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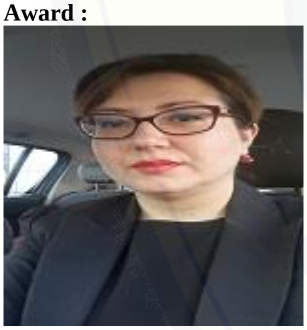

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Marginal gap, Compressive Strength And Bacterial Inhibition Of Gurami Fish (Osphronemus gouramy) Scales Powder

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Abstract
Until now the use of gouramy fish scales, has not been widely used, while the benefits related to restoration. This research aimed to analize the addition of gouramy scales powder (GSP) to Glass Ionomer Cement (GIC) on the marginal gap, compressive strength and inhibition of bacteria. This research divided into 4 groups. K0: the sample is filled with GIC. K1: GIC + 2.5% GSP. K2: GIC + 5% GSP. K3: GIC + 10% GSP. The compressive strength and filling gap width tests were carried out by embedding GIC samples on red wax blocks and cavity preparation and observed with SEM. The inhibition of bacteria by means of GIC and GSP manipulated with GIC fluid and put into wells on agar media that had been cultured by Streptococcus mutans and Lactobacillus acidophilus. The diameter of the inhibition zone was measured at 1x24 hours and 2x48 hours. The ANOVA and LSD test confirmed significant differences for inhibition of bacteria activity, but it did not significant for marginal gap and compressive strength, it caused GSP particle size that is too large. The antibacterial potential of goramy scales caused by chitin, catechins, calcium, flavonoids, amino acids, omega 3, and omega 6.

Keywords: *marginal gap, compressive strength, bacteria inhibition, gouramy fish scale, powder, glass ionomer cement*

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I. Introduction

Glass ionomer cement (GIC) was first introduced by Wilson and Kent in 1972, is a tooth-colored filling material. GIC consist of liquids which are a combination of water and polyacids (polyacrylic, maleic, itaconic, tartaric acids), powder in the form of fluoroaluminosilicate glass (Anang et al., 2015). GIC is used for small Class I and Class II restorations in areas of low load and Class I and Class II restorations in primary teeth (Burke & Bardha, 2013; Nakao, 2016; Nakao, 2020). GIC has the advantage such as biocompatible, better aesthetics than other plastic restorative materials, bonds chemically to dental hard tissues, and being able to release fluoride which can prevent caries by remineralization process and its actibacteria property (Sianturi and riyanti, 2018). On the other hand, it has several disadvantages such as having low compressive strength and really susceptibility to moisture and dehydration that can lead to creating marginal gaps between restorative materials-tooth structure (Khaghani et al., 2016; Asafarlal, 2017). Several GIC modifications have been carried out by adding potential materials, not only to improve the mechanical and aesthetic properties of restorative materials, but also to treat caries by increasing the antibacterial activity of GIC (Zalewska et al., 2014). Streptoccocus mutans (S. mutans) and Lactobacillus acidophilus (L. acidophilus) are the main bacterium associated with caries development. While one of the materials that could potentially be added to GIC is Gouramy scales (Dewanti et al, 2018).

Gouramy scales consist of collagen type 1 fibrils and mineralization with hydroxyapatite (16% to 59% mineral content). 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 (Dzu et al., 2011; Dewanti et al., 2020). The average calcium content in gouramy scales is about 5-7.5%, these calcium form in hydroxyapatite crystal (Suwandi and Yogaswari, 2010). Gouramy scales also contains catechins, amino acids, omega 3, omega 6, and flavonoids which have antibacterial properties (Dewanti et al, 2018).

