



ANTIBACTERIAL EFFECTIVENESS TEST AGAINST *STAPHYLOCOCCUS AUREUS* BACTERIA IN PERIODONTAL DRESSING ZOE WITH AND WITHOUT CHITOSAN BSF (BLACK SOLDIER FLY)

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Abstract

Periodontal dressings has an important role in reducing the risk of infection and assisting in the wound healing processed after periodontal surgery. One of the materials used is ZOE-based. BSF chitosan is one of the ingredients that can be added to periodontal dressings. Periodontal dressing must have antibacterial properties, to increased effectiveness during the healing process. This study aims to determine whether there is antibacterial effectiveness against Staphylococcus aureus bacteria in ZOE periodontal dressing with and without BSF chitosan. Antibacterial testing was carried out using the well diffusion method with test groups using the ZOE control group, ZOE with 10% and 20% BSF chitosan. The results was analyzed using the Shapiro Wilk test, Levene test, One Way Anova followed by the Post Hoc Tukey HSD test ($p>0.05$). The results shows that ZOE periodontal dressing with and without BSF chitosan has antibacterial effectiveness in each treatment, but there is no significant difference between each group.

Keywords: *Antibacterial, Periodontal Dressing, ZOE, Chitosan BSF, Staphylococcus aureus.*

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INTRODUCTION

Indonesia is a country that still has dental and oral health problems that are the concern of health workers. According to Riskesdas 2018, 57.6% of the population has dental and oral health diseases, including periodontal disease and caries (Kemenkes RI, 2018; Soni et al., 2020; Surya et al., 2019). Periodontal disease is among the highest in the world after caries, and in Indonesia the prevalence of periodontal disease reaches 74.1% (Rohmawati & Santik, 2019). Periodontal disease is an inflammatory condition in the supporting tissues of teeth due to microorganisms and can damage tissues such as, gingiva, alveolar bones, periodontal ligament, and sementum (Gofur et al., 2021).

The main treatments in periodontal disease are skeling and root planing, with the aim of eliminating the causative bacteria. If the treatment is unsuccessful, usually in a clinical examination marked with a pocket depth of > 3 mm, then periodontal surgery is performed. Periodontal surgery is one of the treatments in dentistry to eliminate periodontal disease which will produce open wounds such as curettage, gingivectomy and flaps. The wound healing process has several phases, namely, the hemostasis phase, the inflammation, and the proliferation phase. Therefore, to assist in the healing process, periodontal dressing is needed to close the wound, to reduce bleeding, protect the wound from trauma at the time of chewing, and prevent the occurrence of pain caused by surgical contact of the wound at the time of chewing (Monje et al., 2016; Newman et al., 2011; Saputri & Masulili, 2015).

One of the periodontal dressing materials used is made from zinc oxide eugenol-based because it has antibacterial properties, in previous studies researched by Rahman and Christiono that ZOE can inhibit the growth of *Enterococcus faecalis* bacteria, in addition to having antibacterial properties, ZOE has healing properties, but the eugenol ingredient has a drawback that it can cause hypersensitivity reactions to gingiva, therefore to increase the effectiveness of healing The addition of chitosan is one of the ingredients that can be added to periodontal dressings, because it has antibacterial properties, does not have an irritating effect, can help speed up the healing process, chitosan also has biocompatible, biodegradable, anti-inflammatory and non-toxic properties (Ananda & Ervina, 2022; Zerlinda D et al., 2020).

Chitosan is a type of natural polysaccharide cellulose, which is often found in crustean shells, fungal cell walls, and insects, one of which is the black soldier fly sleeve waste. The BSF fly or *Hermetia illucens*, known as the black soldier fly, is an insect that comes from America and its larvae are widely used for waste treatment, especially organic waste. The life phase of prepupa until it becomes a pupa, black fly can be used as a potential source of chitin because the exoskeleton of the black fly contains 35% chitin. Chitosan is also known as non-toxic soluble chitin. Therefore, chitosan can be used as an ingredient in periodontal dressing (Arbi et al., 2019; Sulistyawati et al., 2022; Wahyuni et al., 2021).

Periodontal dressing plays an important role in reducing the risk of infection. It is known that the sterility of surgical tools and threads can cause infection due to contamination by microorganisms that interact with foreign objects in surgical wounds. Therefore, to increase effectiveness during the healing process, periodontal dressing must have antibacterial properties. One of the bacteria involved in the occurrence of infection after surgery is *Staphylococcus aureus* bacteria with a prevalence of 40% (Arbi et al., 2019; Ranjan et al., 2013).

Staphylococcus aureus is a normal flora bacterium that is in the oral cavity, gram positive, spherical in shape to form colonies irregularly like grapes. *Staphylococcus aureus* is an anaerobic facultative bacterium, can grow and develop optimally at a temperature of 37°C. These bacteria can turn into pathogens if there are predisposing factors, such as contamination from the oral cavity that is not good (Pratiwi et al., 2022).

