



Activity of *Averrhoa Bilimbi L* Leaf Extract as Denture Cleaner Towards The Number of Colonies of *Candida Albicans* in Heat Cured Resin with a 30 Minutes Period

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ABSTRACT

Denture stomatitis is a chronic inflammatory condition of the oral mucosa that generally occurs in users of removable dentures. The cause is multifactorial but is often associated with the presence of *Candida albicans*. The mucosa which is covered by the denture base also reduces the function of saliva which functions as a cleanser for the oral cavity and results in food waste accumulating and microorganisms such as *Candida albicans* can increase in number. *Candida albicans* is a normal flora of the oral cavity which is an opportunistic pathogen. The starfruit plant (*Averrhoa bilimbi L*) is a natural ingredient that can be used as an antifungal agent. The aim was to determine the ratio of the effectiveness of starfruit leaf extract (*Averrhoa bilimbi*) between the concentrations of 12.5%, 25%, 50%, 100%, control + (Clorhexidine) and control - (Aquadest) in reducing the number of *Candida albicans* colonies. The test method used the dilution method, the colony count of *Candida albicans* using a spectrophotometer. The results of the one way ANOVA statistical test resulted in a significance value of $p = 0.000$ ($p < 0.05$) which means that there is a difference in the average concentration of *Candida albicans* between the extract concentrations of 12.5%, 25%, 50%, 100%, control + and control - namely 6.78 ± 0.35 , 6.27 ± 0.21 , 4.89 ± 0.29 , 1.01 ± 0.18 , 0.59 ± 0.19 and 9.13 ± 2.28 in inhibiting growth *Candida albicans* and this was confirmed by the Tukey HSD posthoc test. The conclusion of this study is that there is a difference between the antifungal effectiveness of starfruit leaf extract (*Averrhoa bilimbi*) concentrations of 12.5%, 25%, 50% and 100% in inhibiting the growth of *Candida albicans* colonies.

1. Introduction

According to the Basic Health Research (Riskesdas) in 2018, the national reported prevalence rate of dental and oral diseases in North Sumatra was 54.6%. Tooth loss at the age of 35-44 years amounted to 1.7% and increased by 10.1% at the age of 65 years and over¹. Continuous use of dentures accompanied by a low level of hygiene resulted in an increase in the number of growths of *Candida albicans*. the formation of plaque and will adhere to the teeth around the denture, antagonist teeth, and denture base that covers the mucosa. The mucosa which is covered by the denture base also reduces the function of saliva, which

functions as a cleanser for the oral cavity, resulting in the accumulation of food residue and microorganisms such as *Candida albicans* to increase in number.

Candida albicans is a normal flora of the oral cavity which is an opportunistic pathogen. Will turn into pathogenic microorganisms if there is a disturbance in the balance of life of the normal oral flora, such as changes in external factors that affect temperature, pH, energy sources². *Candida* growth in the oral cavity can cause candidiasis. Candidiasis is an infection that occurs due to the presence of fungi and can occur due to the support of several predisposing factors, among

others; changes in normal oral flora due to wearing dentures, poor oral hygiene, pregnancy, decreased immunity due to AIDS, diabetes mellitus, leukemia, chemotherapy and radiation, malabsorption and malnutrition³ As many as 65% of cases like this were found in parents who used full dentures. The cause of candidiasis can also occur due to continuous and unclean denture wear. This results in an increase in the number of *Candida albicans* growths. Therefore, an awareness of the importance of maintaining denture hygiene is needed by cleaning regularly and appropriately and removing dentures while sleeping and can also be helped by using denture cleaning fluids.

Indonesia has many types of plants that have many benefits and can be used by the community as food and medicine. Wuluh starfruit (*Avverhoa bilimbil* L) is a plant that can be found in Indonesia. It was reported in Andayani's research, that the extract of star fruit wuluh (*Avverhoa bilimbil* L) could inhibit the interaction between *S. sanguinis* and *S. mutans*, because of the interaction between antibacterial compounds contained in the extract of star fruit wuluh⁴. It is not only fruit that can be used, the leaves from belimbing are also known to be used as an anti-fungal. Wuluh starfruit (*Avverhoa bilimbi* L) can affect the growth of *Candida albicans* with a concentration of 12.5%, 25%, 50%, 100% and the results of his research show that starfruit also has anti-fungal properties⁵.

2. Method

This type of research used an in-vitro laboratory experimental study with a post-test only control group design. This research was conducted at the Laboratory of Physiology and Plant Tissue Culture of USU and the Laboratory of the Faculty of Medicine of UNPRI from February to March 2021.

