Potential of chitosan as anti candida albicans in denture bases made from thermoplastic nylon

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ABSTRACT

Background: *Candida albicans is main cause of fungal infections in denture users. Prevention is needed by soaking dentures using a disinfectant. Chitosan is an alternative disinfectant emulsion because it is biodegradable, biocompatible, and does not cause discoloration. This research objective is to determine the effect of chitosan emulsion on the number of C.albicans on base plate thermoplastic nylon.*

Method: *This is a true experimental study using post-test only design by using 25 base plates divided into 5 groups namely treatment I, treatment II, treatment III, positive and negative control. Plate was incubated in C.albicans suspension for 24 hours in McFarland and 10^{-3} series dilution. The plates was rinsed with aquadest, then soaked with 0.3% chitosan emulsion, 0.8% chitosan emulsion, 2% chitosan emulsion, 0.2% chlorhexidine gluconate, and aquadest for 15 minutes. The attached C.albicans colony in the plate was released to the SDB by using centrifuge device, then proliferated on the SDA and incubated (24 hours). The results of the C.albicans colony were counted visually by using a colony counter. The number of Candida albicans colonies data were analyzed using One-Way ANOVA and then Post Hoc Tukey HSD test.*

Result: *The results showed that there were significant mean differences between each group ($p < 0.05$)*

Conclusion: *It is concluded that there is an effect of various concentrations of chitosan on the number of C. albicans on thermoplastic nylon base plates.*

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INTRODUCTION

Chitosan is a derivative made from the shells of marine animals such as shrimp, crab and oyster shells. Chitosan is a carbohydrate polymer derived from chitin with antimicrobial and antifungal effects. The general formula of chitosan polysaccharide is (C6H11NO4)n or β-(1-4)-2-amino-2deoxy-D-glucopyranosa1.

According to research by Ismiyati (2017), the use of chitosan as a mixture of acrylic resin materials can cause the number of colonies of Candida albicans fungi to decrease so that it can be said that chitosan inhibits the growth of Candida albicans2. According to research by Evelyna (2017), the use of chitosan baths with a concentration of 2% shows that the average Candida albicans colony on heatcured acrylic dentures is 45.33 CFU/plate, using distilled water as a control is 365.56 CFU/plate, and oxygenizing denture cleaning solution (polident) is 178.33 CFU/plate3. These results show that chitosan has a higher antifungal effectiveness than the oxygenizing denture soaking solution on acrylic resin denture plates.

Based on research by Costa (2014), chitosan was able to reduce the number of Candida albicans colonies at a concentration of 0.8% (b/v)4. Based on research by Pu (2014), the concentration of chitosan that is effective on Candida albicans starts from a concentration of 0.313% which can kill fungi> 50%5. Based on Ismiyati's research (2014), using chitosan nanoparticles mixed with 2% chitosan solution shows that chitosan is fungistatic6.

Many denture immersion studies have been conducted on heat cured acrylic resin materials. In addition, this material is used as a mixture of anti-fungal materials before being made into plates. Research on chitosan solution used as a disinfectant on thermoplastic nylon has never been done. Therefore, researchers wish to observe the effect of chitosan solution when used as a disinfectant solution on thermoplastic nylon base plates with concentrations of 0.3%; 0.8%; and 2% based on effective concentrations from other studies.

This study aims to determine the effect of soaking various concentrations of chitosan on the number of Candida albicans colonies on the thermoplastic nylon base plate. The hypothesis of this study is that there is an effect of soaking various concentrations of chitosan on the number of Candida albicans colonies on the thermoplastic nylon base plate.

METHODS

This study is a true experimental analytic study using post test only design. This research was conducted at the Central Laboratory of Microbiology, Faculty of Medicine, Diponegoro University Semarang in November 2019. The study sample amounted to 25 which were divided into 5 treatment groups.

Chitosan solution was made according to the selected concentration by mixing 0.3 grams of chitosan powder for 0.3% solution, 0.8 grams for 0.8% solution and 2 grams for 2% solution, each of which was mixed with 2% acetic acid until the volume reached 100 ml, then stirred using a magnetic stirrer until the chitosan powder dissolved and there was no sediment3. Sterilize the tools to be used into an autoclave at 37o C for 24 hours. Soak the thermoplastic nylon base plate using sterile distilled water for 24 hours to release the remnants of the monomer and sterilized into a 70% alcohol solution for 60 seconds7. Plant Candida albicans colonies with sabouraud dextrose agar media into petri dishes, then incubate for 24 hours at 37o C in an incubator8.

