DENTINO
JURNAL KEDOKTERAN GIGI
Vol II. No 1. Maret 2017

MINIMUM INHIBITORY CONCENTRATION OF WHITE GINGER AND CHLORHEXIDINE GLUCONATE ON ACRYLIC PLATES TOWARD *Candida albicans*

Debby Saputera, Gusti Aulia Nalar, Lia Yulia Budiarti
Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

ABSTRACT

Background: *Acrylic resin-based dentures are commonly used nowadays.* The hygiene of *denture base can be maintained by soaking the denture base into 0.2% chlorhexidine gluconate. Unhygienic denture base can lead to denture stomatitis. Flora accumulation, such as Candida albicans, may occur. White ginger is active towards Candida albicans, with antifungal properties due to its phenol compound.* ***Aim:*** *This study aims to determine the Minimum Inhibitory Concentration value (MIC) of the ethanol extract of white ginger towards Candida albicans growth in heat cured removable acrylic dentures.* ***Methods:*** *This is an experimental study with a post test only control group design. Acrylic resin were soaked in treatment extract of 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 100% concentration,0.2% chlorhexidine gluconate and 70% ethanol as the control. One Way ANOVA test and Bonferroni Post Hoc test with 95% confidence level were used.* ***Results:*** *The result of the study shows that the MIC value of 40%, 45%, 50%, 60%, 70%, 80% 90% and 100% of treatment extract were respectively 13:07%; 18:36%; 23.67%; 28.87%; 36.84%; 42.10%; 49.98%; 52.61%.* ***Conclusion:*** *This study concluded that the treatment extract can reduce the amount of Candida albicans,100% concentration made the strongets antifungal effect compared to lesser concentrations and 0.2% chlorhexidine gluconate.*

Key words*:* *Candida albicans, chlorhexidine gluconate, denture stomatitis, heat cured acrylic resin, MIC, white ginger.*

Correspondent: Debby Saputera, Faculty of Dentistry, Lambung Mangkurat University, Jalan Veteran No. 12B, Banjarmasin, South Kalimantan, email: debbysaputera@gmail.com

INTRODUCTION

Denture is a prosthesis used for replacing some or all of the lost natural teeth and surrounding tissues. Denture base is part of the denture in contact with the oral mucosa, which provides surface for positioning and supporting the elements of the denture, distribute occlusal forces to support surrounding tissues networks and provide retention and stability of the denture [1.] Acrylic resin has been widely used as denture base material since the mid 1940s and to up now remains a viable choice. Uncleaned buildup of food residue on the denture

acrylic-based resins can increase the number of microorganisms in the oral cavity, such as the fungus *Candida albicans* [3.] The increasing number of *Candida albicans* can change its properties from a commensal to a parasite factors, showed by the change from *yeast* into *hyphae.* Forms of *hyphae* is an initiator of invasion into tissues that can lead to *denture stomatitis* [4.] *Denture stomatitis* or also known as *denture afternoon mouth,* chronic atrophic candidiasis, or chronic erythematous candidiasis is an inflammation process that affects users of denture characterized by erythema and edema under the denture [4, 5.]

To achieve a good oral and denture hygiene by preventing contamination of *Candida albicans,* users can soak the dentures in denture cleaning agents such as chlorhexidine gluconate 0.2% for 15 minutes every day [6.] Now a days, the price of denture cleansers on the market are rather expensive, therefore it is necessary to find then materials as a replacement which are cheaper [3.] One of the materials that can be used as an alternative material denture cleanser that exist in Indonesia is white ginger *(Zingiber officinale var. Amarum).*