So far antibacterial tests against *Staphylococcus aureus* bacteria in periodontal ZOE dressings with and without BSF chitosan have never been studied. Therefore, researchers are interested in conducting this study to find out whether there is antibacterial effectiveness against *Staphylococcus aureus* bacteria in periodontal ZOE dressings with and without BSF chitosan.

METHOD

This research was conducted at the Aretha Medika Utama Bandung Laboratory. It was carried out in December 2023. The ethical approval was obtained through a letter of approval from the ethics commission of the Faculty of Medicine, Padjajaran University, Bandung, with ethic number 1251/UN6. KEP/EC/23. This research

uses a type of laboratory experimental research with post test only control group design research, with a hole diffusion method. The samples in this study consisted of ZOE with and without chitosan Bsf 10 and 20% in the bacterium *Staphylococcus aureus* ATCC BAA-1556 with a number of samples on each treatment of 6 repetitions, obtained from a paired numerical analytical formula. The inclusion criteria in this study are periodontal dressings with thick consistency, BSF chitosan and *Staphylococcus aureus* bacteria that have been re-identified by the Aretha Medika Utama Bandung lab.

The preparation in this study is to make BSF chitosan, with several processes including, demineralization, deproteinization, depigmentation and the final stage of deacetylation.

Periodontal dressing ZOE is manipulated in the Aretha Medika main Bandung laboratory, by being weighed according to a 6 mm diameter hole with a total weight of 0.050gr, then weighing the sample added by chitosan BSF 10% as much as 0.005 gr and 20% as 0.010 gr.

Then the inoculation process on the plate so that the test is carried out using the swab method. A sterile cotton swab is dipped in a suspension of *Staphylococcus aureus* bacteria whose turbidity has been pre-adjusted with a standard McFarland 0.5 solution. The cotton swab is pressed against the tube wall to remove excess suspension, then applied to the MHA surface evenly and allowed to stand at room temperature for 3 to 5 minutes until the suspension is absorbed into the agar. After that, the plate to be perforated with a diameter of 6 mm and as much as 20 µL of the concentration used. Then the sample is inserted into the hole. The plate is incubated at a temperature of 37oC for 24 hours, then the barrier zone diameter measurement is carried out and the data is analyzed using SPSS, One way ANOVA, and to see the difference between each treatment using Post Hoc Tukey HSD.

RESULT AND DISCUSSION

ZOE periodontal dressing effectiveness test results data with and without chitosan BSF against the growth of *Staphylococcus aureus* bacteria in the test and control groups can be seen in the description of table 1.

Table 1. Results of the measurement of the inhibition zone diameter

Group	Average	Interpretation
Zinc Oxide Eugenol	18.50	Curentyl
ZOE + 10% Chitosan BSF	17.93	Curentyl
ZOE + 20% Chitosan BSF	20.38	Strong

According to table 1, there is an antibacterial resistance against *Staphylococcus aureus* bacteria, which shows that ZOE periodontal dressings plus chitosan BSF have antibacterial effectiveness in concentration. 20% which is 20.38 mm, while at 10% concentration which is 17.93 mm and the control group using ZOE has an average of 18.50.

Table 2. Normality test

Variable	Normality test (p* value)	Interpretation
Zinc oxide eugenol	0.237	Normal
Zinc oxide eugenol + Chitosan BSF Concentration 10%	0.302	Normal
Concentration 20 %	0.208	Normal

In table 2 it is explained that the periodontal dressing ZOE variable with BSF chitosan with a concentration of 10%, 20% and ZOE is obtained with normal distributed data (p>0.05). Then the levene test is carried out to find out the homogeneity of the data used.

Table 3. Homogeneity test

	Levene Statistic	Df1	Df2	Sig
Zinc Oxide Eugenol with and without chitosan BSF	0.601	2	15	0.561

In table 3 regarding the data homogeneity test on periodontal dressing ZOE with BSF chitosan, the data is not homogeneous because the p-value or sig value is obtained. <0.05. Based on the results of the data testing above, a One Way ANOVA statistical test can be carried out.

Table 4 One Way Anova Test

Group	Mean ± St.dev	P-Value
ZOE	18.50 ± 2.00	
ZOE with chitosan BSF 10%	17.93 ± 1.62	0.111
ZOE with chitosan BSF 20%	20.38 ± 2.23	

In table 4, the calculation results for the antibacterial power between each ZOE periodontal dressing treatment with BSF chitosan for each concentration and control show that the probability value (p-value) is 0.111 because this value is greater than 0.05, meaning there is no significant difference. meaningful and significant between each treatment group.

