In vitro study *sargassum sp.* Effervescent towards antifungal and compressive strength silicone based softliner

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ABSTRACT

Background: *Softliner is commonly used to provide convenience for edentulous patients. However, the use of softliner can cause denture stomatitis due to colonization of Candida albicans. Sargassum sp. contains active compounds of steroids, alkaloids, phenols, triterpenoids, act as antibacterial and antifungal. The use of Sargassum sp. in the form of effervescent as a denture cleanser can prevent denture stomatitis, and it's expected not to affect the compressive strength. This study aimed to study the concentration variation of Sargassum sp. effervescent towards antifungal and compressive strength silicone based softliner*

Method: *20 sampels of silicone based softliner (10x3x2mm) divided into 5 groups. K(-) soaked in sterilled aquadest; K(+) in Polident solution; P1 in 10% effervescent Sargassum sp. solution; P2 20% ; P3 40%, for 15minutes. Candida calculated by colony counter (CFU/ml). Compressive strength measured by Universal Testing Machine.*

Result: *Candida albicans in the K+ group is the lowest, while the K- group shows the highest value. In P1, P2, P3 group the value of Candida albicans is higher than K+ group, but much lower than K-. The compressive strength of K+ group shows the lowest value but in P3 group shows the highest value compared to other groups.*

Conclusion: *The 10% concentration of effervescent extract Sargassum sp. is effective in reducing the number of Candida albicans in silicone based softliner compared to the K- group. The most effective concentration of effervescent extract Sargassum sp. in reducing the Candida albicans' growth is 40%. The higher concentration of Sargassum sp. resulting in higher compressive strength.*

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INTRODUCTION

Softliner is popular as materials of choice in prosthodontics to treat edentulous patients who often experience trauma due to hard denture bases. Inflammation and swelling of soft tissues under ill-fitting dentures are common cases among patients who wear denture.[1] Softliners can be divided into two groups according to their composition, namely acrylic based and silicone based.[2] Silicone based softliner are made of rubber silicone based materials and has better stability compared to acrylic based softliner because they do not contain volatile plasticizers.[3] Past in-vitro studies proven that softliner increased the formation of fungal biofilms, which is *Candida albicans*. Fungi easily penetrated into the material because of porous characteristic of softliner.[4] Colonization of *Candida albicans* in patient wearing removable denture can cause candidiasis in the oral cavity. Khrishnamurthy and Hallikerimath (2016) comparing samples manufactured from heat cured and self cured denture liners (Molloplast B, Permaflex, GC SoftLiner, Ufi Gel Hard C) and concluded that *Candida albicans* on acrylic based softliner had less adhesion than to silicone based softliner.[5]

Sanitation of acrylic denture can be maintained by soaking dentures in denture cleaning agents at night for ±15 - 20 minutes.[6] Ghazal, *et al.* (2019)[7] concluded that brushing and soaking in a soluble tablet cleaning solution (effervescent tablets), or a combination of both, significantly reduced *Candida albicans* in dentures. Studies reported that fungi and bacterial species can enter porous space within denture liner and that may reduce the intraoral life of the material. This porosity allows water absorption and diffusion of nutrient materials that support the growth of oral yeasts.[5] Therefore, the hygienity of denture liner material needs to be maintained.