Candida albicans fungal suspension was prepared by dilution to adjust the turbidity according to Mc Farland standard solution no. 1 (1 x 108 CFU/ml). To obtain the standard test concentration (1 x 108 CFU/ml), the suspension that has been adjusted to the Mc Farland standard solution no. 1 is taken 1 ml and added 9 ml of Nacl solution to obtain a concentration of 1 x 108 CFU/ml. Then serial dilutions were carried out until reaching a Candida albicans suspension of 10-3 CFU / ml. Insert the thermoplastic nylon base plate into the Candida albicans suspension to be incubated in an incubator at 37o C for 24 hours. Each test tube was filled with 1 thermoplastic nylon plate9. Rinse the

thermoplastic nylon base plate by flushing it with distilled water to ensure that the fungus on the thermoplastic nylon base plate is the fungus that grows on the plate10. Thermoplastic nylon base plate contaminated with Candida albicans, grouped into 5 (5 pieces) which are the same to get different soaking treatments, namely each group getting 10 ml of chitosan solution with different concentrations (0.3%, 0.8%, and 2%) in 3 different test tubes, 1 test tube of distilled water, and 1 test tube of chlorhexidine gluconate 0.2% for 15 minutes11.

The thermoplastic nylon base plate was removed from each treatment group bath and then inserted into the thermoplastic nylon base plate using sterile tweezers containing 10 ml of SDB media and shaken for 30 seconds 3 times in a centrifuge. The results of Candida albicans fungal release were planted on SDA media by dripping 0.1 ml of fungal release solution in a petri dish containing SDA media and leveled using a hockey stick, incubated for 24 hours at 37o C8;10. Counting the number of Candida albicans colonies using a colony counter.

Data were then tested for normality using Saphiro-Wilk and then the Levene Test for homogeneity. Normally distributed data were subjected to parametric One Way ANOVA test to find differences in the effect of chitosan on the growth of Candida albicans, to determine the magnitude of differences between groups, Tukey's HSD test was performed. If there is data that is not normally distributed, it is tested with Kruskal-Wallis, if the significant value of Kruskal-Wallis P <0.05, then a Post-hoc test with Mann Whitney is carried out to determine differences within groups.

RESULTS

Table 1. Calculation results of the mean and standard deviation of the number of Candida albicans fungal colonies

Groups	Mean ± SD
I	28.0 ± 5.916
II	97.80 ± 8.468
III	55.8 ± 12.112
IV	142.0 ± 9.695
V	7.80 ± 4.147

Based on the mean number of Candida albicans fungal colonies in table 1, it is known that the negative control group has the highest number of colonies with an average of 142.0 and a standard deviation of ± 9.695. Based on the mean number of Candida albicans fungal colonies, it is also known that the positive control group (chlorhexidine 0.2%) has the least number of colonies, with a mean of 7.80 and a standard deviation of ± 4.147.

Table 2. Normality Test Results

Groups	Mean ± SD
I	0.672
II	0.675
III	0.399
IV	0.700
V	0.754

Based on the normality test, the group results were normally distributed because p> 0.05. Significance of 0.096 (p > 0.05) was obtained in the homogeneity test, so the data variance was homogeneous. One-Way ANOVA test showed a significance value of 0.000 (p < 0.05) which means there is a significant effect between treatment groups. This result is in accordance with the researcher's hypothesis. Then the Post Hoc test, namely Tukey HSD, was carried out to determine significant differences in means in each group.

DISCUSSION

The results on the immersion of energy and carbonated drinks showed that the average value of the negative control group (aquadest) had the highest number of colonies, namely 142 CFU/ml. The 0.3% chitosan group was used as the first treatment group which showed the most effective results in reducing the number of Candida albicans fungal colonies, namely 28 CFU/ml after the positive control group treatment of chlorhexidine 0.2%, namely 7.8 CFU/ml. Based on these results, it is concluded that the three treatment groups have antifungal effects against Candida albicans. This indicates the presence of compounds that have antifungal effects on these materials.

The use of dentures can trigger the attachment of Candida albicans on the surface of the denture, especially on surfaces facing the oral mucosa. Base plate thermoplastic nylon is a denture plate that is hygroscopic so that it can absorb specific molecules of saliva and form a thin organic layer called pellicle. The pellicle contains proteins that result in microbial attachment so that microbes can adhere to the surface of the denture. The attachment of Candida albicans to the denture plate is due to the galvanic conductivity that occurs due to the presence of salivary pellicles that experience differences in electronegativity so that Candida albicans can interact with receptors mediated by fimbriae12. The interaction of the positive charge of chitosan with phospholipid components causes electrostatic interactions that lead to changes in cell wall membrane permeability and changes in osmotic balance that can inhibit microbial growth. Hydrolysis of peptidoglycan in microbial walls results in the loss of intracellular electrolytes, proteins, and nucleic acids in microbes so that chitosan can inhibit the growth of Candida albicans on thermoplastic nylon base plates13. The highest number of colonies from this study was 0.8% chitosan as much as 97.8 CFU / ml. The number of colonies in the 2% chitosan solution treatment group of 55.8 CFU / ml showed that the colony results were more than the 0.3% chitosan solution group.

