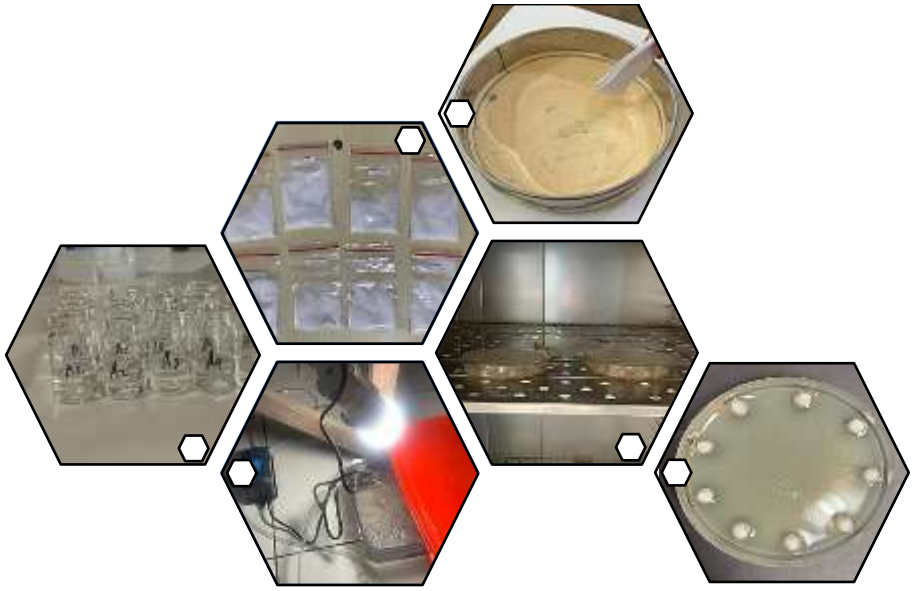
EFFECT OF TYPE VI GLASS IONOMER POWDER COMPOSITION FROM DIATOM NANOSILICA, CHITOSAN AND HYDROXYAPATITE FROM CRABS SHELLS ON THE TOXICITY AND ANTIBACTERIAL PROPERTIES OF CORE BUILD-UP



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THESIS

EFFECT OF TYPE VI GLASS IONOMER POWDER COMPOSITION FROM DIATOM NANOSILICA, CHITOSAN AND HYDROXYAPATITE FROM CRABS SHELLS ON THE TOXICITY AND ANTIBACTERIAL PROPERTIES OF CORE BUILD-UP

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STATEMENT OF THESIS AUTHENTICITY AND COPYRIGHT TRANSFER

I hereby declare that, the thesis entitled "Effect of Glass Ionomer Type VI Powder Composition From Diatom Nanosilica and Chitosan and Hydroxyapatite From Crabs Sheels on the Toxicity and Antibacterial of Core Build-Up" is my true work under the guidance of my supervisor doctor Prof. Moh. Dharmautama, drg., Ph.D., Sp.Pros., Subsp.PKIKG (K). This scientific work has not been submitted and is not being submitted in any form to any university. Sources of information originating or quoted from published or unpublished works from other authors have been mentioned in the text and are included in the Bibliography of this thesis. If in the future it is proven or can be proven that part or all of this thesis is the work of someone else, then I am willing to accept sanctions for such actions based on applicable regulations.

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ABSTRACT

ANDI AURA BUTSAINAH P **Effect of Glass Ionomer Type VI Powder Composition From Diatom Nanosilica, Chitosan and Hydroxyapatite From Crabs Sheels on the Toxicity and Antibacterial Properties of Core Build-Up** (Supervised by Prof. Moh. Dharmautama, drg., Ph.D., Sp.Pros., Subsp.PKIKG (K))

Background: Core build-ups are restorations placed on severely damaged teeth. One attempt to improve the mechanical properties of GIC core build-ups is to replace the silica content using natural nanosilica from diatoms and additives such as hydroxyapatite and chitosan which can be obtained from mud crab (Scylla serrata) shells. Assessment of core build-up restoration materials should also include their biocompatibility and antibacterial properties. Measurements include cytotoxicity, one of the initial methods for toxicity testing is the Brine Shrimp Lethality Test (BSLT) using Artemia salina. In addition, the preferred restoration materials are those that can prevent bacterial growth and surface colonization. To prove the antibacterial effect of GIC core build-up against Streptococcus mutans bacteria, it is necessary to conduct an antibacterial test. **Research Objective:** to determine the effect of Glass Ionomer Type VI powder composition of diatomaceous nanosilica as well as chitosan and hydroxyapatite from crab shells on the toxicity and antibacterial effect of core build-up. **Research Methods:** The research used is Experimental Laboratories research. **Results:** The formation of inhibition zones around the wells in each group of modified GIC formulations and the death of Artemia salina Leach at each toxicity test concentration. **Conclusion:** From the results of the modified GIC antibacterial test of the F1-F8 group, it is known that the highest inhibition is in the F8 group with an average inhibition zone of 13.77 mm and is classified as strong in inhibiting Streptococcus mutans bacteria. The results of toxicity testing using the BLST method on GIC group F8 against Artemia salina Leach are known to be non-toxic and obtained an LC50 value of 2219.051 ppm.