Addition GSP that contains high calcium may increase the chemical reaction. Calcium is one of the components to make the intermediate layer that is made by the fusion process of calcium, aluminium phosphates, and polyacrylates. This layer forms in the interface between the restorative cement and tooth structure. The intermediate layer is essential for a strong adhesive interface that may decrease the marginal gap (Arita et al., 2011; GC South East Asia, 2015). Furthermore, more calcium ions will better bond calcium and aluminium and fill the gaps between the glass particles in the GIC (Mozartha dkk., 2015). Adding GSP to GIC powder, more salt bridges and cross-links are formed as powders mixed with liquid, making restorative cement stronger and may increase the compressive strength of GIC (Astrid et al., 2019). Until now, the application of gouramy scales has not been widely used, while the benefits related to restoration filling gap width, compressive strength and inhibition of bacteria on glass ionomer are not yet available. This research needs to be done to prevent the occurrence of secondary caries.

This study aimed to analyze the effect of the addition of Gouramy Scale Powder (GSP) to glass ionomer cement (GIC) on the marginal gap, compressive strength, and bacteria inhibition.

II. Materials and Methods

2.1. Materials

Materials of this research such as Gouramy scales, GC Gold Label IX HS Posterior Extra, GC Corporation, Tokyo, Japan, Aquadest (ROFA), BHI-B (Brain-Heart Infusion Broth) (Himedia), BHI-A (Brain-Heart Infusion Agar) (Himedia), L. acidhopilus (ATCC 4356 (from Lab. Microbiology Research Centre Faculty of Dentistry Unair), Elements of the maxillary or mandibular premolars, red wax (Cavex), GC Dentin conditioner (GC, Tokyo, Japan), GC Fuji varnish, Micro brush (TPC).

2.2 Instruments

Petridish (Pyrex), Autoklaf (Sanyo), Inkubator (Espec), test tubes (Pyrex), Centrifuge (Dlab), Erlenmeyer tubes (Pyrex), Sprectrofotometer (Dlab), Freeze Dryer (Zirbus Technology Vaco 5-II-D Serial No. 11/3184), Microscope (Olympus), Desicator (Merc), Scanning Electron Microscope (Hitachi TM3030Plus), Plastis filling instrument, Celluloid strip, Micromotor and handpiece (NSK, Japan).

2.3. Gouramy Scales Powder

Gouramy scales powder from the gouramy cultivation (Den Bagus Gurami, Kencong, Jember) made by freeze-drying process for 21 hours. Dried fish scales are ground with a blender then refined with a sieve 200 mesh to get gouramy scales powder (GSP).

2.4. Research Groups

Conventional glass ionomer cement was used in this study as the control and restorative materials. 2,5%, 5%, and 10% of the powder was replaced by GSP. GIC powder and GSP were hand mixed before the addition of the liquid of GIC.

There are four groups for marginal gap and compressive strength test : group 1 (GIC), group 2 (GIC + 2,5% GSP), group 3 (GIC + 5% GSP), group 4 (GIC + 10% GSP). Meanwhile, there are five groups for the bacterial inhibitory test : group 0 (GIC), group 1 (GSP), group 2 (GIC + 2,5% GSP), group 3 (GIC + 5% GSP), group 4 (GIC + 10% GSP). The prepared powder was mixed with the liquid of GIC with a ratio of 3:1.

2.5 Marginal Gap

Six teen extracted human maxillary premolar teeth were used for this experimental study. Teeth without any caries, fracture, and restoration were selected. All the teeth were disinfected with alcohol 70% and stored in distilled water. Class I cavities (3 mm in length and 2 mm in depth) on the buccal surface were prepared for each sample with bur fissure flat end (SF-R11) using low speed handpiece. After tooth preparation, apply with dentin conditioner to the cavity surfaces for twenty seconds using a micro brush. After that, rinse thoroughly with water, then dry the surface with a cotton pellet. The cavities were restored with GC Gold Label IX HS Posterior Extra (A3 Shade). All samples were stored in a sealed vial for 24 hours at room temperature. All the teeth were sectioned longitudinal in a buccolingual directioned in the middle of the restoration using a diamond disk under a water spray. The sectioned teeth were examined under a scanning electron microscope at 500x.