Denture cleansers that commercially used were chemicals products such as alkaline peroxide, sodium hypochlorite, sodium bicarbonate, and chlorhexidine gluconate.[8] Chemicals had a bad effect in long-term use due to the residue could attached to the dentures and might be swallowed through the saliva.[9] Brown algae (*Sargassum sp*.) is a natural resources that can be easily found and used as a denture cleanser.[10] Pakidi and Suwoyo (2016)[11], have found that brown algae (*Sargassum sp*.) contains alginate and iodine which usually used in the food, pharmaceutical, cosmetic and textile industries. In addition, *Sargassum sp.* contains active compounds of steroids, alkaloids, phenols, and triterpenoids that function as antibacterial, antiviral, and antifungal agents. Octavin (2017)[10], using an extract solution of *Sargassum sp*. with concentrations of 100 mg/ml, 200 mg/ml, 400 mg/ml and distilled water as a negative control in the immersion of heat cured acrylic resin to see the inhibition of *Candida albicans*, the lowest number of colonies was found in the *Sargassum sp.* extract group 400 mg/ml (38 CFU/ml).[10] This proven that the extract of *Sargassum sp*. at the mentioned dose capable to inhibit the growth of *Candida albicans*, consequently, these doses considered to be used in this research. Preparat of *Sargassum sp*. in several studies mostly in the extract form. However, the extract spent a long time of manufacturing and not practical to be used comparing to the tablet form.[12,13] Hence, we used the effervescent granules preparat in this research.is the instantaneous preparation of a solution containing the correct dose.[14,15]

A good denture base materials should provide several mechanical properties, including compressive strength, tensile strength, shear strength, flexural strength and surface roughness. The higher mechanical properties of a denture base will improve the physical properties of the denture.[16]

Mohammed *et.al* (2016)[17], found that immersion of denture liners (acrylic based and silicone based softliner) on general denture cleansers (Polident® and Efferdent®) can reduce the surface roughness and hardness of denture liners material but their effect on compressive strength has not been studied. This research aimed to study the effect of variation concentration of *Sargassum sp*. effervescent towards *Candida albicans* growth and its effect on the compressive strength of silicone based softliner. A good denture base material expected to have less *Candida albicans* colonization and high mechanical properties, such as compressive strength.

MATERIAL AND METHODS

20 Samples of silicone based softliner (SOFRELINER TOUGH® MEDIUM - Tokuyama Dental Corporation, Tokyo, Japan) with following criteria's: smooth surface, not porous, size 10x3x2mm, were divided into 5 groups with the number of samples in each group was 4 (Fig.1). This material sets in 20 minutes at room temperature (23^0C/ $73,4^0$F). Medium hardness was chosen due to its used more often by dentists. SOFRELINER TOUGH® are one of the silicone based soft denture liner which available in the market. Divided into negative control group (K-) immersion with sterile distilled water, positive control (K+) immersion with Polident® solution, treatment group 1 (P1) immersion with effervescent solution *Sargassum sp*. concentration of 100 mg/ml (10%), treatment group 2 (P2) concentration of 200 mg/ml (20%), treatment group 3 (P3) concentration of 400 mg/ml (40%).

Effervescent preparat form was chosen because it has the advantage which is the instantaneous preparation of a solution containing the correct dose.[16] The recommended soaking time for cleaning dentures is 10-20 minutes.[6]

Samples that have been contaminated with *Candida albicans*, were placed into reaction tubes. Then the immersion was done into 5 groups : K(-), K(+), P1, P2, and P3 (Fig.2 & 3).

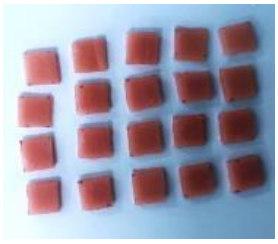


Figure 1. Samples of 20 silicone based softliner

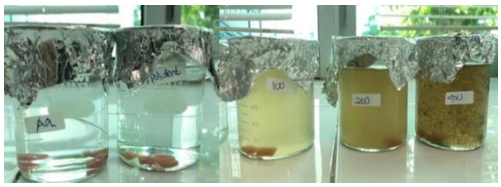
(a) **(b)**

Figure 2. Samples immersion in control groups. (a) immersion in sterile distilled water (K-), (b) immersion in Polident® cleanser solution (K+)

(a) (b) (c)

Figure 3. Samples immersion in treatment groups. (a) immersion in *Sargassum sp*. 10% (P1), (b) immersion in *Sargassum sp.* 20% (P2), (c) immersion in *Sargassum sp*. 40% (P3)

Candida albicans was planted on *Sabouraud Dextrose Agar*, then incubated for 24 hours at room temperature (37ºC). After 24 hours, *Candida* counts were performed using a colony counter (CFU/ml).



