DENTINO JURNAL KEDOKTERAN GIGI Vol VI. No 1. Maret 2021

THE EFFECT OF ULIN (*Eusideroxylon zwageri*) STEM BARK EXTRACT ON THE GROWTH OF *Candida albicans* ON ACRYLIC RESIN DENTURE PLATES

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ABSTRACT

Background: Candida albicans is the main microorganism that causes denture stomatitis, thus denture soaking in cleansing solution is needed to protect them from Candida albicans contamination. The 0.2% Chlorhexidine gluconate is one of denture cleansers that induce side effects at prolonged use. An alternative ingredient that can be used as a denture cleanser is ulin stem bark extract. **Objective:** To determine the effect of ulin stem bark extract at 20%, 40%, 60%, 80%, 100% concentration on the growth of Candida albicans on acrylic resin denture plates. **Method**: True Experimental with post-test only with control group design was employed under 7 treatment groups consisting ulin stem bark extract at 20%, 40%, 60%, 80%, 100% concentration, 0.2% Chlorhexidine gluconate, and aquadest in a total of 28 samples. Acrylic resin samples that had been exposed to Candida albicans were soaked in respective treatment for 15 minutes. **Results:** The average of Candida albicans colonies on acrylic resin denture plates gluconate, and aquadest were 29.5 CFU/ml, 13.0 CFU/ml, 0 CFU/ml, 0 CFU/ml, 0 CFU/ml, 0 CFU/ml, and 155 CFU/ml. Based on Mann Whitney test, there was no significant difference when ulin stem bark extract at 20%, 40%, 60%, 80%, and 100% concentration have been proven to reduce Candida albicans colonies on acrylic resin denture plates, and the 60% concentration have been proven to reduce Candida albicans colonies on acrylic resin denture plates, and the 60% concentration is equivalent to 0.2% Chlorhexidine gluconate.

Keywords: Candida albicans, Eusideroxylon zwageri, Heat cured acrylic resin. Ulin stem bark extract. Correspondence : Annisa Fitri Yuniar, Faculty of Dentistry, Lambung Mangkurat University, Veteran street 128B, Banjarmasin, South Kalimantan, E-mail: annisa.f.yuniar@gmail.com

INTRODUCTION

A common dental and oral health problem that often occurs among Indonesian society is tooth loss. Riskesdas data in 2018 showed that the prevalence of tooth loss at the age of 15-24 years old was 8.4% and increased to 30.6% above the age of 65 years old. Tooth loss may lead to several functional disorders, including over-eruption, decreased aesthetic and masticatory function, tooth rotation, and tooth migration. The use of denture is a solution to avoid the impact of tooth loss.¹

The most common material that used for denture plate is acrylic resin. Around 95% denture plates are made of heat cured acrylic resin. The advantages of this material include good aesthetics, lightweightness, unexacting repairability, easy manufacturing, and cheap. On the other hand, the disadvantages of this material include proneness to fractures, poor thermal conductivity, susceptibility to abrasion during cleaning or usage, and capability to absorb liquid. Therefore, when acrylic resin is placed into oral environment, it will absorb saliva that will cover denture surface with protein-rich saliva; hence, pellicles will be formed. The pellicles will promote the colonization of microorganisms that will gradually grows and cause an increase in the attachment of microorganisms to dentures, and one of them is *Candida albicans*.^{1,2,3}

Candida albicans is a species of fungi found in the oral cavity. In fact, it is normal flora of the mouth. It can transform into an opportunistic pathogen when the environment is suitable, resulting in disturbances. Dirty dentures will increase the number of *Candida albicans* colonies which cause inflammation of the oral mucosa, known as denture stomatitis.⁴ *Candida albicans* is the main microorganism that causes denture stomatitis. 1

Soaking denture in denture cleanser at night is a prevention of denture stomatitis, hence it has an important role in reducing the number of Candida albicans.5,6 Denture cleanser usually have a chemical base, one of them is 0.2% Chlorhexidine gluconate. It can be used by soaking denture in the solution for 15 minutes. The impact of long-term use of 0.2% Chlorhexidine gluconate involves teeth discoloration, a relatively expensive price, and decolorization of denture plate, therefore alternative ingredients for denture cleaners are needed. There are many types of traditional plants in Indonesia that can be used as an alternative ingredient for denture cleaners, thus many researchers have begun to explore the use of these ingredients as disinfecting agents.5,7,8