Keywords: diatom nanosilica, hydroxyapatite, chitosan, Anti-bacterial, toxicity, Streptococcus mutans, Artemia salina Leach, Glass Ionomer Cement

ABSTRAK

ANDI AURA BUTSAINAH P. ***Pengaruh Komposisi Powder Glass Ionomer Type VI dari Nanosilika Diatom Serta Kitosan dan Hidroksiapatit dari Cangkang Kepiting Terhadap Toksisitas dan Antibakteri Pada Core Build-Up*** *(Dibimbing oleh Prof. Moh. Dharmautama, drg., Ph.D., Sp.Pros., Subsp.PKIKG (K))*

Latar Belakang: *Core build-up adalah restorasi yang ditempatkan pada gigi yang rusak parah. Salah satu upaya dalam meningkatkan sifat mekanis dari GIC core build-up yaitu dengan mengganti kandungan silika menggunakan nanosilika alami dari diatom dan bahan tambahan berupa hidroksiapatit serta kitosan yang dapat diperoleh dari cangkang kepiting bakau (Scylla serrata). Penilaian bahan restorasi core build-up juga harus mencakup biokompatibilitas dan antibakterinya. Pengukurannya meliputi sitotoksisitas, salah satu metode awal untuk uji toksisitas adalah Brine Shrimp Lethality Test (BSLT) menggunakan Artemia salina. Selain itu, bahan restorasi yang lebih disukai adalah bahan yang dapat mencegah pertumbuhan bakteri dan kolonisasi permukaan. Untuk membuktikan adanya efek antibakteri pada GIC core buil-up terhadap bakteri Streptococcus mutans, maka perlu dilakukan uji antibakteri.* ***Tujuan Penelitian:*** *untuk mengetahui pengaruh komposisi powder Glass Ionomer Type VI dari nanosilika diatom serta kitosan dan hidroksiapatit dari cangkang kepiting terhadap toksisitas dan antibakteri pada core build-up.* ***Metode Penelitian:*** *Penelitian yang digunakan yaitu penelitian Experimental Laboratories.* ***Hasil Penelitian:*** *Terbentuknya zona hambat disekitar sumur pada masing-masing kelompok formulasi GIC modifikasi dan terjadi kematian Artemia salina Leach pada tiap konsentrasi uji toksisitas.* ***Kesimpulan:*** *Dari hasil uji antibakteri GIC modifikasi kelompok F1-F8 diketahui yang memiliki penghambatan tertinggi ada pada kelompok F8 dengan rata-rata zona hambat sebesar 13,77 mm dan tergolong kuat dalam menghambat bakteri Streptococcus mutans. Hasil pengujian toksisitas menggunakan metode BLST pada GIC kelompok F8 terhadap Artemia salina Leach diketahui tidak toksik dan diperoleh nilai* LC_{50} *sebesar 2219,051 ppm.*

Kata Kunci: *nanosilika diatom, hidroksiapatit, kitosan, Anti bakteri, toksisitas, Streptococcus mutans, Artemia salina Leach, Glass Ionomer Cement.*

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CHAPTER I
INTRODUCTION

1.1 Background

Caries or cavities are a disease of the tooth structure characterized by damage to enamel and dentin caused by the metabolic activity of bacteria in plaque, resulting in demineralization through interaction of microorganism products and tooth enamel (Lobobun *et al*., 2023). Dental caries is commonly found in communities in Indonesia with a high prevalence of severity. Based on data from the Basic Health Research (Riskesdas) in 2018, it states that the largest proportion of dental problems in Indonesia is cavities (45.3%). Left untreated caries then progresses to pulp disease which will then become periapical disease (Rumate *et al*., 2023). One of the treatments for pulp and periapical disease is endodontic treatment.