(a) (b) (c) (d) (e)

Figure 4. Sample immersion before measuring compressive strength. (a) K(-) Group, (b) K(+)Group, (c) P1 Group, (d) P2 Group, (e) P3 Group.

Samples were then prepared for the compressive strength measurement (Fig.4). Measurement of compressive strength done by *Universal Testing Machine* (UTM) with a load cell of 1kN and loading speed of 1mm/minute. Compressive strength is calculated using the following formula:

$$T = F/A$$

T: Compressive strength (MPa),

F: Force obtained from the measurement results using UTM (N),

A: Surface area of the test plane (mm^2).

Candida albicans and compressive strength data results then were tested by ANOVA analytic statistical test and continued by Post Hoc LSD test.

RESULTS

Candida albicans colonies formation showed at the following pictures (Fig.5-9).

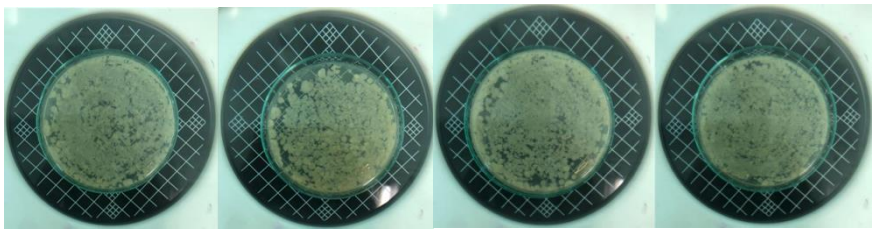


Figure 5. *Candida albicans* colonies in K(-) group

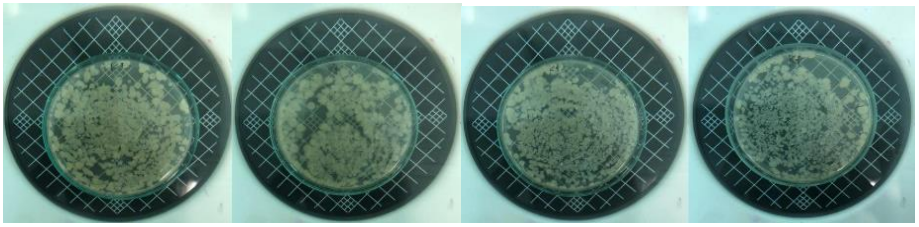


Figure 6. *Candida albicans* colonies in K(+) group

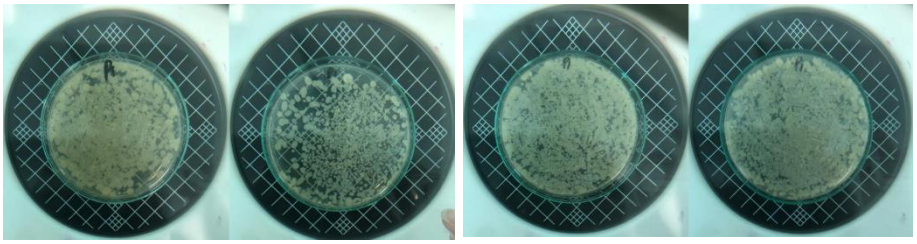


Figure 7. *Candida albicans* colonies in P1 group

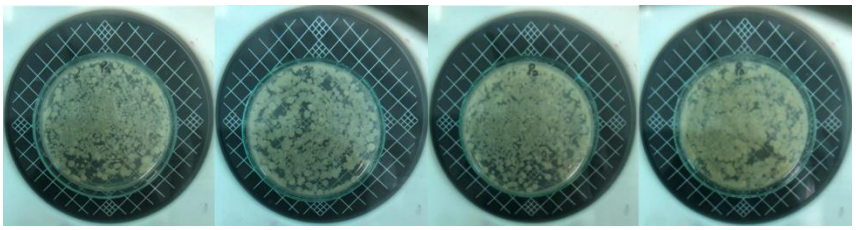


Figure 8. *Candida albicans* colonies in P2 group

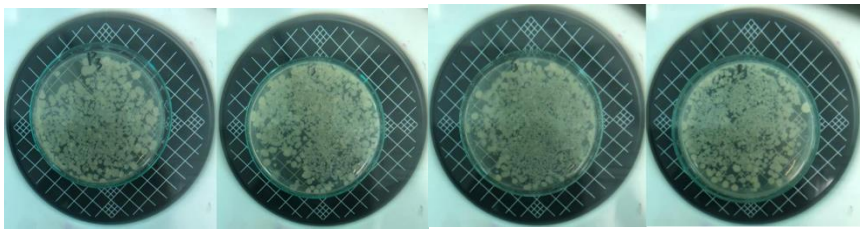


Figure 9. *Candida albicans* colonies in P3 group

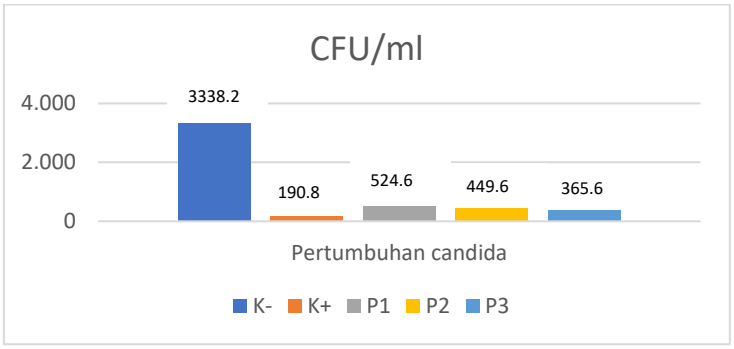


Figure 10. Bar chart results of *Candida albicans* colonies in each study group