Based on the results of phytochemical tests, ulin (*Eusideroxylon zwageri*) stem bark extract contain a large amount of tannins, flavonoids, and phenols and there are moderate amounts of alkaloids, saponins, terpenoids,. Several studies showed that phenols, flavonoids, saponins, terpenoids, alkaloids, and tannins had an antifungal effect and might inhibit the growth of *Candida albicans*.^{10,11}

Up until now, there has been no study on natural ingredients that uses ulin bark to inhibit the growth of *Candida albicans* yet, therefore the study of the effect of ulin *(Eusideroxylon zwagei)* stem bark extract on the growth of *Candida albicans* on acrylic resin dentures plates was conducted.

MATERIALS AND METHODS

This study was conducted at the Laboratory of Mathematics and Natural Science Faculty Lambung Mangkurat University, Industrial Cosultation Research Center Surabaya, and Microbiology Laboratory of Faculty of Dentistry Research Center Airlangga University. This study has obtained research and ethical approval from the Ethical Committee of Faculty Dentistry Lambung Mangkurat University No. 013/KEPKG-FKGULM/EC/I/2020.

This study used a true experimental with a posttest only with control group design. The sample used in this study was heat cured acrylic resin with a size of 10 mm x 10 mm x 2 mm. The sampling process was obtained by simple random sampling technique that used 7 treatment groups, that were ulin stem bark extract at 20%, 40%, 60%, 80%, 100% concentration, 0.2% *Chlorhexidine gluconate* as control positive, and aquadest as control negative with a total of 28 samples.

Ulin stem bark extract was made using maceration method. Two kilograms of ulin stem bark were cleaned and dried. First, ulin stem bark was cut into small pieces and then processed into powder. After that, the powder was filtered through a screen mesh. Ulin stem bark powder was then immersed in 96% ethanol solvent for 1 x 24 hours and stirred with the help of shaker. Furthermore, the extract was filtered, and the filtrate was evaporated using a rotary evaporator at a temperature of 59-60°C until a concentrated extract was obtained, then it was heated on a water bath so that the entire solvent was evaporated to produce 14 grams of brownish liquid residue with 100% concentration. A few drops of potassium dichromate (K₂Cr₂O₇) were added to the sample of ulin stem bark ethanol extract for free ethanol test. If there was no color change, then ulin stem bark extract stated as free ethanol.

Ulin stem bark extract with a concentration of 100% was diluted to various concentrations of 20%, 40%, 60%, 80% with formula as follow:

$V_1.M_1 = V_2.M_2$

- $V_1 = Volume of diluted solution (ml)$
- M_1 = Concentration of ulin stem bark extract (%)
- $V_2 =$ Volume of solution (water and extract) desired (ml)
- M₂ = Concentration of ulin stem bark extract to be made (%)

Acrylic resin plate samples were immersed in sterile distilled water for 48 hours to reduce the residual monomers, then the sterilization was performed. Furthermore, the acrylic resin plates were soaked in sterile saliva for 1 hour to facilitate the attachment of *Candida albicans*. Next, the acrylic resin plates were rinsed with PBS (*Phosphate Buffer Saline*) solution twice. Then, acrylic resin plates were inserted into test tubes containing *Candida albicans* suspensions and incubated for 24 hours at 37°C.

Acrylic resin plates that had exposed to *Candida* albicans are inserted into a test tube containing a solution of ulin stem bark extract (*Eusideroxylon zwageri*) of 20%, 40%, 60%, 80%, 100%, negative control (Aquadest), and positive control (0.2% *Chlorhexidine gluconate*) for 15 minutes. Next, acrylic resin plates were rinsed with PBS solution twice. Then the acrylic resin plates were inserted into the BHIB (*Brain Heart Infusion Broth*) and vibrated using a vortex mixer for 30 seconds. A total of 0.1 ml BHIB was taken and dropped into SDA (*Sabouraud Dextrose Agar*). The media was subsequently incubated for 48 hours at 37°C. The next step was counting the number of *Candida albicans* colonies.