Endodontic treatment is one of the dental conservation treatments that aims to treat teeth that are damaged and pulp necrosis so that the teeth continue to function properly. Making dental restorations after endodontic treatment is done to restore the aesthetic function of the teeth and determines the success of endodontic treatment (Kalalo *et al*., 2022). Failure in endodontic treatment is closely related to inadequate restorations. A good post-endodontic restoration should provide an adequate coronal seal and protection for the remaining tooth structure, and restore tooth shape and occlusal function as well as aesthetics. Full crowns are the restoration of choice for teeth that have experienced extensive crown loss due to endodontic procedures. Restorations with pegs and core build-up are made mainly on teeth that have lost a lot of crown structure, requiring additional retention (Faizarani and Prisinda, 2021).

Core build-ups are restorations placed on severely damaged teeth to restore structural balance, functional efficiency and aesthetic harmony of the teeth including oro-facial structures (Singh *et al*., 2019). Core build-up provides retention and resistance to the crown and acts as a transitional restoration before tooth preparation (Sharma *et al*., 2022). The ideal core build-up material should have similar physical properties to the tooth structure as restored teeth tend to transfer stress differently than intact teeth and flexural strength to prevent loosening during function. To achieve these criteria, several factors must be considered such as: protecting the remaining tooth from fracture, having sufficient compressive strength to withstand intra-oral forces, sufficient flexural strength to prevent removal of the core during function, biocompatibility with surrounding tissues, ease of manipulation, dimensional stability and ability to bond with tooth structure, pins and posts. Various dental materials such as amalgam, composite and Glass Ionomer Cement (GIC) have been used for core build-up procedures (Singh *et al*., 2019).

Glass Ionomer Cement (GIC) is an alternative restoration material because it is anticariogenic/antibacterial which is able to release fluoride thus protecting teeth

from caries, binds well to tooth structure, has the same thermal coefficient as teeth, has high compatibility, and is easy to manipulate. However, GICs are brittle and have poor mechanical properties, making them less suitable for restorations in high-stress areas (Utama *et al*., 2022). GIC consists of two main components, namely Ionomer Glass Powder and Poly Methyl Vinyl Ether-Malaic Anhydride (PVME-MA) liquid. The powders used are silica (SiO_2), alumina (Al_2O_3), calcium fluoride (CaF_2), and aluminum phosphate ($AlPO_4$). Silica in GIC acts as the main framework for oxide networks in developing bonds between glass and networks by making calcium side deposits (Rudyardjo *et al*., 2020). One of the efforts in improving the mechanical properties of GIC is to replace the silica content using natural nanosilica from diatoms (Trilaksana and Murniati, 2020; Tambaru *et al*., 2023).

The incorporation of diatom (*Thalassiosira sp*.) nanosilica in GIC powder causes a deeper particle distribution because it can occupy the empty space between GIC particles so that the mechanical value is higher (Trilaksana and Murniati, 2020). The characteristic of diatoms is shown by the presence of certain sculptures on their cell walls consisting of silica which has high resistance to environmental stress (Umiatun *et al*., 2017). Previous research by (Haribowo *et al*., 2021) conducted on Kotok Besar Island, Kepulauan Seribu showed that the abundance of diatoms in the dry and rainy seasons reached 89%, while in the rainy season it reached 100%. Diatom abundance is an indication of fertility in a water environment (Gurning *et al*., 2020). However, an increase in the abundance of diatom species Chaetoceros spp. and Nitzchia spp. can endanger marine ecosystems because they can become blooming (uncontrolled population explosion) which has the potential to cause health problems in fish and also aquatic ecosystems (Haribowo *et al*., 2021). Another study by Tambaru *et al*. (2023), conducted in the waters of Tompotana Takalar, said that the highest abundance was dominated by diatoms, which amounted to 85%. Therefore, the abundance of this diatom population can be utilized as nanosilica in materials in the manufacture of GIC.

The replacement of silica with nanosilica is not enough, so additional materials in the form of hydroxyapatite and chitosan are needed to improve the mechanical properties of GIC as a core build-up restoration. Hydroxyapatite and chitosan can be obtained from crab shells, one of which is mangrove crab (*Scylla serrata*) shell. Mangrove crab shells contain 15.60% - 23.90% protein, 53.70% - 78.40% calcium carbonate as a calcium precursor in hydroxyapatite synthesis and 18.70% - 32.20% chitin which can be converted into chitosan (Malau and Azzahra, 2020).

According to Fajri, *et al*. (2019), crabs that have been consumed produce as much as 80% waste which can cause various environmental problems such as unpleasant odors that have the potential to be a source of disease carriers, disrupt human activities and damage the environment aesthetically. The results of mangrove crab cultivation in Bone district in 2022 were 1,223 tons and the potential waste was around 978.4 tons (Anton *et al*., 2022). Therefore, the accumulation of mangrove crab shell waste can be utilized as a precursor in the manufacture of hydroxyapatite powder and chitosan.

