table 1). This showed that the addition of propolis to auto-polymerized silicone SDL can inhibit the growth of *Candida albicans* and has an antifungal effect. These results are consistent with Abdelfattah (2017) which also showed that propolis has an antifungal effect.[21] Santos' research (2005) also found that 20% propolis ethanol extract was effective as oral candidiasis treatment.[22] In this study, 80% propolis extract showed the highest inhibition zone diameter compared to the other three groups.

These results are in accordance with the results of Hasanah (2012) and Hartini (2017), who conducted a propolis antifungal activity test using the disc diffusion test method and obtained propolis extract had an antifungal effect, with propolis concentration 80% had largest inhibition zone diameter, followed by 60%, 40%, and 20% concentration.[14] This is because the greater the extract's concentration, the greater its ability to kill microorganisms, due to more concentration of bioactive components contained in the extract.[23] Hasanah's research (2012) reports that the main bioactive components of propolis are flavonoids and phenolic acids, including Caffeic Phenethyl Ester (CAPE) that reaches 50% of the entire composition and is very effective in inhibiting *Candida albicans*. Halim's research (2012) also shows that the ethanol extract of Indonesian propolis contains twice as many flavonoids as compared to Brazilian propolis.[24]

The tensile strength value of heat-polymerized acrylic resin with auto-polymerized silicone SDL in the group without propolis addition was used as a negative control because various studies showed that auto-polymerized silicone SDL had weak tensile strength on the HPAR base due to its molecular differences between the two that made them not chemically bonded. An SDL material is clinically acceptable if it has a tensile strength equal to or greater than the standard value of 0.44 MPa. The auto-polymerized silicone SDL group with nystatin addition was used as a positive control following the inhibition zone test.

Table 2 showed the tensile strength of auto-polymerized silicone SDL with HPAR after adding 76% propolis, 80% propolis, nystatin, and without adding propolis. In group A1, the mean value of tensile strength was 0.16 ± 0.02 MPa, which is the lowest value among all test groups. The value of tensile strength in this study is similar to Gundogdu (2014) which noted the value of the tensile strength of auto-polymerized silicone SDL (Ufi Gel P) was 0.20 ± 0.05 MPa.[26]This study resulted in the value of Febriani's (2019) research on auto-polymerized silicone SDL and HPAR base without surface treatment with a mean value of 0.150 MPa. This value is lower than the optimal value suggested by Craig and Gibbson. Craig and Gibbons assessed the tensile strength of the soft liner and stated that the value of 0.45 MPa is an adequate adhesive value for optimal bonding that is clinically acceptable.[26] The low-value results in this study were related to the type of silicone SDL used, namely auto-polymerized silicone SDL, where auto-polymerized silicone SDL had a weak bond in HPAR due to its molecular differences between the two materials.[2] In addition, the sample before the tensile strength test with Universal Testing Machine was immersed in a solution containing *Candida albicans* for 24 hours. Immersion of the soft liner in the solution causes water absorption by the polymer accompanied by the entry of *Candida*

albicans into the SDL and HPAR attachment areas. This process can cause changes in the physical properties of the soft liner. This condition can affect the value of the tensile strength in this study.[27,28]These results are different from Bayati's (2012) study which reported the tensile strength value of soft liner (Mollosil) with HPAR, which was 1.07 ± 0.16 MPa.[16] The difference in the results of this study could be due to differences in the size of the sample. This study used HPAR with size 83x10x3 mm and SDL with 3x10x3 mm, while Bayati used HPAR samples with size 20x20x4 mm and SDL with diameter 10 mm and height 3 mm.[16]

In group B1 (with the addition of nystatin) the mean value of tensile strength was 0.36 ± 0.01 MPa. This mean value was higher than the mean value in group A1 (without the addition of propolis) (Table 2). The addition of material to the soft liner has been shown to change the physical properties and tensile strength of the soft liner. The results of this study are different from the research of Alcantara (2012) which found that tensile strength value in the acrylic SDL group with the addition of 500.000 units of nystatin and 1.000.000 units, respectively 0.049 ± 0.017 MPa and 0.043 ± 0.012 MPa, was lower than the control group (0.054 ± 0.017 MPa).[29] In Aliaga's (2016) study, the tensile strength of acrylic SDL to HPAR after the addition of 0.032 g/mL nystatin also showed that nystatin had a lower tensile strength value than the control group.[20] This difference in results may be due to differences in sample type where Aliaga and Alcantara used acrylic SDL while this study used silicone SDL.