Table 3. Tukey HSD Test Results

Groups	I	II	III	IV	V
I	-	0,000	0,000	0,000	0,010
II	0,000	-	0,000	0,000	0,000
III	0,000	0,000	-	0,000	0,000
IV	0,000	0,000	0,000	-	0,000
V	0,010	0,000	0,000	0,000	-

The results of the Post Hoc test table above can be obtained the significance value between one treatment group and another treatment group is p = 0.000 and 0.010. The results of the significance value show a significant difference in means between each group.

The 0.2% chlorhexidine group was used as a positive control because 0.2% chlorhexidine is the gold standard of denture plaque cleaning14. Post Hoc test results in the 0.2% chlorhexidine positive control group had a significant difference with all groups and had the greatest antifungal effect compared to other groups. The antifungal mechanism possessed by chlorhexidine 0.2% is what causes the greatest antifungal effectiveness compared to the chitosan treatment group in this study.

CONCLUSION

Based on the results of the study, it can be concluded that there is an effect of soaking various concentrations of chitosan on the number of Candida albicans colonies on the thermoplastic nylon base plate, 0.3% chitosan solution is the highest concentration inhibiting the growth of Candida albicans colonies, namely 28 CFU / ml and 0.8% chitosan solution is the least concentration inhibiting the growth of Candida albicans colonies, namely 97.8 CFU / ml.

REFERENCES

1. Elieh, D. and Komi, A. “Chitin and Chitosan : Production and Application of Versatile Biomedical Nanomaterials.” *IJAR*. 2016;4(3):411-27.
2. Ismiyati, T., Siswomihardjo, W., Soesatyo, M. H. N. E*.,* and Rochmadi, R. “Campuran Kitosan dengan Resin Akrilik sebagai Bahan Gigi Tiruan Penghambat Candida albicans.” *MKGI*. 2017;3(3):139–45.
3. Evelyna, A., Sutanto, D. and Tiffany, E. “Chitosan 2 % effect on prohibiting the growth of candida albicans on heatcured acrylic resin.” *JMKG*. 2017; 6(2):17-24.
4. Costa, E. Silvia, S. Tavaria, F. and Pintado, M. “Antimicrobial and Antibiofilm Activity of Chitosan on the Oral Pathogen Candida albicans. *J Pathogens*.” 2014;3(4):908-19
5. Pu, Y. Liu, A. Zhieng, Y. and Ye, B. “In Vitro Damage of Candida albicans Biofilms by Chitosan.” *Experiment Therapeutic Med*, 2014;8(3):929-34
6. Ismiyati, T. and Setyahadi, S. “Antifungal of Thermoplastic Nylon Denture Base Plate Incorporate with Nanoparticles High-Density Chitosan.” *J Chitin Chitosan Sci,* 2014;2(3): 216-22.
7. Ma'at, S. “Sterilisasi dan Disinfeksi.” Cet. 1. Surabaya: Airlangga University. 2009. p. 10
8. Brooks, G. F., Janet, S. B., Stephen, A. M. Jawetz, Melnick and Adelbergs, “*Mikrobiologi Kedokteran Edisi 23”,* Alih bahasa oleh Mudihardi, E., Kuntaman, Wasito, E.B., Mertaniasih, N.M., Harsono, S., dan Alimsardjono, L. Jakarta: Penerbit Buku Kedokteran EGC; 2007. p. 163, 170.
9. Greenwood, D., Slack, R., Peutherer, J. and Barer, M. “Medical Microbiologi A Guide to Microbial Infection: Pathonesis, Immunity, Laboratory Diagnosis and Control.” Churchill Livingstone Elsevier. Edinburgh; 2007. p. 596, 602-4,614-16
10. Indah, Y.F., Marsono, Yusuf, M. “Efektifitas Ekstrak Lengkuas Putih (Alpina Galanggal L Stuntz Varalba) dan Kunyit (Curcuma Domestica L) Terhadap Pertumbuhan Candida Albicans pada Plat Resin Akrilik.” *Medali Jurnal.* 2015;2(1):37-41.
11. Lee, H., Li, C., Chang, H. and Yang, Y. “Effects of Different Denture Cleaning Methods to Remove Candida albicans from Acrylic Resin Denture Based Material.” 2011;6(4):216-20
12. Soesetidjo, FX, A., Prijatmoko, D. and Hidajati, L. “Biocompatibility of Thermoplastic Nylon Flexible Removable Partial Denture : A Review.” *Int J Curr Res Aca Rev,* 2016;4(10):75-83
13. Ismiyati, T. “Effectiveness of High Molecular Weight Chitosan on the Growth of Candida albicans in Thermoplastic Nylon Denture.” ISBN 978-602-9461-16-9. Yogyakarta : International Joint Symposium. 2012. p. 281-84
14. Mathur, S. Mathur, T. Srivastava, R., and Khatri, R. Chlorhexidine: “The Gold Standard in Chemical Plaque Control.” *N J Physiology & Pharmacology,* 2011;2(2): 45-50