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THE EFFECTIVENESS OF BILIMBI JUICE ON THE GROWTH OF CANDIDA ALBICANS ON ACRYLIC RESIN

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Abstract: -

Background: Acrylic resin is the most common material used as a denture base that contacts directly with the oral mucosa. Acrylic resin has a porous surface and surface roughness that allow the attachment and formation of *Candida albicans* colonies that trigger denture stomatitis. Bilimbi (*Averrhoa bilimbi L.*) juice contains saponins, phenols, tannins, alkaloids, flavonoids, and triterpenoids, which have antifungal activity against *Candida albicans*.

Purpose: To determine the effectiveness of bilimbi juice on the growth of *Candida albicans* on heat-cured and cold-cured acrylic resin.

Material and method: Experimental laboratory research with a post-test only control group design. 24 heat-cured and cold-cured acrylic resin samples, sizes 10x10x2 mm were divided into 4 groups. Groups I and II were heat-cured and cold-cured acrylic resins immersed in 100% bilimbi juice. Groups III and IV were heat-cured and cold-cured acrylic resins immersed in Aqua®. Each group was immersed for 48 hours, and then the number of colonies was calculated.

Results: There was a significant difference in the amount of *Candida albicans* in heat-cured and cold-cured acrylic resins immersed in 100% bilimbi juice compared with Aqua® ($p < 0.05$).

Conclusion: Bilimbi juice is effective in decreasing the growth of Candida albicans on heat-cured and cold-cured acrylic resins.

Keywords: acrylic resin; bilimbi juice; *Candida albicans*.

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INTRODUCTION

A denture is an artificial substitute for missing natural teeth and adjacent tissues. Denture functions are to restore masticatory ability, maintain residual tissues in the mouth, improve jaw relations, improve aesthetics, and improve quality of life. The denture base is the part of a denture that rests on the foundation tissues and to which teeth are attached.[1] Most denture bases are made of acrylic resin because it has many advantages. But acrylic resin also has porosity and surface roughness that need to be considered. This property increases the potential for the accumulation of microorganisms, especially *Candida albicans*. da Silva et al. (2016) evaluate the influences of surface roughness (SR) of denture bases on *Candida albicans* biofilm formation. He stated that reduced surface roughness resulted in decreased *Candida albicans* biofilm accumulation on poly (methyl methacrylate) acrylic resin denture bases.[2] *Candida albicans* colonies can be found in removable denture users who never remove and clean their dentures. *Candida albicans* is a normal flora that is often found in the oral cavity, digestive tract, and vagina. As a normal flora, *Candida albicans* can live commensally with the host without harm, but it can become dangerous if the host does not maintain good oral hygiene. The increase in the number of *Candida albicans* colonies occurred due to the closure of the oral mucosa by the denture base, which was accompanied by poor oral hygiene. This increase in colonies triggers denture stomatitis. A literature study conducted by Mawei et al. (2020) showed that there is a relationship between denture hygiene's level and denture stomatitis in full denture users.[3]

Inflammation and erythema of the oral mucosal areas covered by the denture are characteristics of denture stomatitis. According to epidemiological research, denture stomatitis affects 15% to over 70% of those who wear dentures. The incidence of denture stomatitis is higher among elderly denture users and among women. Poor denture hygiene, wearing dentures all day and night, the accumulation of denture plaque, and bacterial and yeast contamination of the denture surface are etiological factors. Additionally, poor-fitting dentures can increase mucosal trauma. All of these factors seem to make *Candida albicans* more likely to colonize the oral mucosal surfaces and dentures and act as an

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opportunistic pathogen.[4] This increase in *Candida albicans* colonies will interfere with the comfort, health, and quality of the patient's oral cavity, so an effective method of cleaning the acrylic resin denture base is needed.