The highest average value of tensile strength was obtained in the C1 group (with the addition of 76% propolis), which was 0.51 ± 0.03 MPa, while for the D1 group (with the addition of 80% propolis), only 0.24 ± 0.01 MPa were obtained (Table 2). The results of this study are different from the research of Jaboinski (2015) which reports that 100% concentration of propolis (75mg/4g=1.87%) has the same tensile strength value with the control group (0.11 ± 0.02 MPa), while propolis concentration 100% (150 mg/4g and 300 mg/4g) reduced the tensile strength to 0.08 ± 0.01 MPa and 0.10 ± 0.01 MPa, respectively. The difference in the results of this study was due to the different types of propolis used. This study used propolis ethanol extract while Jaboinski's research used propolis dry powder extract. Apart from the propolis type, this difference may be due to the difference in sample size where Jaboinski uses a sample size of 5x8x3 mm.[5]

Table 3 showed the effect of adding propolis, both 76%, and 80% concentrations can inhibit the growth of *Candida albicans*. By increasing the concentration of propolis, it will produce a larger inhibition zone value as well. One-way Anova test showed that there was a significant effect of adding 76% and 80% propolis to auto-polymerized silicone SDL on the inhibition zone of *Candida albicans* with p-value = 0.019 (Table 3). The results of this study are in accordance with the research of Abdefattah (2017), Hartini (2017), Capistrano (2013), Oliveira (2006), and Santos (2005) which showed that there was an effect of using or adding propolis to the inhibition zone.[14,21,22,30,31] The antifungal effect of propolis comes from flavonoids and phenolic acids, including Caffeic Acid Phenethyl Ester (CAPE), which contains up to 50% of the total composition. Flavonoids cause damage in cell membranes, whereas CAPE plays a role in inhibiting the 12-lipoxygenase required by *Candida* for the enzymatic pathway to invade human endothelial cells.[14] The higher the propolis concentration, the larger the inhibition zone. This corresponds to an increase in the chemical composition of an extract.[14,23]

Yuliana's research (2015) stated that propolis extract had the potential as a natural anti-microbial to inhibit and kill the growth of *Candida albicans*. The active compounds that play a role in inhibiting the growth of pathogenic microbes are hydroxyl groups that cause changes in organic components and transport nutrients resulting in toxic effects and inhibiting pathogenic spores in fungal. It is known that hyphae formation helps *Candida albicans* to penetrate the host tissue and the subsequent invasion leading to the appearance of infection. Propolis can prevent the formation of hyphae by 90% of the form of hyphae fungi in *Candida albicans*. Hyphal transition prevention by propolis demonstrated its ability to control the growth of *Candida albicans*, so that the morphological process from fungi to hyphal cells is disrupted which plays an important role in biofilm formation.[32] Propolis is a natural product, so it is safe to use and until now there have been no reports of its side effects, so propolis can be used as an alternative treatment for oral Candidiasis and is effective in reducing the amount of *Candida albicans* when added to soft liners.[21,30]

Table 4 showed the effect of propolis addition on tensile strength and it is obtained that both 76% and 80% concentrations can increase tensile strength value. Unlike the inhibition zone, an increase in propolis concentration does not increase the tensile strength. One-way ANOVA test showed a significant effect of 76% and 80% propolis addition to auto-polymerized silicone SDL on its tensile strength with HPAR with p-value = 0.001 (Table 4). These results are consistent with Jaboinski's research which showed that the addition of 150 mg and 300 mg of propolis to the soft liner affected the tensile strength of the soft liner on the HPAR base, but there was a reduction in tensile strength. In addition, different results were found in the 75 mg propolis group where this group did not show any difference in tensile strength.[5] This similar results in decreased tensile strength value after the addition of propolis weight or concentration is due to the higher the propolis concentration, the more propolis components in SDL with different molecules which causes a reduction in SDL tensile strength.

In the results of this study, 76% propolis increased the tensile strength of auto-polymerized silicone SDL with HPAR. This value is greater than the optimal tensile strength value proposed by Craig and Gibbson, which was 0.45 MPa, and is the only group to achieve this value.[26] The significant value of tensile strength is a sign of none or reduced microleakage in the attachment area. The reduction in microleakage in the attachment area is one of the causes of the absence of *Candida albicans* colonization with the presence of propolis in SDL which reduces the formation of biofilm, reduced water absorption, and reduced the accelerated aging process of the material so that there wasn’t swelling and damage to the SDL attachment area with RAPP so that in the sample of this study, which was contaminated with a solution containing *Candida albicans*, it was found that the tensile strength with the addition of propolis was higher than the group without the addition of propolis and the group with the addition of nystatin.[26]

The increase in tensile strength value in this study may also be related to the use of ethanol as the extracting solvent. One of the soft liner compositions is ethanol.35 The study of Bayati (2012) which compared the use of three types of primers to tensile strength showed that the ethyl acetate content in the primer affected the tensile strength of the soft liner with HPAR base.[16] SDL adhesion with HPAR depends on the use of volatile solvents which soften the surface of the HPAR, when the solvent evaporates the SDL molecules will penetrate the micro-gap of HPAR, resulting in an increase in the

mechanical tensile strength during the SDL curing process. Extract added into soft liner will increase the amount of ethanol content in the soft liner and this may lead to an increase in the tensile strength value.

The limitation in this study is that the sample for testing the inhibition zone and the tensile strength were a different sample, so the correlation about the inhibition zone of *Candida albicans* with tensile strength remains unknown. Another limitation of this study is the time used to assess tensile strength. In this study, the tensile strength assessment was carried out one day after the sample was immersed in *Candida albicans* solution so that these results have not matched clinical use for a long time.

V. Conclusion

Propolis with 76% concentration added to the soft liner is the best concentration to inhibit the growth of Candida albicans and has the best tensile strength value.

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