The fresh starfruit leaves are taken and selected then washed and cleaned. Then the starfruit leaves are cut into pieces to facilitate the drying process. After the leaf sample has dried, the material is mashed using a blender until it becomes a powder. Then it is sieved using a 30 mesh sieve in order to obtain the same

degree of smoothness so that the extraction can run more optimally. This research was conducted using the maceration method. The maceration method in this study used a ratio of 10 mg: 250 ml of solvent for 48 hours⁶. The results of maceration are filtered using a liquid extraction that is free from the dregs of starfruit leaves. The liquid extract was concentrated by means of a rotary evaporator at a temperature of $\pm 80^\circ\text{C}$ for ± 1.5 hours in order to obtain a thick extract. The thick extract that has been obtained is made into four concentrations by mixing into the DMSO solution, namely 12.5%, 50%, 75% and 100%.

Preparation of the suspense was started by taking 1 ose *C. albicans* from the available pure culture and put it on Saborauds Broth 5 ml medium then incubated for 48 hours at 37°C . The turbidity that has been made is then adjusted according to the standard Mc. Farland no. 1 (3×10^8 CFU / ml). Furthermore, the suspension was diluted by taking 1 ml of the adjusted suspension plus 2 ml of Saboraud's broth to obtain a final concentration of 1×10^6 CFU / ml. After obtaining the standard concentration of candida, the 40x10x20 mm acrylic resin plate was immersed. Before the resin plate was contaminated to the *Candida* suspension, the acrylic resin plate was sterilized using an autoclave 121°C for 18 minutes. Then the acrylic resin plate was immersed in sterile saline for 1 hour and then rinsed using PSB (Phosphate Buffer Saline) solution 2 times.

The acrylic resin plate that has been exposed to *Candida albicans* is taken and then inserted into the reaction tube containing the extraction solution of Starfruit (*Avverrhoa bilimbi*) 12.5%, 25%, 50%, 100%, positive control (Chlorhexidine 0.2%) and negative control (Aquadest) for 30 minutes. Then the heat cured acrylic resin plate was inserted into the Saboraud broth 10ml and vibrated using a vortex mixer for 30 seconds to knock out *Candida albicans* which was sticking to the acrylic resin plate. Calculation of the number of *Candida albicans* using a spectrophotometer to determine the absorbance value of each broth media by taking 2 ml from each treatment tube then transferred to the cuvet and then read the absorbance value with a wavelength of 560nm and a

blank solution of Aquadest. The final result is obtained with the formula used according to Pudjiastuti⁷ as follows.

The data obtained were analyzed using the Kolmogorov-Smirnov test to determine normally distributed data. Followed by the homogeneity test using the Variance test (Levene S test of Varians). If the data is normally distributed and homogeneous, then a statistical test is carried out using the ANOVA test to see any differences between treatment groups. Furthermore, the tuckey-HSD (High Significant Different) test was carried out to determine whether there was a more detailed effect between the treatment groups.

3. Results

Research to test the ability of starfruit leaf extract (*Averrhoa bilimbi* L) on the growth of *Candida albicans* in denture users by measuring *Candida albicans* colonies measured using a spectrophotometer. The results of *Candida* colony measurements in each treatment group can be seen in the following table:

Based on table 1, it shows that the average concentration of *Candida albicans* in starfruit leaf extract (*Averrhoa bilimbi* L) with a concentration of 12.5%, 25%, 50%, 100%, Control + and control - is 6.78 ± 0.35 , 6.27 ± 0.21 , 4.89 ± 0.29 , 1.01 ± 0.18 , 0.59 ± 0.19 , and 9.13 ± 2.28 .

The results of research on differences in the concentration of *Candida albicans* colonies in all treatment groups can be seen in the table below:

Based on table 2 above the treatment group of starfruit leaf extract (*Averrhoa bilimbi* L) with a concentration of 100% had the least number of colonies compared to other extract treatments of 1.01 ± 0.18 and the least number of colonies among all treatment groups was in the control group. + where the number of *Candida albicans* colonies was 0.59 ± 0.19

4. Discussion

From the results of the phytochemical test, starfruit leaves were positive for phenolics, flavonoids, alkaloids,

saponins, tannins and triterpenoids. Based on the findings of active compounds in this study, it can be seen that the leaf extract of starfruit (*Averrhoa bilimbi* L) can have an effect in inhibiting the growth of *Candida albicans*. This research is in line with what was done by Putri, this is because the results of these studies indicate that the active substances found in plants are able to inhibit the growth of starfruit leaf extract (*Averrhoa bilimbi* L) with a concentration of 12.5%, 25%, 50%, 100% proven to have an effect in inhibiting the growth of *Candida albicans* 8. The higher the concentration of starfruit leaf extract used, the greater the inhibition power on the growth of *Candida albicans*. This can be seen in graph 1