Lee et al. (2016) investigated the effectiveness of six different denture cleaning techniques. The following six cleaning techniques were examined: mechanical brushing with a toothbrush, chemical soaking in a commercial cleansing tablet solution, combined brushing and chemical soaking in a commercial mouthwash solution, irradiation in an ultraviolet (UV) light box, and chemical soaking in distilled water. Following a single cleaning session, the efficiency of the denture cleaning techniques in lowering *Candida albicans* was assessed. As a result, brushing, soaking in a commercial cleansing tablet solution, or combining both approaches reduces the adherence of Candida albicans to denture samples when compared to other methods.[5] Most commercial cleansing tablet solution are made from chemicals, but nowadays the use of herbal ingredients is widely used by the community, in addition to advances in technology and science regarding chemicals. There are some herbal ingredients that prove to be effective against *Candida albicans*, such as pomegranate (*Punica granatum*) flowers[6], edamame (*Glycine max (L.) Merrill*)[7], parsley (*Petroselinum crispum*)[8], hibiscus flower (*Hibiscus rosa sinensis L.*)[9], etc.

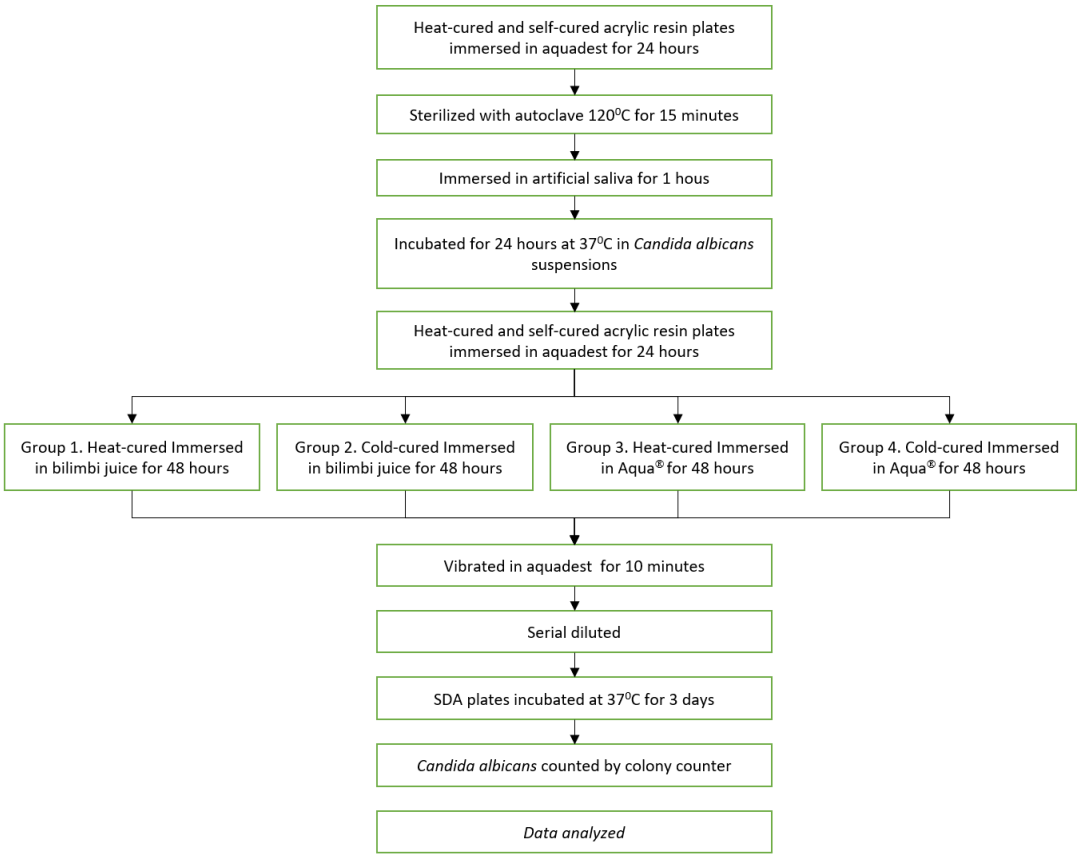
One of the plants that has long been used by the community as traditional medicine to treat disease is *Averrhoa bilimbi*, or in Indonesia, it is called Belimbing Wuluh. The fruits, flowers, and leaves of the Averrhoa bilimbi plant are useful. The fruit is used to make desserts, syrups, and itching paste. Inflammation, mumps, rheumatism, coughing, bleeding gums, tooth ache, good digestive function, minimizing hemorrhoids, and managing obesity are among the conditions it can help with. *Averrhoa bilimbi* is also useful for removing fabric stains, overcoming fishy odors and cleaning and shining brass.[10] Bilimbi is widely used by the community because it is generally safe, affordable, and accessible. Bilimbi contains a variety of phytochemicals, such as saponins, phenols, tannins, alkaloids, flavonoids, and triterpenoids. A previous study showed bilimbi have fungistatic or antifungal properties. Octaviani (2018) stated that bilimbi juice at 100% concentration can inhibit the growth of *Candida albicans* colonies by 55%.[11] However, there are no studies about the effectiveness of bilimbi juice on the growth of *Candida albicans* on acrylic resin. Based on that, we are interested in testing the antifungal effectiveness of bilimbi juice (*Averrhoa bilimbi L.*) on the growth of *Candida albicans* on heat-cured and cold-cured acrylic resin.

