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Antibacterial Activity of Nano-Sized Gourami Fish Scales Powder (Osphronemus Gourami) Added to Conventional Glass Ionomer Cement

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ABSTRACT

Background: Gourami scales contain antibacterial compounds, e.g., catechins, chitin, calcium, omega 3, omega 6, and flavonoids. Our previous studies have shown that millimicrons-sized gourami fish scales powder (GFSP) added to Glass ionomer cement (GIC) acts as an immunomodulator against Streptococcus mutans both in vitro and in vivo. However, the antibacterial activity of the material was affected by the particle size, and the effects of smaller GFSP, which is nano-sized (nGFSP) on oral biofilms are largely unknown.

Materials and Methods: This study analyzed the potential of nGFSP added to conventional GIC in inhibiting the growth of Streptococcus sanguinis, Streptococcus mutans, and Staphylococcus aureus. nGFSP were divided into five groups, 0% (control), 0.5%, 1.5%, 2.5%, and 3.5% (4 samples for each group). The antibacterial activity was evaluated using the agar diffusion method. Then, the diameter of the inhibition zone was measured and analyzed using ANOVA followed by the LSD test.

Results: Antibacterial activity against S. sanguinis, S. aureus, and S. mutans significantly increased with nGFSP. The largest zone of inhibition was measured at a concentration of 3.5%, that is, 4.02 mm (S. sanguinis) and 2.29 mm (S. aureus). Meanwhile, nGFSP is more effective in inhibiting the growth of S. mutans in an optimum concentration of 0.5% with a zone of inhibition is 3.54 mm.

Conclusion: The addition of nGFSP improved the antibacterial activity of GIC against gram-positive pathogenic bacteria, such as S. sanguinis, S. aureus, and S. mutans.

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I. INTRODUCTION

Conventional Glass Ionomer Cement (GIC) is a restorative material commonly used in dentistry. This material has several advantages in terms of aesthetics, good adhesion to the tooth surface, and antibacterial properties. The antibacterial properties of GIC are due to the release of fluorine before setting.[1] However, other studies have found the presence of Streptococcus mutans (S. mutans), at the interface between the edge of restoration and the tooth surface.[2] The antimicrobial activity of conventional GIC can be increased by adding an antibacterial agent. Some researchers add ingredients to GIC with a particular composition ratio to increase their inhibition against cariogenic bacteria.[3,4,5]

Gourami scales (Osphronemus gourami) are fish waste that can be utilized due to the content of type-I fibril collagen and minerals (about 16-59%).[6] The outer layer (bone layer) of fish scales is more mineralized, containing higher inorganic materials, while the inner layer (basal/collagen layer) is higher organic materials content.[7] The average calcium content in gourami scales is about 5-7.5%, and they form in hydroxyapatite crystals. Gourami scales also contain catechins, amino acids, omega 3, omega 6, and flavonoids which have antibacterial properties. [8] Gourami can absorb minerals and calcium from their food and aquatic environment. The absorbed calcium is then stored in the bones and skin.[9]

Our previous study showed that the paste of gourami's bones and scales is biocompatible and has immunomodulatory activity against S. mutans, both in vitro and in vivo. [3] In addition, we also found decreased expression of Toll-like Receptor 2 (TLR-2) and TLR-4 in the dental pulp of Wistar rats.[10] However, particle size dramatically determines the antibacterial activity of a material,[11] and that study used a millimicron-sized powder, larger than the GIC powder. Therefore, this research will examine the antibacterial power of nano-sized gourami scale powder. The addition of nanoparticles GFSP is expected to improve the antibacterial activity of GIC.

II. MATERIAL AND METHODS

This laboratory experimental studies study was conducted at the Bioscience Laboratory of the Faculty of Dentistry, University of Jember, Jember, Indonesia from Juli 2022 to December 2022. A total of 20 samples were used in this study. The sample size was calculated based on Federer's formula. The minimum sample that we obtained for this study was four for each group, as described below.