The leaf extract of starfruit (*Averrhoa bilimbi* L) concentrations of 12.5%, 25% and 50% did not have a significant difference in inhibiting the growth of the number of *Candida albicans* colony. This can be seen in the post hoc Tuckey-HSD test where the significant value is $p > 0.05$. This finding is not in line with the research conducted by Puspitasari and Ardiansyah⁵. This is due to differences in the duration of immersion and the differences in research methods conducted by researchers where in this study conducted trials using the dilution method by calculating the diameter of the inhibition zone while in this study the researcher used the dilution method by calculating the absorbance value with a spectrophotometer to see the concentration of *Candida albicans*. This can happen because the UV-Vis spectrophotometer has one of the limitations where the light absorbed by the spectrophotometer cannot distinguish between living and dead microbial cells¹⁰.

In this study, the concentration that had the greatest effect in inhibiting the number of *Candida albicans* colonies was a concentration of 100% compared to a concentration of 12.5%, 25%, 50%, this is also in line with the research of Rakhmatullah and colleagues who said that the concentration of 100 % is more effective than the concentration below, this is because there are similarities in the use of starfruit leaf extract (*Averrhoa bilimbi* L) where the most important compounds are flavonoids, tannins and saponins¹¹. However, it is still less effective when compared to

positive controls (Clorhexidine 0.2%). The results obtained are in line with research conducted by Permatasari, et al. who said that Clorhexidine has a chlorodenol ring in its formula structure which has lipophilic properties so that clorhexidine works by penetrating into the cell wall so that it makes it easier for it to enter the fungal cell membrane which is predominantly composed of lipids and then causes intracellular leakage. Clorhexidine binds directly through ions, therefore clorhexidine has a high antimicrobial effect so it is more effective in inhibiting fungal

growth¹¹.

Based on the description above, it can be seen that the leaf extract of starfruit (*Averrhoa bilimbi* L) has an effect in inhibiting the number of *Candida albicans* colonies. The concentration of starfruit leaf extract (*Averrhoa bilimbi* L) 12.5%, 25%, 50% and 100% can inhibit the growth of *Candida albicans*. Leaf extract of starfruit wul-uh (*Averrhoa bilimbi* L) has the ability to inhibit the number of *Candida albicans* colonies, but it needs to be tested for cytotoxicity so that it can be used as a denture cleaning agent.

Calculation formula for the number of *Candida albicans* colonies

$$X = \frac{(\text{absorbance value of media + candida}) - (\text{absorbance value of media}) \times (3.10^9)}{(\text{The absorbance value of the Mc Farland No.1 standard solution})}$$

Information :

X = Concentration of fungi from Mc.Farland standard solution no.1

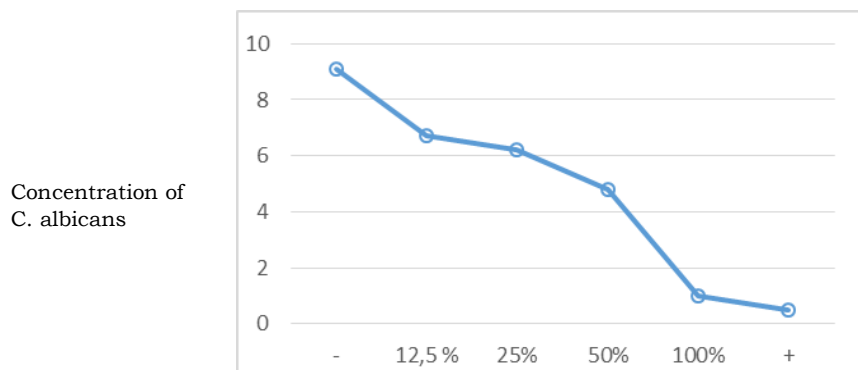
Table 1. Summary of the results of calculation of the number of candida colonies and the normality test

Group	Concentrations of Candida albicans				Average ± SD	Shapiro Wilk test
	Sample					
	1	2	3	4		
12.5 %	0.408	0.403	0.385	0.369	6.78 ± 0.35	0.606
25 %	0.375	0.375	0.355	0.358	6.27 ± 0.21	0.089
50 %	0.298	0.317	0.290	0.282	4.89 ± 0.29	0.748
100 %	0.095	0.115	0.097	0.103	1.01 ± 0.18	0.404
Chlorhexidine	0.81	0.093	0.070	0.083	0.59 ± 0.19	0.881
Aquades	0.588	0.431	0.392	0.624	9.13 ± 2.28	0.328

Table 2 Differences in the number of candida albicans colonies in all treatment groups to inhibit the growth of candida albicans.