White ginger, or small white ginger, acts as an antifungal agent. White ginger contains several chemical substances that act as antifungal such as the phenolic compounds *gingerol, shogaol,* and *zingeron,* which are known to denature the protein binding the cell membrane *of Candida albicans* [7.] The results of previous studies suggests that the mentioned antifungal efficacy of ethanol extract of small white ginger (EEJPK) of 30% is more effective against *Trichophyton mentagrophytes* and *Cryptococcus neoforrmans* compared to lower concentrations. The increase of EEJPK concentration will in turn increase the inhibition of fungal growth [8.] Results of other studies mentioned that the antifungal activity of 30% EEJPK is high enough [7] to inhibit the growth of *Candida albicans.* Results of other studies that examine the content of ginger essential oil *(Zingiber officinale)* at a concentration of 5 mL, 10 mL, 15 mL, 20 mL, and 25 mL states that ginger can inhibit the growth of *Candida albicans* and *Aspergillus flavus.* In that research, the increased concentration of essential oil can increase inhibitory effect on both funguses [9.] Testing the activity of antimicrobial agents *in vitro* can be done by using the dilution method, in which the determinination of the lowest concentration of an antimicrobial material is done by observing the clearence of the suspended culture started which is referred to as the minimum inhibitory concentration (MIC) [10.]

It is necessary to further study on the increased EEJPK concentration in order to determine the optimum antifungal effect in inhibiting the growth of *Candida albicans.* In this study we tested the effect of EEJPK with the concentrations of 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% in their the antifungal activity towards *Candida albicans,* which are compared with 0.2% chlorhexidine gluconate by assessment of the MIC values from each concentration. We expect these results can be used as the basis of using this alternative denture cleanser material.

MATERIALS AND METHODS

This study is a pure laboratory experimental *(true experimental)* study with *post test only control group design* using ten treatments, EEJPK 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%, with 0.2% Chlorhexidine gluconate and 70% ethanol as the control. Samples in this study are heat-cured acrylic resin plates with a size of 10 mm x 10 mm x 1mm polished with a number of 2000 rubbing paper three times outwards on the edges and surface. The number of repetitions for each treatment is three times, which was obtained from the calculation using the *Federer* formula.

Instruments used in this study are : *bowl* (rubber bowl), spatula, model knives, cuvette, test tubes, beakers, *mixing jar, hydraulic bench press,* incubators, *syringe,* ose, *petri dish, autoclave,* analytical balance scale, mortar and pestles, *Buchner* funnel, *vortex, evaporator,* a pH meter and a *stopwatch.* Materials used in this study are : heat-cured acrylic resin, white gypsum, blue gypsum, red wax evening, number 2000 rubbing paper, *distilled* sterile saline solution, EEJPK concentration of 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%, chlorhexidine gluconate 0.2%, 70% ethanol, *Sabouraud Dextrose Agar, Candida albicans* suspension, *Brain Heart Infusion* (BHI) medium and CMC-Na.

Making the acrylic resin plate. Model acrylic resin mold plates were made using red wax with a measurement of 10 mm x 10 mm x 1 mm by 30 pieces. The model is implanted in the cuvette bottom by using gypsum. After it was harden, the entire surface of the model is wiped with vaseline. Make a counter by placing a cuvette top and filling it with gypsum, and seperate it after it became hardens. Boiling is carried out thoroughly until a mold is formed, which is then filled with acrylic resin.

Polymers and monomers from the heat-cured acrylic resin are mixed in a stellon pot with a 2: 1 ratio. When the dough is formed, add the acrylic resin which was previously wiped with CMS into the mold. The pressing is done with a *hydraulic bench press* with a pressure of 22 kg / cm Hg. Acrylic resin is processed by heating the cuvette containing acrylic resin in boiling water for 20 minutes. Acrylic resin plate were removed from the cuvette, with a size of 10 mm x 10 mm x 1 mm and then at the edges and surface are polished with a number of 2000 rubbing paper three times outwards on the surface.