METHODS

This study was an experimental laboratory with a post-test only control group design. It was conducted at the Testing and Research Services Laboratory (Qlab), Faculty of Pharmacy, Pancasila University. The materials consisted of: 1) *Candida albicans* colony; 2) fresh bilimbi (*Averrhoa bilimbi L.*) juice with 100% concentration; 3) Aqua® (negative control); 4) heat-cured acrylic resin plates with a size of 10x10x2mm; and 5) cold-cured acrylic resin plates with a size of 10 x 10 x 2 mm. All plates of acrylic resin are the same size and shape. Based on Federer's formula, the sample size obtained is six plates in each group. So, the total size was 24, which was divided into four groups and immersed for 48 hours:

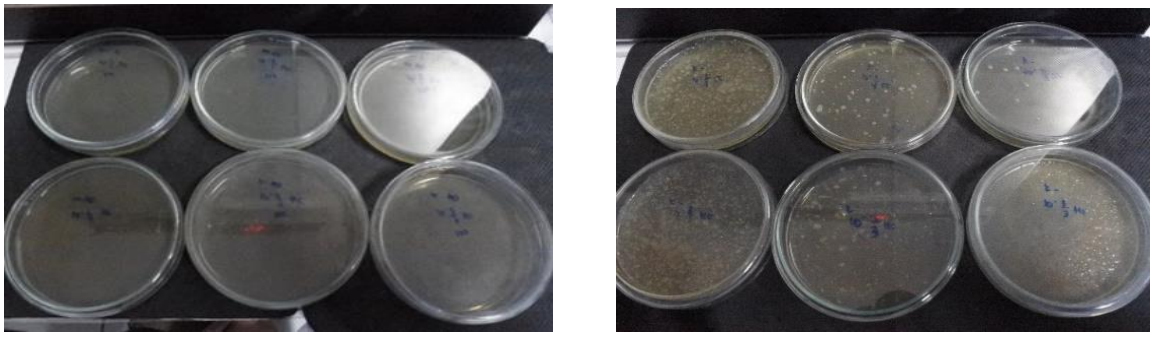
- Group I: treatment group with a heat-cured acrylic plates immersed in 100% bilimbi juice.
- Group II: treatment group with a cold-cured acrylic plates immersed in 100% bilimbi juice.
- Group III: control group of heat-cured acrylic plates immersed in Aqua®.
- Group IV: control group of cold-cured acrylic plates immersed in Aqua®.

The research flow diagram is in Figure 1. Data analysis in this study was carried out with SPSS®, with the data first being tested using Shapiro-Wilk to determine whether the data were normally distributed or not. If the data is normal, the independent t-test is used. If the data is not normal, the Kruskal-Wallis test is used.