The mean value of *Candida albicans* colonies in K(+) group showed the lowest value, while the K(-) group showed the highest value compared to other groups (Fig.10). The results of the treatment group (P1, P2, and P3) showed that

the mean number of *Candida albicans* was higher than K(+) but much lower than K(-).

Table 1. ANOVA test results of *Candida albicans* colonies

Candida albicans colony

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28199853.832	4	7049963.458	396.003	.000
Within Groups	267041.846	15	17802.790		
Total	28466895.678	19			

The results of the ANOVA test showed p <0.05, so it was concluded that there were significant differences in the 5 groups of data tested (Table 1).

Table 2. Post Hoc LSD test results

	K-	K+	P1	P2	P3
K-	-	0.000	0.000	0.000	0.000
K+	0.000	-	0.003	0.015	0.084
P1	0.000	0.003	-	0.439	0.113
P2	0.000	0.015	0.439	-	0.388
P3	0.000	0.084	0.113	0.388	-

The post hoc LSD test showed that there was a significant difference in the mean value of *Candida albicans* between K(-) and K(+), K(-) and P1, K(-) and P2,K(-) and P3, K(+) and P1, and K(+) and P2, while in K(+) and P3, P1 and P2, P1 and P3, and P2 and P3 there were no significant difference (Table 2).

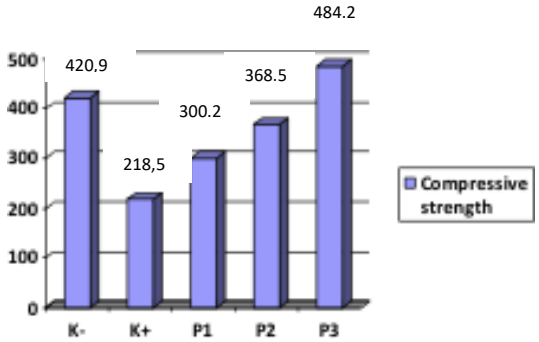


Figure 11. Bar Diagram of Compressive Strength (MPa) in each study group

The mean value of compressive strength at K(+) showed the lowest result, while P3 shows the highest result (Fig.11). From the results of P1, P2, and P3 can be seen that the mean of compressive strength tends to increase in immersion with a higher concentration of *Sargassum sp*.