The rhizomes of the white ginger were washed, dried and weighed. The ginger were sliced into small pieces and dried naturally (without direct sunlight), then crushed and weighed. Making EEJPK 100% in this study, the extraction method used is maceration. A total of 500 grams of powder samples were inserted in the maceration instrument. A solution of 70% ethanol is poured slowly into the maceration instrument containing the sample, and stirred until evenly distributed. Filter solution is poured up to 1 cm above the sample surface. The filtrate is filtered and replaced every 1x24 hours with a new solvent while stirring it occasionally. Replacement of the solvent is done until the solution is clear. Extract were collected and evaporated with a *rotary evaporator* under reduced pressure with a temperature of 40 ° C to obtain the ethanol extract of the condensed *water bath*, which are then evaporated to obtain the fixed weights. Condensed extract is then dissolved in CMC-Na to obtain the desired concentration.

This study uses a dilution method. Acrylic resin with a size of 10 mm x 10 mm x 1 mm by 30 pieces (numbers 1-30) are sterilized with 70% alcohol and then taken with sterile tweezers and immersed in saline for approximately one hour. Acrylic resin is taken using tweezers and then soaked in 10 ml suspension of Candida albicans for 24 hours at 37 ° C in test tubes. Thirty acrylic resin plates were divided into ten groups. The ten treatment groups were immersed for 15 minutes in the ethanol extract of l white ginger with different concentrations at room temperature. Group I: acrylic resin 1-3 soaked in 10 ml EEJPK 30%. Group II: acrylic resin 4-6 soaked in 10 ml EEJPK 40%. Group III: acrylic resin 7-9 soaked in 10 ml EEJPK 50%. Group IV: acrylic resin 10-12 soaked in 10 ml EEJPK 60%. Group V: acrylic resin 13-15 soaked in 10 ml EEJPK 70%. Group VI: acrylic resin 16-18 soaked in 10 ml EEJPK

80%. Group VII: acrylic resin 19-21 soaked in 10 ml EEJPK 90%. Group VIII: acrylic resin 21-24 soaked in 10 ml EEJPK 100%. Group IX: acrylic resin 24-27 soaked in 10 ml *Chlorhexidine gluconate* 0.2%. Group X: acrylic resin 28-30 soaked in 10 ml of 70% ethanol. All treatment groups were mixed using a *a vortex mixer* for 1 minute. Each test tube of all treatments underwent serial dilutions of up to 10 $^{10.}$ P1 dilution (10 ^{1}) obtained from 1 ml tube number 1 is inserted into the tube containing 9 ml of sterile *distilled water* and homogeneously mixed. P2 dilution (10 ^{2}) obtained from 1 ml of solution P1 was added to a tube containing 9 ml of sterile *distilled water* and homogeneously mixed. This is done up to P10 dilution (10 ^{10}) . 1 ml solution of P10 were then taken and discarded. The same is done in the test tube numbers 2-30.

When the solution begins to clear, 0.01 ml are taken on dropped on 1 Petri dish with Sabouraud Dextrose agar, which is incubated for 48 hours at 37 ° ° C. If the serial dilution of the obtained EEJPK reached 30%, it is used as a treatment concentration. The calculation of the number of colonies of Candida albicans at each concentration and the control solution EEJPK were made after incubation for 48 hours at a temperature of 37 ° C. The number of colonies of Candida albicans obtained, used for calculating the concentration of each mushroom EEJPK and control solution, is determined by the following formula: Fungal count : (colony count x dilution factor) / solution volume. Fungal count can be used to determine antifungal properties power at each concentration by calculating the MIC.

MIC : (100%) – [(treatment fungal count x 100%)/control fungal count)]

Information: Treatment fungal count: Fungal count at certain concentrations in units of CFU / ml. Control fungal count: Fungal count on the control solution in units of CFU / ml.

RESULTS

Based on the results of the calculation of the minimum inhibitory concentration of small white ginger and chlorhexidine gluconate on acrylic plate against *Candida albicans*, we obtained data of the inoculum turbidity on each tube at each treatment after soaking the acrylic resin plates. Inoculum tubes showed that the *Candida albicans* is still present.