RESULTS

The results for the effect of ulin stem bark extract on *Candida albicans* on acrylic resin plates were obtained by counting the number of *Candida albicans* colonies after the soaking process. The average number of *Candida albicans* colonies found on acrylic resin denture plates after the soaking process in ulin stem bark extract, 0.2% *Chlorhexidine gluconate*, and aquadest can be seen in the table below.

Table 1. The Avarage of Candida albicansColonieson Acrylic Resin Denture Plates After the
Soaking Process in Ulin Stem Bark
Extract, 0.2% Chlorhexidine gluconate,
and Aquadest.

Group	N	Mea n	Std. Devia tion
EKBU 20%	4	29.5	2.08
EKBU 40%	4	13.0	0.82
EKBU 60%	4	0	0
EKBU 80%	4	0	0
EKBU 100%	4	0	0
K (+)	4	0	0
K (-)	4	155	2.16

Table 1 shows the average results of various treatment groups. The number of *Candida albicans* colonies on acrylic resin denture plates after soaking in ulin stem bark extract at 20%, 40%, 60%, 80%, 100% concentration, 0.2% *Chlorhexidine gluconate*, and aquadest have an average of 29.5 CFU/ml, 13.0 CFU/ml, 0 CFU/ml, 0 CFU/ml, 0 CFU/ml, 0 CFU/ml.

Data obtained from each treatment was tabulated and a normality test was performed using Saphiro Wilk test. Normality test results obtained that p<0.05, thus it can be concluded that the data were not normally distributed and based on Levene's homogeneity test showed a significance value of p < 0.05 revealing that the data were not

homogeneous. Furthermore, the data were analyzed using Kruskal Wallis non-parametric test.

Based on Kruskal Wallis non-parametric analysis test results, the value of p=0,000 (p < 0.05) which shows differences in the number of *Candida albicans* colonies when assessed on the treatment given; therefore, the analysis was continued by the Mann Whitney test to find out which groups impart the difference.

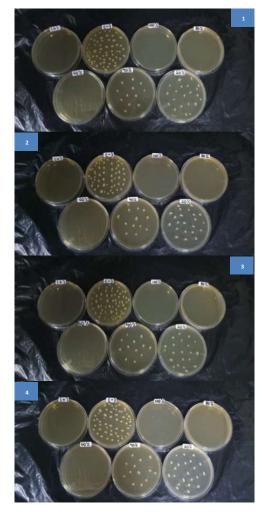


Figure 1. Results for the Effect of Ulin Stem Bark Extract, 0.2% *Chlorhexidine Gluconate* as Positive Control, and Aquadest as Negative Control on the Growth of *Candida albicans* on Acrylic Resin Dentures with 4 repetitions.

G	Growth on Acrylic Resin Denture Plates.								
Group	EKBU 20%	EKBU 40%	EKBU 60%	EKBU 80%	EKBU 100%	K (+)	K (-)		
EKBU 20%		0.020*	0.014*	0.014*	0.014*	0.014*	0.021*		
EKBU 40%			0.013*	0.013*	0.013*	0.013*	0.020*		
EKBU 60%				1.000	1.000	1.000	0.014*		
EKBU 80%					1.000	1.000	0.014*		
EKBU 100%						1.000	0.014*		
K (+)							0.014*		
K (-)									

 Table 2. Mann Whitney Test Results of Ulin (*Eusideroxylon zwageri*) Stem Bark Extracts on Candida albicans Growth on Acrylic Resin Denture Plates.

*Significant differences (p<0.05)

Table 2 represents the results of Mann Whitney test for each treatment group. Ulin stem bark extract at 20% concentration had p<0.05 when compared to ulin stem bark extract at 40%, 60%, 80%, 100% concentration, positive control of 0.2% Chlorhexidine gluconate, and negative control of aquadest; thus, a significant difference was found. Ulin stem bark extract at 40% concentration had p<0.05 when compared to ulin stem bark extract at 60%, 80%, 100% concentration, positive control of 0.2% Chlorhexidine gluconate, and negative control of aquadest; thus, a significant difference was found. Ulin stem bark extract at 60% concentration had a value of p=1.000 when compared to ulin stem bark extract at 80%, 100% concentration, and positive control 0.2% Chlorhexidine gluconate; thus, no significant difference was found, whereas a significant difference was found when compared to negative control aquadest which had p<0.05. Ulin stem bark extract at 80% concentration had a significance value of p=1.000 when compared to ulin stem bark extract at 100% concentration and positive control of 0.2% Chlorhexidine gluconate; thus, no significant difference was found, whereas a significant difference was found when compared to negative control of aquadest which has a value of p<0.05. Ulin stem bark extract at 100% concentration had p=1.000 when compared to positive control 0.2% Chlorhexidine gluconate; thus, no significant difference was found, whereas a significant difference was found when compared to negative control aquadest which had of p<0.05.