Table 3. ANOVA test results of compressive strength

ANOVA

Compressive strength

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	171781.910	4	42945.477	1.751	.191
Within Groups	367852.102	15	24523.473		
Total	539634.012	19			

Table 4. Post Hoc LSD test results of compressive strength

	K-	K+	P1	P2	P3
K-	-	0.088	0.293	0.643	0.572
K+	0.088	-	0.472	0.196	0.030
P1	0.293	0.472	-	0.547	0.116
P2	0.643	0.196	0.547	-	0.310
P3	0.572	0.030	0.116	0.310	-

The results of ANOVA test showed p=0.191 (p>0.05), so it was concluded that there was No. significant difference in the 5 groups of data tested (Table 3). Post Hoc LSD test showed that there was a significant difference between K(+) and P3. While there were no significant differences in another groups (Table 4).

DISCUSSION

The results showed that there was a significant difference between *Candida albicans* at K(-) and K(+), this can be explained by the longer the denture material immersed in distilled water, the more microbes adhered, considering that the water (K- group) did not have antifungal effect.

The lowest number of *Candida albicans* was found in K(+) (immersion by Polident® solution). The effectiveness of denture cleaning tablets in inhibiting the growth of *Candida albicans* was obtained from the citric acid ingredients. Citric acid acts as a chemotherapeutic agent that can eliminate biofilms through the mechanism of calcium ions. This mechanism caused citric acid to damage Ca-bridges and then lead to the biofilm matrix degradation resulting in biofilm activity.[8]

From the results data, there were significant differences between *Candida albicans* in K(-) and P1, K(-) and P2, and K(-) and P3. The

colony count of *Candida albicans* in immersion of silicone based softliner with *Sargassum sp*. effervescent showed that higher concentration of *Sargassum sp*. extract, the more effective it was in reducing *Candida albicans* colonies in silicone based softliner. This due to the active antifungal compounds (tannins and phenols) which found in *Sargassum sp.* extract increased proportional to the higher concentration, so it is more effective in inhibiting *Candida albicans*. This result in line with the research by Triastinurmiatiningsih *et.al* (2015)[18], which concluded that the higher of *Sargassum crassifolium* extract concentration resulting in wider inhibiting zone area of *Candida albicans*. Distilled water was used as K(-) groups to show *Candida albicans* colonization in denture liners which is not cleaned with antifungal agent, meanwhile Polident® was used as K(+) groups as the golden standard of denture cleaning materials, which had antifungal and antibacterial potential.

Sargassum sp. extract contains chemical active components such as tannins and phenolic compounds. Tannins are phenolic compounds with high molecular weight proteins containing hydroxyl and carboxyl to form effective complexes with proteins and macromolecules.[19] According to the research of Arifin *et.al* (2018)[20], tannin have effectiveness in inhibit the growth of *Candida albicans*.

Phenol can kill vegetative cells of fungi and spore-forming bacteria by denaturing proteins and lowering surface tension so that the permeability of bacteria and fungi increased. The mechanism of action is firstly, the reaction with protein cells is the process of inhibition or killing by destroying the colloid system by coagulation and protein precipitation. The presence of microbial cell protein coagulation causes metabolic disorders.[21] Secondly, changing the permeability of the cell membrane by lowering the surface tension which increase the permeability of the cell membrane so that water enters and results in the death of microbes.[22] The high solubility of lipids is the ability of phenols to combine with lipid cell components. Fungal cell membranes are composed of phospholipids which can cause the permeability to be disturbed so that the fungus is inhibited. The research by Triastinurmiatiningsih (2015)[18] showed that the ethanolic extract of *Sargassum crassifolium* inhibited the growth of *Candida albicans*.