The results of this study showed that *Candida albicans* can still grow at a concentration of inoculum tube less than 40% and at 70% Ethanol as a negative control. The growth of *Candida albicans* can be inhibited by EEJPK 40%, 45%, 50%, 60%, 70%, 80%, 90%, and 100%, as shown by the clear inoculum tubes.

Calculation of the mean and standard deviation of the MIC of *Candida albicans* on acrylic plate after immersion in EEJPK 40%, 45%, 50% 60% 70%, 80%, 90%, 100% and 0.2% chlorhexidine gluconate can be seen in Table 1.

Table 1. Average and standard deviation of *Candida albicans* MIC on acrylic plates after immersion in EEJPK at various concentrations and 0.2% chlorhexidine gluconate.

Group	Sample/ repetition (n)	MIC (%) Mean±SD
EEJPK 40%	3	13,07±4,92
EEJPK 45%	3	18,36±4,21
EEJPK 50%	3	23,67±2,41
EEJPK 60%	3	28,87±4,51
EEJPK 70%	3	36,84±2,31
EEJPK 80%	3	42,10±2,30
EEJPK 90%	3	49,98±1,32
EEJPK 100%	3	52,61±1,25
KG 0,2% (positive control)	3	39,43±3,75

Table 1 showed that the highest mean MIC of *Candida albicans* in EEJPK is at the 40% concentration, which is 13.07%, and lowest at 100%, which is 52.61%. *One way ANOVA* test results (α = 0.05) shows that there are differences in the effects of each treatment which means that the hypothesis is accepted (p = 0.000; p <0.05). Furthermore, to determine any treatment that gives a different effect, we followed up by a further test using *Bonferroni Post Hoc* test.

Post Hoc Bonferroni test results showed that chlorhexidine gluconate (positive control) had a significant difference with EEJPK concentrations of 40%, 45%, 50%, 60%, 90, and 100% (p <0.05) but did not have significant differences with EEJPK concentration of 70% and 80% (p> 0.05). EEJPK concentration of 40% has significant differences with EEJPK concentration of 50%, 60%, 90, 100%, and 0.2% chlorhexidine gluconate (p <0.05) but did not have significant differences with EEJPK concentration of 45% (p> 0, 05).

EEJPK concentration of 45% has a significant difference with EEJPK concentrations of 60%, 70%, 80%, 90%, 100%, and 0.2% chlorhexidine gluconate (p <0.05) but did not have a significant difference with EEJPK concentration of 40% and 50% (p <0.05). EEJPK concentration of 50% has a significant difference with EEJPK concentration of 40%, 70%, 80%, 90%, 100%, and 0.2% chlorhexidine gluconate (p <0.05) but did not have a significant difference with EEJPK concentration of 45% and 60% (p> 0.05).

EEJPK concentration of 60% has a significant difference with EEJPK concentration of 40%, 45%, 80%, 90%, 100%, and 0.2% chlorhexidine gluconate (p <0.05) but did not have a significant differences with EEJPK concentration of 50% and 70% (p> 0.05). EEJPK concentration of 70% has a significant difference with EEJPK concentration of 40%, 45%, 50%, 90% and 100% (p <0.05) but did not have a significant difference with EEJPK concentration of 60%, 80% and Chlorhexidine gluconate 0.2 % (p> 0.05).

EEJPK concentration of 80% has a significant difference with EEJPK concentration of 40%, 45%, 50%, 60%, and 100% (p <0.05) but did not have a significant difference with EEJPK concentration of 70%, 90% and chlorhexidine gluconate 0, 2% (p> 0.05). EEJPK concentration of 90% has a significant difference with EEJPK concentration of 40%, 45%, 50%, 60%, 70%, and 0.2% chlorhexidine gluconate (p <0.05) but did not have a significant difference with EEJPK concentration of 80% and 100% (p> 0.05).