Based on this study, ulin stem bark extract at 60% concentration is equivalent to 0.2%

Chlorhexidine gluconate. Based on Mann Whitney test, ulin stem bark extract with a concentration of 60% did not show any significant difference when compared to the positive control of 0.2% *Chlorhexidine gluconate.*

DISCUSSION

Based on the results of the study it can be seen that the acrylic resin denture plate soaked in ulin stem bark extract at 20%, 40%, 60%, 80%, and 100% concentration has been proven to reduce the number of *Candida albicans* colonies. Based on statistical test, there was no significant difference when ulin stem bark extract at 60% concentration was compared to 0.2% *Chlorhexidine gluconate*. Therefore, ulin stem bark extract at 60% concentration is equivalent to 0.2% *Chlorhexidine gluconate*.

The number of Candida albicans colonies found on acrylic resin denture plates after the soaking process in ulin stem bark extract at the concentration of 20% obtained an average value of 29.5 CFU/ml. The soaking process in ulin stem bark extract at the concentration of 40% obtained an average value of 13.0 CFU/ml. Meanwhile, soaking process in ulin stem bark extract at the concentration of 60%, 80%, and 100% obtained an average value of 0 CFU/ml. This shows that the higher the concentration of ulin stem bark extract, the lower the number of Candida albicans colonies on the acrylic resin denture plate. This was in accordance with research by Hertanti et al (2015) and Ornay et al (2017) that the higher the concentration, the inhibitory and killing power will

increase because the bioactive components in an extract are increased.^{12,13}

Ulin stem bark extract is proven to reduce the number of *Candida albicans* colonies on acrylic resin denture plates; and ulin stem bark extract at 60% concentration is equivalent to 0.2% *Chlorhexidine gluconate*. This is due to the antifungal content found in the ulin stem bark. Based on the research conducted by Wila *et al* (2018), secondary metabolite compounds contained in ulin stem bark are tannins, phenols, and flavonoids, saponins, alkaloids, and terpenoids. The secondary metabolite compounds present in the ulin stem bark extract can work as an antifungal.^{9,10,11}

Tannins and phenols work as antifungals by inhibiting the synthesis of chitin for the formation of cell walls and damaging cell membranes in fungi.14,15 Tannins damage cell membranes by inhibiting the biosynthesis of ergosterol, whereas phenols can cause fungal cells to become lysis due to denaturation of protein bonds present in cell membranes and phenols can enter the cell nucleus so that the fungi is undeveloped.^{16,17} Flavonoids as antifungals work by interfering with the permeability of cell membrane walls. Hydroxyl groups present in flavonoids cause fungal cells to become lysis due to changes in organic components and nutrient transports.¹⁸ Saponins can reduce the surface tension of sterol membranes and cell walls because of its polar surfactant nature and cause disruption of fungal membrane permeability which results in swelling and rupture of the cell because of the disturbance in inclusion of material or substances needed by the fungus.¹⁹ Terpenoids have toxic characteristics that can inhibit fungal growth by damaging cell membranes so that fungal growth is inhibited.^{15,20} Alkaloids can cause damage and death in fungi due to strong bonds with ergosterol which causes leakage in cell membranes.²¹

This study used 0.2% Chlorhexidine gluconate as positive control. Chlorhexidine gluconate with 0.2% concentration is effective against grampositive bacteria, gram-negative bacteria, virus, and fungi. It takes 15 minutes to eliminate Candida albicans effectively.^{22,23} Chlorhexidine gluconate with 0.2% concentration works as an antifungal by disrupting cell membranes and triggering precipitation.24 cytoplasmic Chlorhexidine gluconate with 0.2% concentration has a high degree of antimicrobial activity which causes changes in the integrity of the fungal cell wall when bound to a fungal cell membrane component, so that the function of the fungal cell membrane will be lost. The chlorophenol ring in the structure of the 0.2% *Chlorhexidine gluconate* formula is lipophilic which works by absorbing into the cell wall so that it can be easily accepted by the cell membrane and causing leakage of intracellular components.²⁵