2.6 Compressive Strength

The compressive strength test was performed using twenty-four specimens, prepared and submitted to compression tests. An acrylic plate with four cylindrical holes measuring 5 mm in diameter x 2 mm in height was used to make the test specimens. The acrylic plate has been smeared with vaseline placed on a glass plate. After mixing, put the homogenized restorative cement into a cylinder with a plastic instrument, then give light pressure to drain the excess cement. Place a celluloid strip on top, then give a load of 0.5 kg above the mould and allow it to set. After setting, the specimens were placed in a sealed vial to avoid contamination with air.

After 24 hours, the samples were tested for compressive strength using a universal testing machine (UTM Mitsubishi GTO1000). A compression load was applied at 0.5 mm/min speed until the test specimens were fractured. The following formula was applied to the results, in newtons (N): CR=4p/πd2, where CR=compression resistance, in megapascals (MPa), p=maximum load applied to the sample, in newtons (N), π=constant 3.1416 and d=sample diameter, in millimeters (mm).

2.7 Antibacterial activity

The antibacterial activity was evaluated against *S. mutans* and *L. acidophillus* by using agar well diffusion method. The number of sample each bacteria was 25 samples divided into 5 groups in 1 petridish. The variable studied was the diameter of the inhibition zone as a sign of inhibited growth of *S. mutans* and *L. acidophilus* on agar plate. GIC and GSP were weighed according to the predetermined concentration group, then manipulated with GIC fluid and inserted into wells on agar media that had been cultured with *S. mutans* and *L. acidophilus* with the consistency of unsetting cement. Samples were incubated in incubator (*Espec*) at 37^o for 48 hours. The diameter of the inhibition zone was measured at 1x24 hours and 2x48 hours with vernier callipers (*Kenmaster*) by calculating the mean diameter of the vertical and horizontal inhibition zones.

2.8. Statistic Analisys

The data collected were tabulated accordingly and were subjected to statistical analysis One way ANOVA test then continued Least Significant Difference test (P < 0,05) was considered to be significant.

III. Results

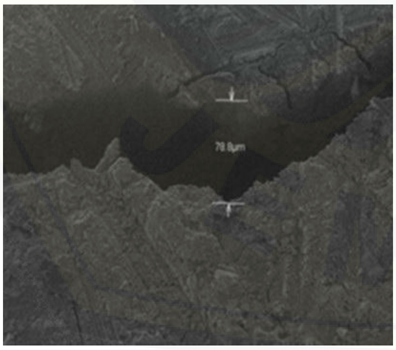
Means and standard deviations for the marginal gap and compressive strength of the control and experimental groups are summarized figure 2. Representative SEM micrographs are shown in figure 1.

3.1 Marginal Gap

Observations and measurements of the largest marginal gap width, the average marginal gap width in each research group was obtained (table 1). The average width of the marginal gap from the smallest to the largest is in group 2 < group 1 < group 3 < group 4.

Table 1. Marginal gap width After Addition Of Gurami Fish Scale Powder and GIC (μm)

Groups	Mean
GIC	74 ± 27,8
GIC + 2,5% GSP	57,2 ± 17,8
GIC + 5% GSP	77,7 ± 9,5
GIC + 10% GSP	94,4 ± 19,4
P-value	0,119



Group 1

Group 2

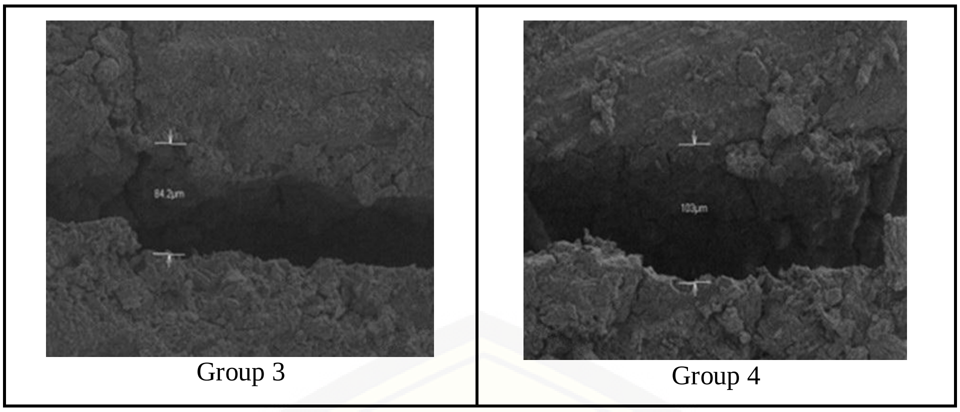


Figure 1. Marginal gap analysis with scanning electron microscope (500x)

