ORIGINAL ARTICLE

Effectiveness of Biduri leaf extract *(Calotropis gigantea)* as a denture cleanser in acrylic immersion against the growth of *Candida albicans*: an experimental laboratory

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ABSTRACT

Introduction: The high number of cases of tooth loss in Indonesia has led to the emergence of dentures. Dentures not appropriately kept clean can trigger cases of denture stomatitis due to *Candida albicans*. *C. albicans* is often detected on denture plates and causes denture stomatitis. Cleaning the dentures can prevent denture stomatitis, and chemical cleaning often uses synthetic materials such as alkaline peroxide. However, alkaline peroxide can cause side effects in the form of increased porosity on the surface of the acrylic plate, so an alternative natural ingredient, namely Biduri leaves (*Calotropis gigantea*), is needed because it has various active compounds such as alkaloids, flavonoids, phenols, saponins, terpenoids and tannins which have antifungal effects. This study aimed to determine the effective inhibitory power of Biduri leaf extract as a denture cleanser in acrylic immersion against the growth of *C. albicans*. **Methods:** Biduri leaves were extracted using the maceration method with 70% ethanol solvent to produce varying concentrations of 20, 30, 40, and 50%. The sample used in this research was an acrylic plate measuring 10x10x1 mm, soaked in artificial sterile saliva for 1 hour, then soaked in each sample group for 8 hours, after that it was placed in 10 ml of Sabouraud Dextrose Broth (SDB) media, and vibrated for 30 seconds using a mixing vortex, spreading 0,1ml SDB on Saboraud Dextrose Agar (SDA) and incubated for 48 hours. The growth of C. albicans was observed and counted using the colony counting method. **Results:** This research showed an inhibitory power of Biduri leaf extract in concentrations of 20, 30, 40, and 50% on the growth of *C. albicans*, with a concentration of 50%, having the most significant inhibitory power. **Conclusion:** Biduri leaf extract as a *denture cleanser* has an inhibitory power on the growth of *C. albicans*.

KEYWORDS

Biduri leaves, *Calotropis gigantea*, heat cured acrylic, *Candida albicans*

INTRODUCTION

Dental and oral health problems often found in Indonesia are cases of tooth loss. The results of Riskesdas (2018) prove that the average Indonesian population has a DMF-T index value of 7.1, meaning tooth decay occurs on average in 7-8 teeth per person.[1] Tooth loss is a condition where some or all of the teeth in the jaw arch are missing. That condition can affect functional, aesthetic, social damage, and impact a person's quality of life.[2] Treatment for cases of tooth loss can be done by making artificial teeth. A well designed denture will replace missing teeth and tissues to restore mastication and maintain arch integrity.[3]

Dentures must be clean to support oral health. Dentures that are not kept clean can adversely affect oral health, such as denture stomatitis due to *C. albicans*

infection.[4] Denture stomatitis due to *C. albicans* infection is a condition where the oral mucosa becomes inflamed due to contact with the anatomical base of the denture. In 70% of denture stomatitis sufferers, *C. albicans* fungus can be found, especially in the porous and undercut areas.[5] *C. albicans* is a 45-65% commensal microorganism in the oral cavity which almost always linked to denture stomatitis and the prevalence will increase up to 60-100% in the uses of denture.[6] One of the treatments for denture stomatitis due to *C. albicans* infection is maintaining cleanliness of the teeth and mouth along with the dentures. Denture cleaning can be done either mechanically or chemically. According to Research by Daaniyal *et al*[7]., there are still people who are not cleaning their dentures at all. That behavior can happen due to a lack of understanding about cleaning dentures. People usually clean dentures using soap/detergent, which may not be compatible with denture materials.[8]

The denture cleanser ingredient that is currently widely marketed is alkaline peroxide, which can be used by dissolving it in water and has a tablet dosage form.[9] The alkaline peroxide in denture cleansers produces oxygen bubbles to remove staining and kill bacteria that can create plaque on the surface of the teeth. Research by Dewi *et al.,*[9] found that heat cured acrylic resin plates experienced changes in roughness values after being soaked in alkaline peroxide. When a denture cleanser tablet is dissolved in water, sodium perborate will decompose into alkaline peroxide. Then, this compound releases oxygen, which cleans the denture plate.[10] Alkaline peroxide, capable of strong oxidation, can cause physical changes to heat cured acrylic resin plates, namely changes in surface roughness.[11] Alkaline peroxide material also has a relatively expensive price, so not all denture users can afford it.[12]