Ulin stem bark extract was studied to be used as an alternative ingredient to chemical denture cleansers. The raw material of the extract used is ulin which is a typical plant of South Kalimantan and has the potential as an herbal denture cleansers because it contains antifungal compounds that can reduce the number of *Candida albicans* colonies that cause denture stomatitis, and is expected to reduce the side effects of long-term use of denture cleaning chemicals such as 0.2% *Chlorhexidine gluconate*.

Based on the results, it can be concluded that ulin stem bark extract at 20%, 40%, 60%, 80%, and 100% concentration have been proven to reduce the number of *Candida albicans* colonies on acrylic resin denture plates, and ulin stem bark extract at 60% concentration is equivalent to 0.2% *Chlorhexidine gluconate*.

REFERENCES

- Junaedi DR, Salim S , Soekobagiono. Efektivitas Ekstrak Daun Senggani (Melastoma candidum D. Don) dalam Menghambat Pertumbuhan Candida albicans pada Resin Akrilik Heat Cured. Journal of Prosthodontics. Jan –Jun 2013; 4 (1): 8-13.
- Diansari V, Fitriyani S, Haridhi FM. Studi Pelepasan Monomer Sisa dari Resin Akrilik Heat Cured Setelah Perendaman dalam Akuades. Cakradonya Dent J. 2016; 8 (1): 61-67.
- Togatorof RS, Rumampuk JF, Wowor VNS. Pengaruh Perendaman Plat Resin Akrilik dalam Larutan Kopi dengan Berbagai Kekentalan terhadap Perubahan Volume Larutan Kopi. Jurnal e-GiGi. 2017: 5 (1); 19-23.
- 4. Dama C, Soelioangan S, Tumewu E. Pengaruh perendaman plat resin akrilik dalam ekstrak kayu manis (*Cinnamomum burmanii*) terhadap jumlah blastospora *Candida Albicans*. Jurnal e-GiGi. 2019; 7 (2): 1-5.
- 5. Fakhriyana E, Rostiny, Salim S. Efektivitas minyak kayu manis dalam menghambat pertumbuhan koloni *candida albicans* pada resin akrilik. Journal of Prosthodontics. Juli-Desember 2010; 1 (2): 19-23.
- 6. Kusmawati FN, Putri TF. Pengaruh Rebusan Daun Sirih Terhadap Penurunan Jumlah

Candida Albicans Pada Plat Resin Akrilik Heat Cured. JITEKGI. 2019; 15 (1): 12-15.

- Pratiwi N, Saputera D, Budiarti LY. Efektivitas Ekstrak Metanol Daun Kersen Dibandingkan Klorheksidin Glukonat Terhadap *Candida Albicans* Pada Heat Cured Akrilik. Dentino Jurnal Kedokteran Gigi. April 2017; 1 (1): 89-93.
- Atmaja WD. Kulit Buah Kakao (Theobroma kakao L.) sebagai Bahan Pembersih Gigi Tiruan dan Mencegah Perlekatan Candida albicans pada Basis Plat Akrilik. J.K.G Unej. 2015; 12 (2): 46-50.
- Wila H, Yusro F, Mariani Y. Skrining Fitokimia Dan Aktivitas Antibakteri Ekstrak Kulit Batang (Eusideroxylon Zwageri) Terhadap Escherichia coli dan Salmonella typhi. Jurnal Tengkawang. 2018; 8 (1): 38-49.
- Pulungan ASS. Aktivitas Antijamur Ekstrak Etanol Daun Kunyit (*Curcuma Longa Linn.*) Terhadap Jamur *Candida Albicans*. Jurnal Biologi Lingkungan, Industri, Kesehatan. 2017; 3 (2): 120-124.
- Jalianto, Khotimah S, Raharjo W. Uji Aktivitas Antijamur Ekstrak Etanol Biji Buah Langsat (*Lansium domesticum corr.*) Terhadap Jamur *Candida albicans* Secara In Vitro. Jurnal Untan. 2017; 5 (1): 4-14
- Hertanti SR, Suswati I, Setiawan I. Efek Antimikroba Ekstrak Etanol Daun Pepaya (Carica papaya l) terhadap Shigella dysenteriae secara In Vitro dengan Metode Dilusi Tabung dan Dilusi Agar. Jurnal Ilmu Kesehatan dan Kedokteran Keluarga. 2015; 11 (1): 1-8.
- Ornay AKD, Prehananto H, Dewi ASS. Daya Hambat Pertumbuhan *Candida albicans* dan Daya Bunuh Candida Albicans Ekstrak Daun Kemangi (*Ocimum sanctum l.*). Jurnal Wiyata. 2017; 4 (1): 78-83.
- Christoper W, Natalia D, Rahmayanti S. Uji Aktivitas Antijamur Ekstrak Etanol Umbi Bawang Dayak (*Eleutherine americana* (Aubl.) Merr. Ex K. Heyne.) terhadap Trichophyton mentagrophytes secara In Vitro. Jurnal Kesehatan Andalas. 2017; 6 (3): 685-689.
- 15. Balafif FF, Satari MH, Dhianawaty D. Aktivitas Antijamur Fraksi Air Sarang Semut Myrmecodia Pendens Pada Candida albicans ATCC 10231. MKB; 49 (1): 28-34