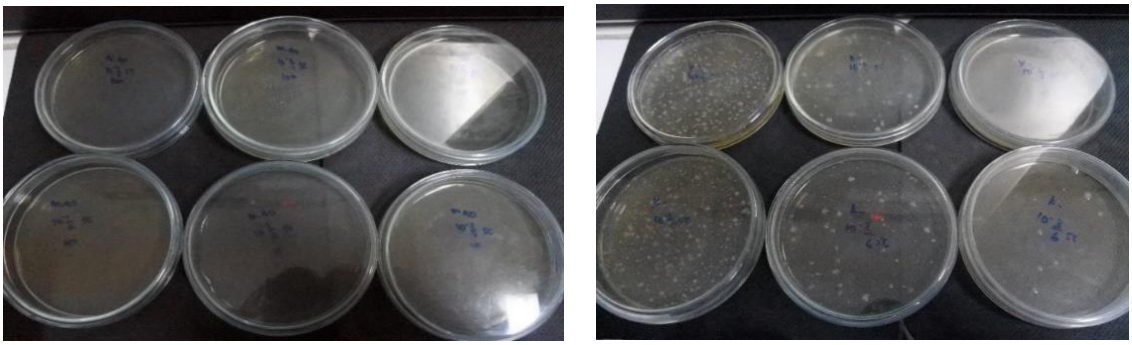
RESULTS

The aim of this study was to determine the effectiveness of bilimbi juice on the growth of *Candida albicans* on heat-cured and cold-cured acrylic resin denture bases. The growth of *Candida albicans* on head-cured and cold-cured acrylic resin after being immersed for 48 hours in bilimbi juice is less than that in Aqua® (Figure 1 and 2, Table 1)



(a) (b)

Figure 1. Candida albicans on heat-cured acrylic resin after being immersed in (a) bilimbi juice and (b) Aqua®



(a) (b)

Figure 2. Candida albicans on cold-cured acrylic resin after being immersed in (a) bilimbi juice and (b) Aqua®

Table 1. The average of *Candida albicans* colonies and standard deviation

Acrylic resin	Immersion	*Candida albicans* (CFU/ml)						Average ± SD
		I	II	III	IV	V	VI	
Heat-cured	Bilimbi juice	29,000	19,000	21,000	19,000	22,000	22,000	22,000.0 ± 3,687.8
	Aqua®	250,000	270,000	440,000	300,000	400,000	250,000	318,333.3 ± 81,833.2
Cold-cured	Bilimbi juice	11,000	14,000	16,000	20,000	20,000	14,000	15,833.3 ± 3,600.9
	Aqua®	520,000	410,000	400,000	320,000	550,000	470,000	445,000.0 ± 85,029.4

The results were tested for normality using the Shapiro-Wilk test because sample < 30. The results obtained a significance value of p>0.05, so the data is not normally distributed. The test was then continued with the Mann-Whitney test to find whether a significant difference between all research groups could be observed. The results showed that there was a statistically significant difference (p<0.05) between all study groups (Table 2).

Table 2. *Mann-Whitney test result*

Comparison of research groups		*p value*
Heat-cured bilimbi juice	Heat-cured Aqua®	0.004*
Cold-cured bilimbi juice	Cold-cured Aqua®	0.004*
Heat-cured bilimbi juice	Cold-cured bilimbi juice	0.024*
Heat-cured Aqua ®	Cold-cured Aqua®	0.03*

* = There were significant differences between all research groups (p<0.05)

DISCUSSION

This study was an experimental laboratory with a post-test only control group design. The aim of this study is to determine the effectiveness of bilimbi juice on the growth of *Candida albicans* on heat-cured and cold-cured acrylic resin denture bases. In this study, Aqua® was used as a control because it is commonly used by people in daily activities and easy to obtain, and some people use Aqua® to immerse their dentures all night. This study used heat-cured and cold-cured acrylic resins because they are still used in dentistry today as denture bases. Heat cured acrylic resin is currently the material of choice because it has good aesthetic quality, is non-toxic, does not irritate tissues, is relatively inexpensive, has no odor or taste, is easy to manufacture, and is easy to clean. The most commonly used acrylic resin is heat-cured, but cold-cured is still widely used for temporary dentures and the repair of broken dentures.

The total size of 24 samples of resin acrylics was divided into four groups, and all samples were immersed for 48 hours.