With increasing roughness and the relatively high price of alkaline peroxide denture cleansers, an alternative denture cleanser with a more affordable price and minimal effect on denture teeth is needed. One natural alternative ingredient that might be used is the Biduri leaf (*Calotropis gigantea*). The Biduri leaf can inhibit the growth of fungi and bacteria because it contains various phytochemical ingredients such as flavonoids, tannins, polyphenols, and saponins, which help inhibit and kill foreign substances in the body, including fungi.[13] Research by Dama *et al.,*[14] showed that concentrations of 20, 30, 40, and 50% cinnamon extract could inhibit *C. albicans* with significant differences and an increase at each concentration due to the presence of phytochemicals such as flavonoids, saponins, and tannins in cinnamon extract which are also found in Biduri leaves.

Based on the description above, Biduri leaves have various benefits, especially as an antifungal agent.[15] However, further research regarding the use of Biduri leaf extract as a denture cleanser ingredient still needs to be carried out. Therefore, it is necessary to research the effectiveness of Biduri leaf extract in concentrations of 20, 30, 40, and 50% as a denture cleanser in inhibiting the growth of *C. albicans* on immersion in heat cured acrylic plates with a soaking time of 8 hours. The soaking time of 8 hours is considered the length of time the denture is soaked when the user rests at night. This study aimed to determine the effective inhibitory power of Biduri leaf extract as a denture cleanser in acrylic immersion against the growth of *C. albicans*.

METHODS

This type of research is an experimental laboratory in vitro with a posttest only control group design. This research was carried out in June-December 2023. The locations of this research were Jember State Polytechnic Plant Laboratory for identification of Biduri leaves, the Biology Laboratory of the Teacher Training and Education Faculty of Jember University for making extracts, the Basic Dentistry Laboratory of Jember University Faculty of Dentistry for *Candida albicans* growth analysis, and the Dental Technology Laboratory of Jember University Faculty of Dentistry for making heat cured acrylic plates.

The acrylic resin used in this research was ADM, England. Making heat cured acrylic resin plate samples began with making a mould space using dental wax measuring 10x10x1 mm, according to ISO guidelines (ISO/DIS 1567: 1997). Then, the cuvette was smeared with vaseline, and a casting mixture was made in proportion according to the manufacturer's requirements in the bowl, and then it was put into the cuvette while vibrating. The dental wax plate was placed on the mixture until partially embedded and left for 15 minutes. After the gypsum mixture had hardened, the surface of the gypsum was smeared with vaseline. The upper cuvette was installed as the gypsum mixture was applied while vibrating it. Then, the cuvette was closed and pressed using a begel press. After the plaster mixture hardened, wax elimination was carried out by boiling water to a temperature of 100°C and inserting a cuvette into it. After that, the cuvette was opened and the remaining dental wax was poured with hot water until clean. After that, mold space would form.[11]

The surface of the mold space was smeared using a cold mold seal (CMS). Then, the heat cured acrylic resin material was stirred in a mixing jar with a ratio of 3:1 according to the manufacturer's instructions. After polymerization reached the dough stage, the dough was put into a mold space smeared with CMS. The upper cuvette was installed and pressed twice using a hydraulic bench press. After that, it was placed in a begel press and soaked in clean water. The cuvette and begel press were then heated (cured) in an aluminum pan with water that covered the entire surface of the cuvette. The water was heated until boiling (±100°C), then the cuvette was kept for 30 minutes.[11] After that, the cuvette was left in the pan until the water temperature returned to room temperature and could be opened. Then, finishing and polishing could be done on one side of the acrylic using sandpaper no. 260 and 550.

Making Biduri leaf extract began with picking leaves that met the inclusion criteria, namely intact leaves free from pests, not rotten, fresh green in color, not brownish, and semi-old to old leaves marked by a reduced number of dense white hairs on the surface of the leaves. The process was started by washing the leaves with running water, cutting them into small pieces, and air drying them for seven days to reduce the water content in the material. The semi-dried leaves were baked in the oven at 40°C for 48 hours. The oven-dried leaves were ground with a blender and sieved with a 30 mesh sieve to form simplicia powder. After that, simplicia was dissolved in 70% ethanol with a ratio of 1:5 and extracted using the maceration method using a shaker. The extraction results were concentrated using a rotary evaporator at a speed of 100 rpm with a temperature of 50°C until the weight was stable and produced a thick extract. The thick extract was diluted using distilled water to make varying concentrations of 20, 30, 40 and 50%.