Sample	Average number of colonies
12.5 %	6.78 ± 0.35 ^b
25 %	6.27 ± 0.21 ^b
50 %	4.89 ± 0.29 ^b
100 %	1.01 ± 0.18 ^a
Control +	0.59 ± 0.19 ^a
Control -	9.13 ± 2.88 ^c

Note: Data are presented in average ± SD. Different superscript signs (a, b, c, d) indicate different levels of effectiveness based on the Tukey HSD posthoc test.



Graph 1 Concentration of *Averrhoa Bilimbi* L leaf

5. Conclusion

Starfruit leaf extract (*Averrhoa Bilimbi* L) has active compounds including saponins, tannins, flavonoids, phenolics, alkaloids and triterpenoids. There is an effect of the effectiveness of the leaf extract of starfruit (*Averrhoa Bilimbi* L) with a concentration of 12.5%, 25%, 50% and 100% immersed for 30 minutes in inhibiting the number of *Candida albicans* colonies where the optimal concentration is found at a concentration of 100% and at a minimum concentration of 12.5% with mean values of these two concentrations are 1.01 ± 0.18 and 6.78 ± 0.35 , respectively. However, there was no significant difference between groups of starfruit leaves (*Averrhoa bilimbi* L) with a concentration of 12.5%, 25% and 50% in inhibiting the number of *Candida albicans* colonies ($p > 0.05$). Starfruit leaf extract (*Averrhoa Bilimbi* L) proved to be less effective than 0.2% chlorhexidine in inhibiting the number of *Candida albicans* colonies.

6. References

1. Health, B. P. d. P., 2018. Basic Health Research (RISKESDAS), Jakarta: Ministry of Health, Republic of Indonesia.
2. Todd, O. A. & Peters, B. M., 2019. *Candida albicans* and *Staphylococcus aureus* Pathogenicity and Polymicrobial Interactions: Lessons beyond Koch's Postulates. *Journal of Fungi*.
3. Utami, L., 2010. Percentage of *Candida albicans* and *Staphylococcus aureus* in denture stomatitis patients who wear prostheses.
4. Andayani, R., Chismirina, S. & Kumalasari, I., 2014. Effect of Wuluh Starfruit Extract (*Averrhoa bilimbi*) on the In Vitro Interaction of *Streptococcus sanguinis* and *Streptococcus mutans*. *Cakradonya Dent J*, pp. 678-744.
5. Puspitasari, S. A. & Ardiansyah, M., 2017. Inhibition of Ethanol Extract of Belimbing Wuluh Leaves (*Averrhoa Bilimbi* L.0 on the Growth of *Candida Albicans* in Loose Orthodontics. *Dental Journal Incisors*, Volume 6, p. 2.
6. Yulianingtyas, A. & Kusmartono, B., April 2016. Optimization of Solvent Volume and Maceration Time for Uptake of Flavonoid Leaves of Belimbing Wuluh (*Averrhoa Bilimbi* L). *Journal of Chemical Engineering*, Volume 10, p. No.2.
7. Pudjiastuti, P., 1999. The Effect of Biocompatible Concentration of Pineapple Weevil Extract and Contact Time on the Amount of *S. sanguinis* on the Tooth Surface. Surabaya: Graduate Program, Airlangga University.
8. Putri, A. Y., 2018. Activation Test of Anti-fungal and Secondary Metabolite Phytochemicals Endophytic Fungi *Trichoderma* Sp. Against Pathogenic Fungi *Colletotrichum* Sp. And *Fusarium oxysporum* in Chili Plants, Faculty of Science and Technology Uin Maulana Ibrahim Malam: Thesis.

9. Warokka, K. E., Wuisan, J. & J., 2016. Minimum Inhibitory Concentration Test (MIC) of Binahong Leaf Extract (*Anredera cordifolia* Steenis)
10. Rakhmatullah, H., Saputera, D. & Budiarti, L. Y., April 2018. Inhibition Activity of Wuluh Starfruit Leaf Extract with Chlorhexidine Against *Candida Albicans* on Acrylic Plate. *Dentin Journal of Dentistry*, II (No.1), p. 73.
11. Permatasari, D., Budiarti, L. Y. & Apriasari, M. L., 2016. Antifungals Effectiveness of Methanol Extract of Mauli Banana Stems (*Musa acuminata*) and Clorhexidine gluconate 0.2% AGAINST *Candia albicanas*. *Dentino*, 1 (1), pp. 10-14.