- Group I: heat-cured acrylic plate immersed in 100% bilimbi juice.
- Group II: cold-cured acrylic plate immersed in 100% bilimbi juice.
- Group III: heat-cured acrylic plates immersed in Aqua®.
- Group IV: cold-cured acrylic plates immersed in Aqua®.

Based on observations, heat-cured and cold-cured acrylic resins immersed in 100% bilimbi juice showed a lower amount of Candida albicans colonies, with an average Candida albicans colony of 22,000 CFU/ml on heat-cured acrylic resin and 15,833.3 CFU/ml on cold-cured acrylic resin. The statistical results showed that there were significant differences in the amount of Candida albicans colonies between heat-cured in bilimbi juice and Aqua® (*p value* = 0.004), cold-cured in bilimbi juice and Aqua® (*p value* = 0.004), heat-cured and cold-cured in bilimbi juice (*p value* = 0.024), and heat-cured and cold-cured in Aqua® ((*p value* = 0.03).

A denture is an artificial substitute for missing natural teeth and adjacent tissues. The denture base is the part of a denture that rests on the foundation tissues and to which teeth are attached.[1] The denture base has direct contact with the oral mucosa. Most denture bases are made of acrylic resin, but they also have porosity and surface roughness that increase the potential for the accumulation of microorganisms, especially *Candida albicans*.[2] Several research studies have suggested that the materials' surface roughness has a significant impact on how well Candida albicans adheres to them.[12] The increase in the number of *Candida albicans* colonies occurred due to the closure of the oral mucosa by the denture base, which was accompanied by poor oral hygiene. This increase in colonies triggers denture stomatitis. The most common method of cleaning acrylic resin denture bases is by brushing and immersing them in disinfectant. Most commercial disinfectants are made from chemicals, but nowadays the use of herbal ingredients is widely used by the community, in addition to advances in technology and science regarding chemicals.

One of the plants that has long been used by the community as traditional medicine to treat disease is *Averrhoa bilimbi*, or in Indonesia, it is called belimbing wuluh.[10] Previous studies showed bilimbi have fungistatic or antifungal properties.[11] Bilimbi contains a variety of phytochemicals, such as saponins, phenols, tannins, alkaloids, flavonoids, and triterpenoids.[13,14] The mechanism of action of saponins as antifungals is by reducing the sterol membrane, resulting in increased permeability, then the cells swell and burst causing the cells to die. The death of these cells results in inhibited growth and development of the fungus.[15] Tannins may have an antibacterial impact by destroying membranes and obstructing metabolic pathways, which could cause the microorganism to perish.[16] Flavonoids damage cell

membranes, inhibiting fungal adherence, fungal growth, *Candida albicans* proliferation, changes in yeast to hyphal forms, and biofilm formation.[17]

CONCLUSIONS

Based on the results of this research on the amount of Candida albicans on heat-cured and cold-cured acrylic resins after immersion in bilimbi juice, it can be concluded that bilimbi juice is effective in decreasing the growth of Candida albicans on heat-cured and cold-cured acrylic resins. What can be done in the next research are:

1. Conduct research with a lower immersion time;
2. Reduce the percentage of bilimbi juice;
3. Conduct research with a pre-test post-test control group design.