The samples were divided into six treatment groups, with each treatment consisting of 5 heat cured acrylic resin plate samples. The positive control group (K+) used alkaline peroxide, the negative control group (K-) used sterile distilled water, treatment group 1 (P1) used 20% concentration Biduri leaf extract, treatment group 2 (P2) used Biduri leaf extract concentration of 30%, treatment group 3 (P3) used Biduri leaf extract with a concentration of 40%, and treatment group 4 (P4) used Biduri leaf extract with a concentration of 50%. Acrylic resin plates in all treatment groups were soaked for 8 hours, considering the length of time the denture was soaked when the user rested at night. The test started by immersing a heat cured acrylic resin plate in sterile distilled water for 48 hours to reduce residual monomer. The heat cured acrylic resin plate was sterilized using an autoclave at 120°C for 18 minutes. Then, the plate was soaked in artificial sterile saliva for 1 hour to form a pellicle on the surface, then rinsed with phosphate buffer saline (PBS) 2 times. The heat cured acrylic resin plate was then contaminated with *C. albicans* ATCC 10231 suspension (Mc. Farland no. 1) and incubated for 24 hours at 37°C. After contamination, the acrylic resin plate was rinsed with PBS 2 times. After that, the acrylic resin plate was soaked in each treatment group for 8 hours. The plate was then rinsed with PBS 2 times, placed

in 10 ml of Sabouraud Dextrose Broth (SDB) media, and subjected to vibration for 30 seconds using a mixing vortex. After that, the 0.1 ml of *C. albicans* suspension was taken from SDB media, and was dropped onto Sabouraud's Dextrose Agar (SDA) for spreading, then it was incubated at 37°C for 48 hours.[16] The inhibitory power of Biduri leaf extract was calculated against *C. albicans* using the colony counting method with the help of a colony counter.

Calculating the number of colonies using a colony counter (Funke gerber, Germany) began by connecting the colony counter to an electrical power source, turning on the tool by pressing the "On" button, ensuring that the tool calibration was correct, and pressing the calculation button so that it returned to 0 (zero). The petri dishes were placed on a table equipped with a scale to count the colonies; then, the existing colonies were marked with a pen until the calculation button sounded and colonies were counted. If it was not clear enough, a magnifying glass could be used. After counting all the colonies and recording the results, the colony counter was turned off by pressing the "Off" button. The data obtained was then analyzed using the Shapiro-Wilk normality and Levene homogeneity tests. Next, the data were analyzed using the Kruskal Wallis and Mann Whitney non-parametric tests because the data were not normal and homogeneously distributed. Overall data analysis used a significance level of 5% ($\alpha = 0.05$).

RESULTS

The results of research on the antifungal potential of Biduri leaf extract (*Calotropis gigantea*) as a denture cleanser on immersion in heat cured acrylic plates on the number of *C. albicans* colonies showed differences in colony growth in each sample group after soaking for 8 hours, presented in Figure 1.