Group KO/ control (N=4 samples): GIC;
Group K1 (N=4 samples): GIC + 0,5% nano GFSP);
Group K2 (N=4 samples)**:** GIC + 1,5% nano GFSP);
Group K3 (N=4 samples): GIC + 2,5% nano GFSP);
Group K4 (N=4 samples): GIC + 3,5% nano GFSP);

Manufacturing of Gourami Fish Scales Powder

Nano-sized GFSP (nGFSP) was obtained through the freeze-drying method described by Wulandari et.al.[12] The fish scales were then ground using a ball mill.[13] Furthermore, the pH of nGFSP was measured, and particle size was analyzed using a Particle Size Analyzer (Horiba-SZ 100z, California, US).

Sample preparation

This study used GIC powder from Fuji IX Gold Label High Strength Posterior Extra, Japan. The sample was divided into five groups, including the control. GIC and nGFSP were mixed using a vortex (Labinco L46, Breda, Netherland).

Antibacterial assessment

Antibacterial activity against S. mutans and S. aureus was evaluated by the well diffusion method. AntiTryptone soya agar (TSA) and bacterial suspension were prepared. As much as 1 ml of the bacterial suspension was taken using a syringe, then inoculated in TSA media, and leveled with gigaskrin. Bacterial inoculation was carried out by streaking, and scratching in a zig-zag manner across the entire media surface, repeated three times. After inoculation, it was covered for 15 minutes to absorb the bacterial suspension well into the media. Wells were made with a diameter of 5 mm and a depth of 3 mm. The powder and liquid are mixed and fed into the wellbore in an unsetting state. The treated media was put into a desiccator and incubated at 37°C for 24 hours. The inhibition zone was measured using a caliper (in millimeters). The diameter of the inhibition zone was measured using a caliper (in millimeters). We placed the plate on a non-reflective surface and measured directly across the area of inhibition from edge to edge, crossing through the center of the disk.

Statistical analysis

The data obtained were analyzed using Statistical Package for the Social Sciences (SPSS). One-way analysis of variance and Tukey HSD post-hoc tests at a significance level of p=0.05 were used to perform multiple comparison tests.

III. RESULT

Figure 1 shows the inhibition zone formation produced through the reaction between the bacterium and nGFSP added to GIC. In agar media, the zone of inhibition is indicated by a circular area around the spot of the antibiotic in which the bacteria colonies did not grow.