EEJPK 100% concentration has a significant differences with EEJPK concentration of 40%, 45%, 50%, 60%, 70%, 80% and chlorhexidine gluconate 0.2% (p <0.05) but did not have a significant difference with concentration EEJPK 90 % (p> 0.05). In this study, 100% EEJPK have the optimum effect in inhibiting the growth of *Candida albicans.*

DISCUSSION

The results of this research proved that EEJPK and chlorhexidine gluconate has an activity of inhibiting the growth of *Candida albicans.* This is due to EEJPK contains phenolic compounds which acts as antifungals (11). Phenol will denature protein and wrinkled cell walls so that cells can lyse the cell walls of fungi. Phenol compounds also can damage cell membranes, resulting in changes in cell permeability which can result in death or inhibition of cell growth. The mechanism of action through a hydroxy group binds to sulfhydryl groups of proteins in the fungi which leads to an alteration of the target cell membrane proteins (12).

Chlorhexidine gluconate has a broad-spectrum antimicrobial activity (13). Chlorhexidine molecules have an interaction between the molecules and the positive charge with negatively

charged cell walls. This interaction will result in irreversible loss of cytoplasmic constitution, the membrane, and the inhibition of the enzyme. At high concentrations, chlorhexidine gluconate is able to destroy cells, coagulate cytoplasm, and precipitate proteins and nucleic acids (14).

In this study, the lowest inhibition were found in EEJPK with a concentration of 40% with a mean of 13.07%, and the highest in EEJPK 100% with a mean of 52.61%. Phenols in EEJPK 100% are higher than 40%, which leads to a higher inhibition of *Candida albicans.* We observed that higher concentration of EEJPK will increase inhibitory effects. These results are in accordance with the results of Pelczar and Chan, who stated that higher concentration of a substance will have stronger anti-microorganism property, which will accelerate the destruction of microorganism cells or inihibit cell growth. Tyler stated that the ability to inhibit and damage fungal cells is caused by the therapeutic properties (15).

Based on Table 1, we concluded that EEJPK 40%, 45%, 50%, 60%, 70%, 80%, 90%, and 100% are fungistatic. The results are consistent with the opinion of Washington (1985), in which he stated that if MIC reached 99.9%, then the solution is a fungicide, while if the MIC is less than 99.9%, then the solution is fungistatic (16).

Based on data we observed, the MIC of EEJPK 80%, 90%, and 100% are higher than 0.2% chlorhexidine gluconate. The results of this study found that EEJPKl 80%, 90% and 100% can be considered more effective than the 0.2% chlorhexidine gluconate to inhibit the growth of *Candida albicans* in the heat-cured acrylic resin plate. This is because the chlorhexidine used in this study are low-concentration chlorhexidine, which will only cause disturbance of cell transport and form pores in the cell membrane. At higher concentrations, the solution is able to penetrate cells and cause cell lysis of these microorganisms (17). The mechanism of action of phenolic compounds contained in EEJPK of phenols is by interacting with the fungal cell wall, where the phenol at low levels will denaturate proteins while high levels will coagulate proteins causing yeast cells death (18).

The results concluded that the EEJPK antifungal activity of 80%, 90% and 100% concentrations are greater than the antifungal activity of chlorhexidine gluconate 0.2%. In order to be used as an alternative denture cleaning agent, further studies are needed to determine possible influences from EEJPK immersion such as damage, roughness and discoloration of the acrylic resin.