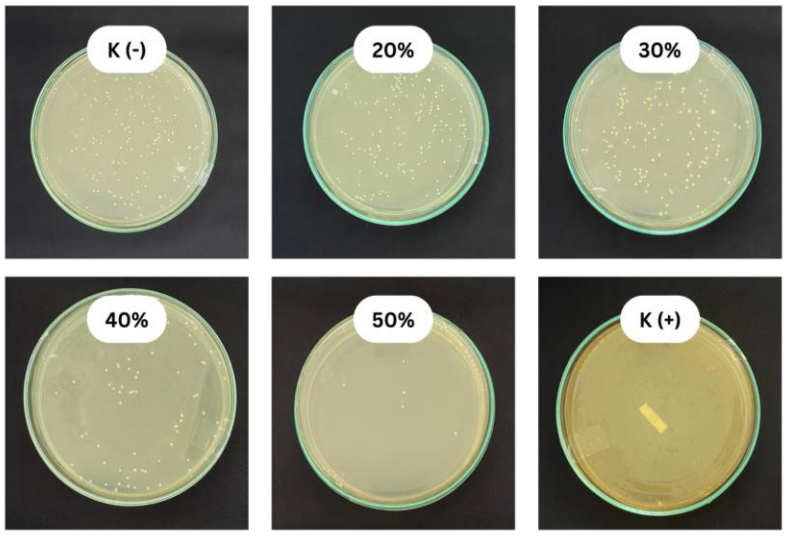

Figure 1. Growth of *C. albicans* colonies growing on SDA media in each sample group.

Figure 1 shows that in the K (+) sample group, 1 colony of *C. albicans* was found to grow in one of the repetitions. At a concentration of 50% Biduri leaf extract, 5 colonies of *C. albicans* were found in one of the repetitions. Meanwhile, in the K (-), 20%, 30%, and 40% sample groups, there was still a lot of growth of *C. albicans* in each sample group and repetition. The results of the average number of *C. albicans* colonies in each sample group are presented in Table 1 and Figure 2.

Table 1. Average results and standard deviation of the number of *C. albicans* growths in each sample group.

Sample group	N	Average	SD
K (-)	5	411,53	66,27
20%	5	353,87	166,43
30%	5	175,73	65,28
40%	5	106,87	70,62
50%	5	23,87	29,17
K (+)	5	42,07	56,95

The results of the Shapiro-Wilk normality test in one of the sample groups showed a significance value of 0.036 ($p<0.05$), which means the data were distributed non-normally. The Levene test homogeneity test results showed a significance value of <0.001 ($p<0.05$), meaning the data were distributed non-homogeneously. Based on the normality and homogeneity tests, the results showed that the data were not normally distributed and not homogeneous, so the data did not meet the parametric test requirements. The data were analyzed further using the Kruskal Wallis and Mann Whitney non-parametric tests. The results of the Kruskal Wallis test showed a significance value of <0.001 ($p<0.05$), so there were significant differences between sample groups. After that, the Mann Whitney test was carried out to see the significance of each sample group (Table 2).

Table 2. *Mann Whitney* test result

Sample group	K(+)	K(-)	20%	30%	40%	50%
K(+)	-	0.009*	0.009*	0.016*	0.175	1.000
K(-)	-	-	0.917	0.009*	0.009*	0.009*
20%	-	-	-	0.076	0.028*	0.009*
30%	-	-	-	-	0.117	0.009*
40%	-	-	-	-	-	0.047*
50%	-	-	-	-	-	-

*: There were significant differences ($p<0,05$)

The results of the Mann Whitney test (Table 2) showed that the differences in the number of *C. albicans* colonies were not significant ($p>0.05$) in the control group (-) with a concentration of 20%, a concentration of 20% with a concentration of 30%, a concentration of 30% with a concentration of 40%, a concentration of 40% with the control (+), and 50% concentration with control (+). This could happen because there was a small distance between each extract concentration so it was possible that the amount of active compound was not much different in inhibiting the growth of *C. albicans*.

DISCUSSION

The results of research on Biduri leaf extract (*Calotropis gigantea*) on immersion in heat cured acrylic plates on the growth of *C. albicans* showed the ability of Biduri leaf extract as a denture cleanser in inhibiting the growth of *C. albicans* for a soaking time of eight hours (Figure 1). The decrease in the average number of *C. albicans* growth in each Biduri leaf extract concentration group could be evidence of the ability (Table 1). That is in line with research that cinnamon extract, with the same phytochemical ingredients as Biduri leaves, can influence the growth of the number of *C. albicans* blastospores on acrylic resin plates and increases with increasing extract concentration.[14] As the extract concentration increased, the number of *C. albicans* colonies decreased. That is due to the increasing number of active substances which play a role in inhibiting fungal

growth.[17]

In this study, Biduri leaf extract at a concentration of 20% compared to control (-) was not significantly different. However, Figure 2 shows a decrease in the average number of *C. albicans* colonies compared to the control (-), meaning that Biduri leaf extract at a concentration of 20% can already inhibit *C. albicans* even though the number decrease is insignificant. That is possible because the small amount of active compounds such as alkaloids, flavonoids, phenols, saponins, terpenoids, and tannins contained in Biduri leaf extract at a concentration of 20% means that they are not able to inhibit the growth of *C. albicans* optimally. That can happen because antifungal activity will increase as the extract concentration becomes high.[17]