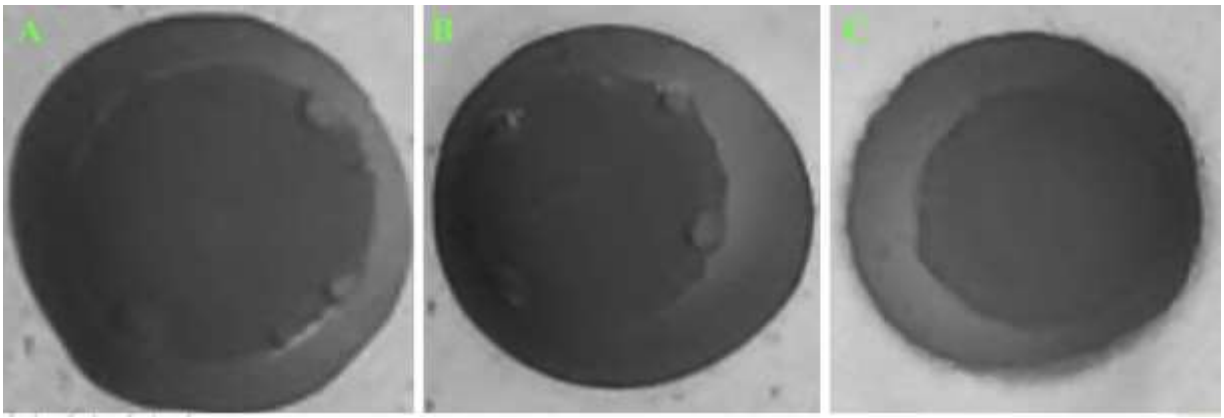
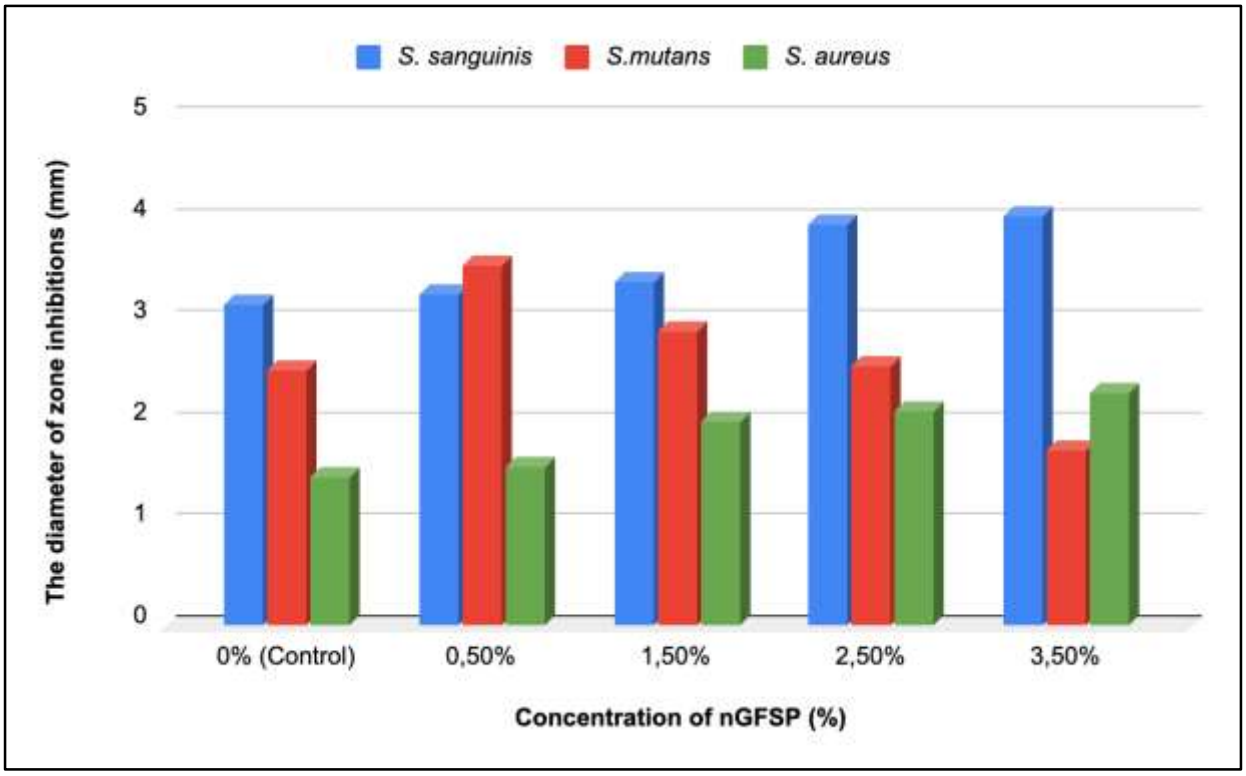


Figure 1. The zone of bacterial inhibition produced by nGFSP against S. sanguinis, S. aureus, and S.mutans

Graph 1 illustrates the inhibition of bacteria growth by nGFSP against S. sanguinis, S. aureus, and S. mutans, which showed good antibacterial activity. The addition of a mixture of GIC and nGFSP in agar media inoculated with (A) S. sanguinis, (B) S. aureus, and (C) S.mutans inhibited the growth of these organisms.



Graph 1. Antibacterial activity of nGFSP against S. sanguinis, S. aureus, and S.mutans

Antibacterial activity against S. sanguinis, S. aureus, and S. mutans significantly increased with the addition of nGFSP ($P<0.05$). The largest zone of inhibition was measured at a concentration of 3.5%, that is, 4.02 mm (S. sanguinis) and 2.29 mm (S. aureus). Meanwhile, nGFSP is more effective in inhibiting the growth of S. mutans in an optimum concentration of 0.5% with a zone of inhibition was 3.54 mm. Moreover, the inhibition of this bacterium declined when the content of nGFSP was 1.05% and above, but it was still higher than the control (except at the concentration of 3.5%).

IV. DISCUSSION

The antimicrobial action of restorative material is clinically important because the oral cavity has the second largest and most diverse microbiota after the gut harboring over 700 species of bacteria.[14] S. sanguinis, S. mutans, and S. aureus are indigenous oral microorganisms found in carious dentin. As a widely used restorative material, GIC can inhibit those bacterium growths because of the presence of fluoride. However, the amount of released fluoride is insufficient to guarantee its antibacterial activity. One way to enhance antibacterial inhibition is by incorporating antibacterial materials, such as nGFSP.