2.2 Compressive Strength

The mean values obtained in the compressive strength test for the GIC after adding GSP, according to the addition of 2.5%, 5% and 10% GSP are shown in Table 2. The average width of the compressive strength from the smallest to the largest is in group 4 < group 1 < group 3 < group 2.

Table 2.2 Compressive strength (MPa)

Groups	Mean
GIC	62,71 ± 22,79
GIC + 2,5% GSP	68,19 ± 19,93
GIC + 5% GSP	65,65 ± 24,38
GIC + 10% GSP	54,41 ± 11,15
P-value	0,670

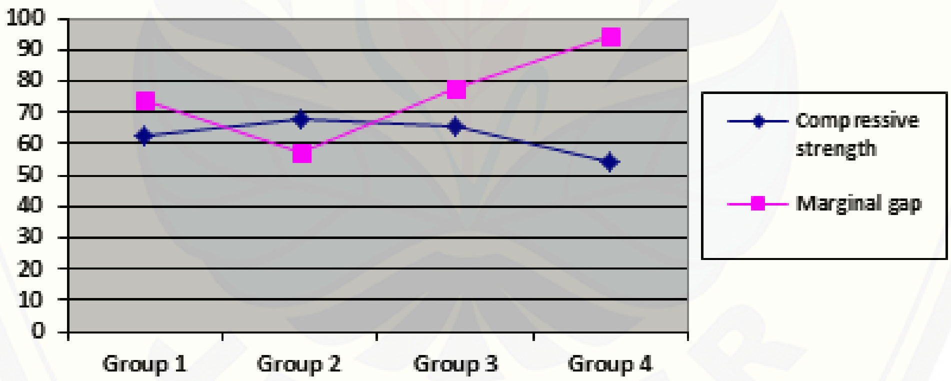


Figure 2. Marginal gap and compressive strength

The first null hypothesis was accepted after statistical data analysis (One way Anova test). The addition of GSP to GIC did not significantly affect the GIC marginal gap and compressive strength compared to the control group (p>0,05).

2.3. Antibacterial Activity

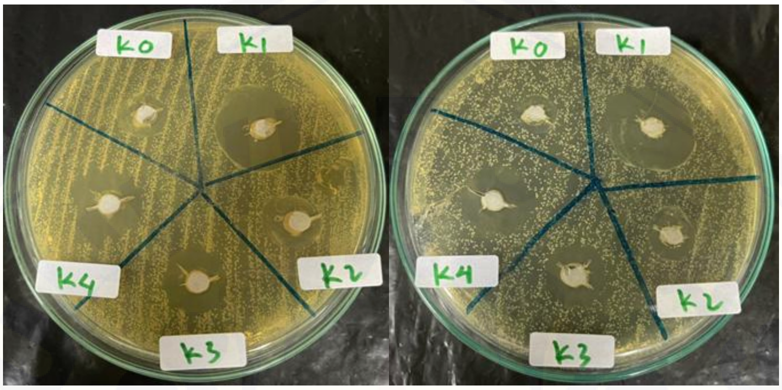
The results showed that there was a clear zone around the wellbore in all groups. The results of the mean diameter of the inhibition zone and the standard deviation in each group can be seen in the following table:

Table 2. Mean Diameter of the Inhibition Zone and Standard Deviation

Group	N	The Mean Diameter of Inhibition Zone and Standard deviation (mm)			
		L. acidophilus		S. mutans	
		24 hours	48 hours	24 hours	48 hours