- Hong LS, Ibrahim D, Kassim J, Sulaiman S. Gallic Acid: An Anticandidal Compound in Hydrolysable Tannin Extracted From the Barks of *Rhizophora apiculata* Blume. J Appl Pharm Sci. 2011;1 (6):75–79.
- Anggraini M, Nazip K, Melinda M. Efektifitas Daya Antijamur Daun Salam (Syzygium polyanthum w) terhadap Pertumbuhan Jamur Candida albicans dan Sumbangannya pada Pelajaran Biologi di SMA. eJournal Unsri. 2014; 2 (1): 138-145.
- Vifta RL, Khotimah SK, Luhurningtyas FP. Uji Aktivitas Antifungi Ekstrak Etanol Biji Timur Sari (*Cucumis melo L.*) terhadap Pertumbuhan *Candida albicans* secara In Vitro. Indonesian Journal Pharmacy and Natural Product. 2018; 1 (1): 10-17.
- 19. Kurniawati A, Mashartini A, Fauzia IS. Perbedaan Khasiat Antijamur Antara Ekstrak Etanol Daun Kersen *(Muntingia calabura L.)* dengan Nistatin terhadap Pertumbuhan *Candida albicans*. Jurnal PDGI. 2016; 65 (3): 75-77.
- Wahjuni S, Puspawati NM, Arista NPRE. Isolasi dan Identifikasi Senyawa aktif Antijamur dari Daun Mimba (Azadiractha indica A. Juss.) sebagai Pengendali Jamur Fusarium sp. Pada Tanaman Buah Naga (Hylocereus sp.). Jurnal Kimia. 2016; 10 (2): 197-203.
- 21. Marantika VM, Trimulyono G. Aktivitas Antifungi Ekstrak *Lichen Parmelia sulcata* terhadap Pertumbuhan Jamur *Alternaria porri*. LenteraBio. 2019; 8 (3): 1-6.
- Rao J. Quick Review Series for BDS 4th Year Vol 1 Second Edition. New Delhi: Elsevier; 2017, p 958.
- 23. Ibrahim I, Jaya F, Luthfia P, Izzati DPA. Pengaruh Lama Perendaman Dalam Larutan *Chlorhexidine* Terhadap Perubahan Warna Resin Akrilik *Heat Cured*. Jurnal Material Kedokteran Gigi. 2016; 5 (1): 7-14.
- 24. Gantait S, Bhattacharyya J, Samiran Das, Biswas S, Ghati A, Ghosh S, Goel P. Comparative assessment of the effectiveness of different cleaning methods on the growth of *Candida albicans* over acrylic surface. Contemp Clin Dent. 2016; 7 (3): 336-342.
- 25. Rakhmatullah H, Saputera D, Budiarti LY. Aktivitas Daya Hambat Ekstrak Daun Belimbing Wuluh dengan Klorheksidin terhadap *Candida albicans* Pada Plat Resin Akrilik. Dentin Jurnal Kedokteran Gigi. 2018; 2 (7): 73-78.