REFERENCES

1. Gunadi HA, Margo A, Burhan LK, Suryatenggara F, Setiabudi I. Buku ajar ilmu geligi tiruan sebagian lepasan. Jilid 1. Jakarta: Hipokrates; 1995. Hal. 12, 215-6.
2. Anusavice K. Philips: Buku ajar ilmu bahan kedokteran gigi. Edisi 10. Jakarta: EGC; 2004. Hal. 197-218.
3. Dama C, Soelioangan S, Tumewu E. Pengaruh perendaman plat resin akrilik dalam ekstrak kayu manis (*Cinnamomum burmanii*) terhadap jumlah blastospora *Candida albicans.* Ejournal Unsrat. 2013; 1(2): 1-5.
4. Gaib Z. Faktor-faktor yang berpengaruh terhadap terjadinya kandidiasis eritematosa pada pengguna gigi tiruan lengkap. Ejournal Unsrat. 2013; 1(2): 1-14.
5. Hadjieva H, Dimova M, Todorov S. Stomatitis prosthetica-a polyetiologic disorder. Journal of IMAB. 2006; 12(2): 38-4.
6. David, Munadziroh E. Perubahan warna lempeng resin akrilik yang direndam dalam larutan desinfektan sodium hipoklorit dan klorhexidin. Maj. Ked. Gigi. (Dent. J.). 2005; 38(1): 36–40.
7. Santoso HD, Budiarti LY, Carabelly AN. Perbandingan aktivitas antijamur ekstrak etanol jahe putih kecil *(Zingiber officinale var. amarum)* 30% dengan C*hlorhexidine glukonat 0,2%* terhadap C*andida albicans* in vitro. Dentino (Jur. Ked. Gigi). 2014; 2(2): 125-9.
8. Gholib D. Uji daya hambat estrak etanol jahe merah *(Zingiber officinale var. rubrum)* dan jahe putih *(Zingiber officinale Var. Amarum)*

terhadap *Trichophyton mentagrophytes* dan *Cryptococcus neoformans*. Seminar Nasional Teknologi Peternakan dan Veteriner. Bogor; 2008. Hal. 827-30.

9. Sustiami. Pengaruh minyak atsiri jahe (*Zingiber officinalle Roxb)* terhadap pertumbuhan *Candida albicans* dan *Aspergillus flavus*. Surabaya: Universitas Airlangga; 1994. Hal. 1.
10. Tortora GJ, Berdell RF, Christine LC. Microbiology An Introduction. San Francisco: Pearson Benjamin Cummings. 2001. Hal. 960.
11. Staf Pengajar Departemen Farmakologi Fakultas Kedokteran Universitas Sriwijaya. Kumpulan kuliah farmakologi. Jakarta: EGC; 2009. Hal. 164.
12. Anggara ED, Suhartanti D, Mursyidi A. Uji aktivitas antifungi fraksi etanol infusa daun kepel (*Stelechocarpus burahol, Hook F&Th*.) terhadap *Candida albicans.* Prosiding Seminar Nasional Hasil - Hasil Penelitian & Pengabdian. Semarang; 2014. Hal. 1-7.
13. Mohammadi Z, Abbot PV. The properties an applications of *Chlorhexidine* in endodontics. International Endodontic Journal 2009: 4.
14. Chanaka S. Sifat kekasaran permukaan lempeng resin akrilik polimerisasi panas setelah direndam dalam larutan desinfektan klorheksidin glukonat 0,2%. Skripsi. Medan: Fakultas Kedokteran Gigi Universitas Sumatera Utara; 2010. Hal. 13-5.
15. Wahyuningtyas E. Pengaruh ekstrak *Graptophyllum pictum* terhadap pertumbuhan *Candida albicans* pada plat gigi tiruan resin akrilik. Indonesian Journal of Dentistry 2008; 1 (3): 187-191.
16. Rahman EF. Efektivitas ekstrak daun dewa (*Gynura pseudochina (Lour.) DC*) terhadap pertumbuhan *Candida albicans* pada plat dasar gigi. Jurnal Majalah Ilmiah Sultan Agung 2010; 48 (123): 1-13
17. Mathur S, Mathur T, Srivastava R, Khatri R. Chlorhexidine: the gold standard inchemical plaque control. National Journal of Physiology, Pharmacy & Pharmacology 2011; 1(2): 45-50.
18. Lidyawita R, Sudarsono, Harsini. Antifungal activities of boiled cashew bark (Anacardium occidentale L.) on c. albicans in acrylic resin. Trad. Med. J. 2013; 18(1): 46-52