Significant differences were also found in K(+) and P1, and K(+) and P2. However, between K(+) and P3 there was no significant difference, this is as previously stated, the content of tannins and phenols in the extract of *Sargassum sp*. proportional to the higher concentration of *Sargassum sp*. extracts resulting in higher effectivity in reducing the *Candida albicans* colonies. As a result, the number of *Candida albicans* colonies on P3 was closest to the number of colonies on K(+) (no significant difference). The amount of *Candida albicans* colonies between treatment groups (P1-P2), (P1-P3), and (P2-P3) did not show a significant difference, this indicated that there is no difference in effectiveness of reducing the *Candida albicans* colonies with a variation concentration of 10%, 20%, as well as 40%. Further research is needed with higher concentrations of *Sargassum sp*. to obtain significant result.

The results of the compressive strength of silicone based softliner immersed in *Sargassum sp.* extract showed that the compressive strength at K(+) had the lowest value, while at P3 was the highest value compared to other groups. This explained as in K(+) (immersion with Polident® solution), Polident® contains alkaline peroxide. Alkaline peroxide works by reducing the surface tension of the polymethylmethacrylate in the softliner. The reduced surface tension causes the molecules in the alkaline peroxide solution to easily

enter the gap between the polymethylmethacrylate molecules and large diffusion occured. This diffusion caused the softliner components, the plasticizers evaporated.[23] In immersion with distilled water, no effect was obtained because of the neutral nature of the water. The results of this study are not in accordance with the research of Hilal *et.al.* (2016)[24] that stated the immersion of softliner in alkaline peroxide increased the hardness of the softliner compared to the group immersed with distilled water. This could be caused by a non homogeneous sample or the difference in the sample size of the softliner used in this research.

In the results of P1, P2, and P3 can be concluded that the compressive strength value tends to increase in immersion with a higher concentration of *Sargassum sp*. This is not in line with the research by Vita (2019)[25] which stated that the higher the concentration of *Sargassum sp.* will further reduce the transverse strength of the materials. This is explained by the higher concentration of *Sargassum sp*. the more phenol components. Phenol is an alcohol derivative with alkali characteristics because it has an OH. Soaking too long in an alcohol solution will caused changes in the physical and mechanical properties of the silicon-based softliner material. These alcohol molecules disrupted the polymer bonds and changed the physical characteristics of the polymer. The polymer chains become separated so that the matrix expansion occurs, then the matrix softened and there will be a decrease in transverse strength.[26] Transverse strength is a combination of compression strength, tensile strength and shear strength. Reduced transverse force means reduced compressive strength as well. The discrepancy between the results of this study and the existing theory could be due to external factors such as the shape of the sample being less homogeneous, the difference in the brand of softliner used, resulting in different results.

Significant differences in compressive strength were seen at K(+) and P3. Polident® solution causes hydrolysis when compared to the phenol content in *Sargassum sp.* so it appears that the compressive strength of K(+) is lower than P3. Immersion in denture cleansers such as Polident®, caused plasticizers and other soluble components to decompose out in some time while the water from the solution will be absorbed. The sodium perborate content of Polident® also causes the plasticizer to decompose in the denture matrix when the sodium perborate contacts to the softliner. Decomposition of plasticizers and water absorption lead to detrimental effect on physical properties of the softliner material where water absorption and solubility caused surface porosity of the softliner. This caused a change in the compressive strength of the material after being immersed in a Polident® solution.[25]

CONCLUSION

The concentration of *Sargassum sp.* effervescent extract in concentration of 10%, 20%, and 40% effective in reducing *Candida albicans* colonies on silicone based softliner compared to K(-) group, but still less effective than K(+) group. The most effective concentration of *Sargassum sp*. effervescent extract in inhibiting *Candida albicans* growth on silicone based softliner was 40%. The highest compressive strength was found in immersion with concentration of 40%.

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