The addition of a mixture of GIC and nGFSP in agar media inoculated with S. sanguinis, S. mutans, and S. aureus significantly inhibited the growth of these organisms ($p < 0.05$). The inhibition activity was more excellent in the treatment groups than in the control. This condition happened because of the antibacterial compounds in gourami scales, such as catechins, chitin, omega 3, omega 6, and calcium. Catechin is a polyphenolic compound. It is bacteriostatic by damaging the bacterial cytoplasmic membrane, thereby preventing the entry of the nutrients needed by the bacteria. As a result, bacteria will experience growth inhibition and even bacterial death.[4] Chitin can change the permeability of the bacterial cell wall through the reactions between its positively charged amino groups and negatively charged lipopolysaccharide groups and proteins on the surface of the bacterial cell wall. This reaction causes the disintegration of the cell membrane and damage to the cell wall, resulting in bacteria lysis.[15,16]

Omega 3 and omega 6 in gourami scales are bactericidal and have a surfactant effect on bacterial cell membranes, so they can inhibit protein synthesis and kill bacterial cells.[10] Both fatty acids can inhibit bacterial growth by changing the hydrophobicity of cell membranes and cell surface charge, which causes electron leakage and results in cell death. The survival of bacteria depends on the phospholipid chains that regulate the viscosity of the cell membrane. Through this viscosity adjustment, the vital functions of the bacterial cell membrane, such as active solute transport, passive permeability of hydrophobic molecules, and interactions between proteins, work well.[17] The inorganic content of gourami scales, calcium in the form of hydroxyapatite, can cause cell death by releasing OH- ions, raising the pH to become alkaline, and damaging the bacterial cell walls.[10]

The inhibition zone formed in the control group is influenced by fluoride in GIC.[18] The released fluoride will control remineralization and has an antimicrobial effect.[7] While it can

enter bacterial cells in an acidic pH environment as HF (hydrogen-fluoride) ions, it will dissociate when exposed to the more neutral bacterial intracellular pH. The released fluoride ions will be toxic to bacteria and cause cell death.[18] Fluoride can reduce the growth rate of bacteria by inhibiting glucose metabolism. In the glycolysis cycle, glucose or lactose metabolism requires the fluoride-sensitive enzyme enolase. Bacterial growth that depends on the glycolysis cycle can be inhibited by fluoride.[19]

Particle size affects the antibacterial activity of a material. The smaller the particle size, the greater the specific surface area so that it is more likely to contact, penetrate the bacterial cell membrane and induce intracellular antibacterial effects.[7] This study used nano-sized GFSP, and the particle size is 51,77 nm. Nanoparticles may induce antibacterial properties via several mechanisms, e.g., alteration of the cell wall, cytoplasm, and membrane or inhibition of respiratory activity.[20] The bacteria's cell wall has a significant role against nanoparticles (NPs). NPs, through electrostatic interaction, can connect to the outer membrane of bacteria and cause to disrupt the membrane, suppressing periplasmic enzymes, rupturing bacterial cells in the culture media, and ultimately restraining DNA, RNA, and protein synthesis.

Staphylococcus aureus, through biofilm formation, creates more resistance against NPs. In this regard, more NPs and retention time may be needed for a better bactericidal effect. Ions released from nanomaterials may react with thiol groups (−SH) of bacteria cell's surface proteins. Some of these cell membrane proteins play a role in transferring minerals from the cell membrane. The effect of NPs on these proteins leads to their inactivation and the cell wall becoming impenetrable. The inactivation of membrane permeability caused the cell's death.[21] Hydroxyl ions are highly oxidant free radicals that show extreme reactivity, reacting with several biomolecules. This reactivity is high and indiscriminate, so this free radical rarely diffuses away from sites of generation. Their lethal effects on bacterial cells are probably due to the following mechanisms: damage to the bacterial cytoplasmic membrane, protein denaturation, and damage to the DNA.[22]

V. CONCLUSION

The addition of nGFSP significantly enhances the antibacterial activity of GIC against S. aureus and S.mutans. The results of this study present marine biota as a new alternative antibacterial candidate to fight against oral microorganisms.

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