0	5	8,74±0,24	8,74±0,24	9,34±0,16	9,34±0,16
1	5	20,23±0,30	20,23±0,30	21,2±0,29	21,2±0,29
2	5	10,51±0,22	10,51±0,22	11,54±0,16	11,54±0,16
3	5	13,41±0,15	13,41±0,15	14,34±0,22	14,34±0,22
4	5	16,34±0,27	16,34±0,27	17,45±0,34	17,45±0,34

The mean diameter of the inhibition zone and the standard deviation from the smallest to largest are group 0> group 2> group 3> group 4> group 1. Representative of inhibition zones of all samples are shown in figure 3



(a) (b)

Figure 3. Representative of The Inhibition Zones GIC added with GSP on (a*) L. acidophilus and (b) S. mutans.*

These results followed by statistical data analysis by one way ANOVA and LSD Test. Both of the Test showed p value <0.05, meaning the mean value of the inhibition zone in each group and between group had a significant differences.

IV. Discussion

4.1 Marginal gap

Marginal gaps are caused by sufficient strength of dental cement to bond to enamel or dentin. Three main factors can create a marginal gap: polymerization shrinkage, wear, and thermal cycling. In addition, the marginal gap can make bacteria, food, debris, saliva enter into the gap that can lead to pulpal irritation due to bacterial products such as lipopolysaccharides (Powers and Sakaguchi, 2012). None of the GIC materials evaluated completely prevented marginal gaps. Group 1 (GC Gold Label IX Extra) exhibited a mean width of 74 ± 27,8 µm. GIC was sensitive to water, leading to the formation of marginal gaps due to shrinkage of the cement (Mabrouk et al., 2012). The dry condition caused the liquid of GIC to evaporate, so it may lead to cracking and shrinkage of cement that may create marginal gaps (Kinasih et al., n.d.)

The less marginal gap was observed at group 2, which added 2,5% GSP to GIC exhibited a mean marginal gap width of 57,2 ± 17,8 µm. GSP contains calcium around 5-7,5%, its form in hydroxyapatite crystal (Suwandi and Yogaswari, 2010). Adding GSP may increase adhesion bonding between restorative materials-tooth structure. Ion calcium of GSP may release then participate in the acid-base reaction. Calcium ions will bind to carbonyl anion of polyacrylic acid of the liquid component of GIC (Arita et al., 2011). It may increase the formation of ionic bonds between restorative material-tooth structures to achieve better bonding that can lead to a less marginal gap (Sharafeddin and Feizi, 2017). Aside from that, a fusion bonding reaction occurred with calcium, aluminium phosphate, and polyacrylate mixed and precipitated, forming an intermediate layer. This layer is located between restorative material-tooth structures, which is very resistant to acid and stronger (Arita *et al.*, 2011; GC South East Asia, 2015). Combining the intermediate layer and the chemical bonding may provide better adhesion that can lead to less marginal gap.

The marginal gap was increased to 77,7 ± 9,5 µm at group 3 (GIC + 5% GSP), and 94,4 ± 19,4 µm at group 4 (GIC + 10% GSP). It can be influenced by different sizes of the particles GIC and GSP. The particle size of GSP is around 74 µm due to the sieving process using 200 mesh sieve. Adding greater concentration of GSP, which has a greater particle size than GIC makes higher viscosity that hamper the wetting of the cavity surface properly, preventing good adhesion of restorative material-tooth structures (Singla et al., 2012).

The addition of GSP affects the width of the marginal gap. The addition of 2.5% by weight of GSP can decrease the width of the marginal gap, but it is not significantly different compared to SIK (p>0,05).