In this research, Biduri leaf extract with a concentration of 30% inhibited the growth of *C. albicans*. Table 2 shows a significant and decreasing number of *C. albicans* between the control group (-) and the 30% concentration. Meanwhile, 40% and 50% concentrations showed increased *C. albicans* inhibition, with decreased *C. albicans* colonies (Figure 2). That is in line with research by Ngegba *et al.*,[17] which stated that as the concentration of the extract increases, its antifungal potential will also increase. That is also possible because there are differences in the number of active compounds in each extract concentration.

In Table 2, it can be seen that Biduri leaf extract concentrations of 40% and 50% are not significantly different from the control (+), meaning that concentrations of 40% and 50% have the same ability as the control (+) in inhibiting the growth of *C. albicans*. However, based on the results of several repetitions of the control (+), it was still found that there was growth of *C. albicans*. That is possible because alkaline peroxide has the side effect of increasing porosity on the surface of the denture base.[18] The rougher and more porosity on the surface of the denture, it can cause an increase in the number of *C. albicans* on the surface of heat cured acrylic resin.

The content of active compounds in each extraction result can vary. That can be affected by several factors, such as the temperature when processing simplicia, the time of maceration, and the type of solvent used. Based on research by Saha *et al*[13]., the Biduri plant can inhibit fungi because it contains various phytochemical ingredients such as alkaloids, flavonoids, tannins, polyphenols, and saponins. Based on the results of phytochemical screening, the active compounds with the highest amounts in Biduri leaf extract are alkaloids. Alkaloids have antifungal capabilities because they can thwart the process of fungal cell wall formation and cause lysis by disrupting the peptidoglycan components of fungal cells.[19] The second active compound contained in Biduri leaf extract is flavonoids. Flavonoids work by denaturing fungal protein bonds so that the cell membrane will lyse so flavonoids will enter the fungal cell nucleus and inhibit its development.[19,20] Biduri leaf extract also contains active phenol compounds, which can inhibit fungal growth by binding to cell membrane proteins, then causing the decomposition of these protein compounds accompanied by penetration of phenolic compounds into the cells, resulting in denaturation of the cell membrane proteins. Damage to the fungal cell membrane can cause disruption of cell permeability and even destruction of the fungal cell membrane.[19,20] The higher the phenol content in a material, the stronger the material will be in inhibiting fungal growth.

The active compound also found in Biduri leaf extract is saponin. Saponin disrupts the stability of the fungal cell membrane by reducing the surface tension of the fungal cell wall so that the stability of the membrane is disturbed and causes the cell to experience lysis.[20] Apart from saponins, Biduri leaf extract also contains terpenoid compounds that can inhibit fungal growth by interfering with the development of fungal spores or through fungal cell membranes.[20] The last active compound is tannin. Tannins can inhibit fungal growth by inhibiting chitin synthesis to disrupt the formation of fungal cell walls, and the cell membrane is damaged, causing inhibition of fungal growth. Tannins also work by inhibiting the vegetative process of *C. albicans* through inhibiting sterols, which are responsible

for membrane fluidity and permeability. When the sterols are inhibited, membrane formation can be disrupted.[20]

The present study showed the antifungal efficacy of the *Calotropis gigantea* extract. However, it requires further assessment of whether the colony counting test method could be improved. *Calotropis gigantea* has a potential future direction in developing denture cleansers. Various microorganism types need to be investigated to confirm whether *Calotropis gigantea* is a potential material to be used as a denture cleanser and to inhibit any bad influence on oral health while using dentures. *Calotropis gigantea* in this study was extracted using the maceration method which needs to be developed with other solvents or modern extraction techniques to obtain sufficient quality and quantity of bioactive molecules.

CONCLUSION

Biduri leaf extract (*Calotropis gigantea*) has the inhibitory power against the growth of *C. albicans* on immersion in heat cured acrylic plates for eight hours which has the potential to be used as a denture cleanser. Based on that, *Calotropis gigantea* is a potential material to be used as a denture cleanser and to inhibit any bad influence on oral health while using dentures.

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