To find out more about the comparison between the concentrations of ZOE periodontal dressing and BSF chitosan, further tests were carried out using the Post Hoc Tukey HSD test. This test was carried out to determine the differences between each treatment group. After testing, it can be seen whether there are significant differences between each group. The results of the Post Hoc Tukey HSD calculation can be seen in table 5.

Table 5. Concentration comparison test

Comparison between concentrations	Average Difference	P-value
ZOE with chitosan BSF 10%	0.57667	0.869
ZOE with chitosan BSF 20%	-1.88000	0.254
Chitosan BSF 10% with ZOE	-0.57667	0.869
Chitosan BSF 10% with 20%	-2.45667	0.111
Chitosan BSF 20% with ZOE	1.88000	0.254
Chitosan BSF 20% with 10%	2.45667	0.111

In the comparison test between concentrations of antibacterial power between periodontal dressing ZOE with and without BSF chitosan, the results showed that there was no significant difference between each treatment concentration of 10%, 20%, and ZOE because $p > 0.05$.

Discussion

The results of the study shown in table 4.1 and figure 4.1 that periodontal dressing of ZOE with chitosan BSF concentration of 10%, 20% and ZOE group have an inhibitory against the growth of *Staphylococcus aureus* bacteria which is characterized by the presence of a clear zone around the pit. Based on the results of the research, it was found that the resistance of periodontal dressing ZOE with chitosan BSF against the growth of *Staphylococcus aureus* bacteria which has an average resistance at a concentration of 20%, with a diameter of 20.38 mm, and at a concentration of 10%, which is 17.93 mm, while the control group had an average of 18.50 mm. Based on the results, it is known that the higher the concentration of the extract, the greater the barrier

zone that forms around the hole. However, in this study, there was no significant difference between all treatment groups.

The results of this study also showed that all groups added by BSF chitosan had an effect on the diameter of the bacterial growth barrier zone in each concentration increase. This research is in line with the research conducted by Anisa and Anna regarding the antibacterial test of freshwater lobster shell chitosan against *Staphylococcus aureus* bacteria, which has the highest barrier zone diameter at a concentration of 100% of 11.01 mm and the lowest at a 30% concentration of 5.4 mm, this is in accordance with the research that has been done, the greater the concentration, the greater the barrier zone that forms around the hole, this is because chitosan has antibacterial properties derived from the amine group (-NH₂) owned by chitosan. This amine group gives a positive charge to chitosan and makes it very reactive, so it can bind to negatively charged bacterial cell walls. In addition, the structure of chitosan resembles peptidoglycan which is the constituent structure of 90% of the cell wall of gram-positive bacteria (Lagat et al., 2021; Pebiansyah & Yuliana, 2022).

In this study, testing was carried out using ZOE samples against *Staphylococcus aureus* bacteria isolated from bacterial cultures after being re-identified in the laboratory. The results show that it has an obstacle zone diameter of 18.50. For comparison, another study conducted by Rahman and Christiono tested the antibacterial properties of ZOE against the bacteria *Enterococcus Faecalis* with the disc paper method showing the presence of antibacterial activity, with a diameter of the barrier zone of 27.7 mm, there was a difference in the diameter of the barrier zone with the research conducted allegedly caused by different bacteria and the methods used (Ananda & Ervina, 2022). These findings show that the content of eugenol is predicted to be very influential against antibacterial, it is known that eugenol has hydrophobic properties, and bactericidal by forming a phenol structure. Phenol works as an antibacterial by completely damaging the plasma membrane. so that the content of eugenol in the extract will enter the lipopolysaccharide contained in the bacterial cell membrane and damage the cell structure (Utami, 2019).

Based on the results of this study, there is antibacterial effectiveness in ZOE periodontal dressings with and without BSF chitosan against the growth of *Staphylococcus aureus* bacteria, but there is no significant difference between all

treatment groups. In the clinical report, it is reported that periodontal dressings with the main ingredient ZOE have a side effect, namely hypersensitivity to gingiva due to the presence of eugenol content that has the potential for irritation to the surrounding soft tissues, but this material has advantages in the presence of antibacterial properties (Zerlinda D et al., 2020). Other ingredients such as chitosan have positive properties that can be used as periodontal dressing, because it has anti-inflammatory properties, biocompatibility, good biodegradability, is hemostatic, has no side effects and is able to accelerate wound healing (Ananda & Ervina, 2022). Based on these things, it is necessary to carry out further tests on ZOE periodontal dressings added with BSF chitosan to see if the irritating properties of ZOE ingredients can be minimized by Addition of BSF chitosan. It is hoped that ZOE periodontal dressing with the addition of BSF chitosan can be redeveloped as a periodontal dressing that is safe to be applied to wounds after surgery.

CONCLUSION

There is antibacterial effectiveness against *Staphylococcus aureus* bacteria in ZOE periodontal dressing with and without BSF chitosan, but there is no significant difference between all treatment groups.

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