4.2 Compressive Strength

The compressive strength is very influential on the brittleness of material and the weakness of material against pressure (Powers and Sakaguchi, 2012). The highest increase was in group 2 with an additional weight of 2.5% GSP, which had an average compressive strength of 68.19 ± 19.93 MPa, followed by group 3 with an additional weight of 5% GSP, which had an average compressive strength of 65.65 ± 24.38 MPa. The increase in compressive strength is caused by addition of GSP that contain calcium and phosphorus which have an important role in development and growth of the framework and several biochemical reactions (Prabu et al, 2017). In theory, the primary setting reaction of GIC is a consequence of acid-base reaction and followed by calcium and aluminum ions from the glass powder into cross-links with polyacrylic acid which forms a cement matrix (Moshaverinia et al., 2011; Astrid et al., 2019). Through adsorption, complex series ion exchange takes place between calcium and ions phosphate from GSP and glass powder with the help of polyacrylic acid. More calcium ions will better bond calcium and aluminium and fill the gaps between the glass particles in the GIC (Mozartha dkk., 2015). Therefore, by adding GSP to GIC powder, more salt bridges and cross-links are formed as powders mixed with liquid, which makes GIC stronger, strengthening restorative cement (Astrid et al., 2019).

Moreover, GIC showed the lowest compressive strength 54,41 ± 11,15 MPa was in group 4 than control group, with an additional weight 10% of GSP. It may be caused by several factors that affect the decrease of the compressive strength. First, the particle size where the particles should not be too small or too large which can cause the reaction of the GIC component with the GSP to be inhibited so that it affects the GIC strength (Arita et al., 2011; Al-hamoy et al., 2018).Then, recent studies reported variations of GIC materials properties depending on powder/liquid ratio, mixing technique and variances in operator will affect the physical properties of the GIC (Zahra et al., 2011; Shaza et al., 2011). In this study, P/L ratio was 3,4:1,0 as the manufacturer suggested and the manipulation was done manually by spatulation, so it may be hard to achieve a maximal incorporation and reaction among GSP and GIC.

In this study, it was found that the addition of 2.5% and 5% GSP to GIC caused an increase in compressive strength. At 10% mass weight decreases. Even though there is an increased compressive strength of the two masses, there is no significant difference between the average compressive strength values in all the sample groups, where P value is more than 0.005 ($P > 0.05$). It shows statistically there is no significant difference.

4.3 Antibacterial Activity

The antibacterial ability of group 0 was caused by the fluoride content of GIC. Microbiologically, fluor has strong antibacterial properties. Fluor contributes directly to bacterial cells by its mechanism of toxicity. Fluor can enter bacterial cells in environments with acidic pH values as HF (*hydrogen-fluoride*) ions and dissociate when exposed to a more neutral bacterial intracellular pH. As a result, the released fluorine ions will be toxic to these bacteria and eventually cause cell death (Breaker, 2012).

The antibacterial potential of goramy scales is obtained from its content including chitin, catechins, calcium, flavonoids, amino acids, omega 3, and omega 6. (Dewanti et al, 2018). Catechins are polyphenolic compounds that are bacteriostatic with the mechanism of damaging the cytoplasmic membrane of bacteria so that they can inhibit bacteria (Dewanti et al., 2019). Flavonoids are able to inhibit the nucleic acid synthesis and inhibit the function of bacterial cell membranes. The result of the interaction between flavonoids and bacterial DNA causes damage to microsomes, lysosomes, and bacterial cell wall permeability (Rijayanti, 2015). The inorganic content contained in goramy fish scales is calcium in the form of hydroxyapatite with an OH group (Nurjanah et al, 2010). The release of OH- ions can raise the pH to alkaline and trigger bacterial cell wall damage (Radeva and Tsanova, 2016). The content of omega 3 and 6 is bactericidal because it has a surfactant effect on bacterial cell membranes so that it will inhibit protein synthesis and kill bacterial cells (Wayan et al, 2012). Those various antibacterial content of goramy scales resulted in the diameter of the inhibition zone in group 1 being the largest of all groups and significantly different from others.

V. Conclusion

The addition of GSP can decrease the width of the marginal gap, but it is not significantly different.

It was found that the addition of 2.5% and 5% GSP to GIC caused an increase in compressive strength. However, it shows statistically there is no significant difference.

The inhibition zone in each group and between group had a significant differences.

Furthermore, the research is needed to be carried out with more varied concentrations and smaller particle sizes.

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