REFERENCES

1. Ferro, K. J., Morgano, S. M., Driscoll, C. F., Freilich, M. A., Guckes, A. D., Knoernschild, K. L., ... & Twain, M. (2017). The glossary of prosthodontic terms.
2. da Silva, W. J., Gonçalves, L. M., Viu, F. C., Leal, C. M. B., Barbosa, C. M. R., & Cury, A. A. D. B. (2016). Surface roughness influences Candida albicans biofilm formation on denture materials. *Revista Odonto Ciência*, *31*(2), 54-58.
3. Mawei, G. T., Wowor, V. N., & Mintjelungan, C. N. (2022). Hubungan Tingkat Kebersihan Gigi Tiruan Penuh dengan Kejadian Denture Stomatitis. *e-GiGi*, *11*(1), 20-25.
4. Gendreau, L., & Loewy, Z. G. (2011). Epidemiology and etiology of denture stomatitis. *Journal of Prosthodontics: Implant, Esthetic and Reconstructive Dentistry*, *20*(4), 251-260.
5. Lee, H. E., Li, C. Y., Chang, H. W., Yang, Y. H., & Wu, J. H. (2011). Effects of different denture cleaning methods to remove Candida albicans from acrylic resin denture based material. *Journal of Dental Sciences*, *6*(4), 216-220.
6. Golfeshan, F., Mosaddad, S. A., Alamdarloo, Y., Motamedifar, M., & Dehno, F. H. (2023). Effect of incorporating Punica granatum extract in acrylic resins on Streptococcus mutans and Candida albicans: a preliminary study. *Journal of Herbal Medicine*, *42*, 100770.
7. Kristiana, D., Hidayati, L., & Nabil, H. R. (2022). The Effectiveness of Edamame (Glycine Max (L.) Merrill) Extract as Acrylic Resin Denture Cleanser on the Number of Candida Albicans.
8. Arismunandar, R. N., Nosartika, I., Purnomo, B. N. R., & Antari, A. L. (2021). The Effectivity of Parsley (Petroselinum crispum) Extract on The Growth Inhibition of Candida Albicans. *Journal of Biomedicine and Translational Research*, *7*(3), 123-128.
9. Syaula, Y., Antari, A. L., & Purbaningrum, D. A. (2021). Pengaruh Perendaman Ekstrak Bunga Sepatu (Hibiscus rosa sinensis L.) terhadap Pertumbuhan Candida albicans pada Plat Resin Akrilik. *e-GiGi*, *9*(2), 159-166.
10. Setyawan, H. Y., Sukardi, S., & Nareswari, B. F. (2021, April). The phytochemical potential of Averrhoa bilimbi–A review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 733, No. 1, p. 012091). IOP Publishing.
11. Octaviani, M., & Fadila, F. (2018). Uji aktivitas antijamur sari buah belimbing wuluh (Averrhoa bilimbi L.) terhadap jamur candida albicans. *Jurnal Katalisator*, *3*(2), 125-133.
12. da Silva, W. J., Leal, C. M. B., Viu, F. C., Gonçalves, L. M., Barbosa, C. M. R., & Del Bel Cury, A. A. (2015). Influence of surface free energy of denture base and liner materials on Candida albicans biofilms. *Journal of Investigative and Clinical Dentistry*, *6*(2), 141-146.
13. Nair, M. S., Soren, K., Singh, V., & Boro, B. (2016). Anticancer activity of fruit and leaf extracts of Averrhoa bilimbi on mcf-7 human breast cancer cell lines: a preliminary study. *Austin J Pharmacol Ther*, *4*(2), 1082.
14. Setyawan, H. Y., Sukardi, S., & Nareswari, B. F. (2021, April). The phytochemical potential of Averrhoa bilimbi–A review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 733, No. 1, p. 012091). IOP Publishing.
15. Yulia, R., Chatri, M., Advinda, L., & Handayani, D. (2023). Saponins Compounds as Antifungal Against Plant Pathogens. *Jurnal Serambi Biologi*, *8*(2), 162-169.
16. Gizaw, A., Marami, L. M., Teshome, I., Sarba, E. J., Admasu, P., Babele, D. A., ... & Abdisa, K. (2022). Phytochemical screening and in vitro antifungal activity of selected medicinal plants against candida albicans and aspergillus niger in west shewa zone, Ethiopia. *Advances in Pharmacological and Pharmaceutical Sciences*, *2022*(1), 3299146.
17. Putri, H. F. W., Khusmitha, Q. N., Mahardhika, G. P. C., Hidayati, D. Y. N., Raras, T. Y. M., & Norahmawati, E. (2022). Comparison of Phytochemical Content and Antifungal Activity of Bajakah Tampala Stem (Spatholobus Littoralis Hassk.) Methanol and Ethanol Extracts Against Candida Albicans. *Asian Journal of Health Research*, *1*(2), 19-24.