The addition of hydroxyapatite can increase the compressive strength and density of GIC. The content of calcium ions in hydroxyapatite will be involved in acid-base reactions with the GIC liquid so that more salt bridges and cross-linking are formed (Hidayati *et al*., 2023). Meanwhile, the addition of chitosan can increase the release of fluorine, thus providing a good antibacterial effect on GIC and preventing secondary caries (Lobobun *et al*., 2023). Chitosan has broad-spectrum activity and a high killing rate against Gram-positive and Gram-negative bacteria. One of the intrinsic factors of chitosan as an anti-bacterial is the polycationic structure of chitosan that can bind and destabilize the bacterial cell wall. In addition, the chelating ability of chitosan can also cause damage to bacterial cells (Wahjunigrum *et al*., 2022).

The assessment of core build-up restoration materials is not only their chemical, physical and mechanical characteristics but should also include their biocompatibility and antibacteriality, understood as the ability of a material to function in a living organism and induce an appropriate tissue response. Measurements include cytotoxicity, which is the effect of the material being studied on cell survival. Cytotoxicity is a complex process, as there are many mechanisms that cause functional and structural changes in cells and tissues (Kolada *et al*., 2017). Meyer, et al. (1982), suggested that one of the early methods for toxicity testing was the *Brine Shrimp Lethality Test* (BSLT) using marine shrimp larvae of the *Artemia salina* type. *Artemia salina* is the simplest and smallest organism from marine biota and has a fairly high sensitivity to toxic compounds (Puspitasari *et al*., 2018). Cytotoxicity test with BLST method can be done quickly, cheaply and easily. The results of the BLST test are in the form of an LC_{50} (Lethal Concentration 50) value which shows the concentration of exposure to toxic substances to 50% of the dead test biota (Andini *et al*., 2021). If it is not toxic, it can be studied again to determine other toxicity properties using other experimental animals that are larger than *Artemia salina Leach* larvae such as mice and rats in vivo (Putri *et al*., 2021).

In addition, the preferred restoration materials are those that can prevent bacterial growth and surface colonization. Because acid-producing bacteria can cause tooth demineralization and ultimately lead to the formation of secondary caries, which occurs at the junction of the restoration and the tooth surface. The microflora of the secondary caries biofilm mostly includes *Prevotella, Veillonella, Lactobacilli, Streptococcus mutans, Neisseriae*, and *Actinomyces*, followed by *Peptostreptococcus, Fusobacterium, Porphyromonas gingivalis* and *occasionally* detected *Capnocytophaga* (Si-su mo *et al*., 2010).

To prove the antibacterial effect of GIC core build-up on *Streptococcus mutans*, it is necessary to conduct an antibacterial test. Methods for antibacterial tests are divided into two, namely diffusion methods and dilution methods, usually for diffusion methods using solid medium. The diffusion method is divided into disk, well and trench methods. The method used in this study is the pitting diffusion method, the pitting diffusion method is by making holes in a solid agar medium that has been inoculated with bacteria. The number of holes is adjusted to the research objectives, then the hole is inserted with the tested extract. After incubation,

bacterial growth is observed to see whether or not there is an area of inhibition around the hole (Retnaningsih *et al*., 2019).

According to Khare, *et al*. (2019), on average 50% of dental restorations fail within 10 years, mainly due to secondary caries. Therefore, core build-up restoration materials must have good antibacterial properties and toxicity.

Based on this theory, the authors are interested in analyzing the Effect of Glass Ionomer Type VI Powder Composition From Diatom Nanosilica, Chitosan and Hydroxyapatite From Crabs Sheels on the Toxicity and Antibacterial Properties of Core Build-Up.

1.2 Problem Formulation

Based on the background that has been described, the problem is formulated, namely whether there is an Effect of Glass Ionomer Type VI Powder Composition From Diatom Nanosilica, Chitosan and Hydroxyapatite From Crabs Sheels on the Toxicity and Antibacterial Properties of Core Build-Up?.

1.3 Research Objectives

1.3.1 General Objectives

The objective to be achieved in this study is to determine the Effect of Glass Ionomer Type VI Powder Composition From Diatom Nanosilica, Chitosan and Hydroxyapatite From Crabs Sheels on the Toxicity and Antibacterial Properties of Core Build-Up.

1.3.2 Special Objectives

1. To determine the LC_{50} value of GIC core build-up toxicity testing on shrimp larvae (*Artemia Salina Leach*).
2. To determine the antibacterial inhibition test of GIC core build-up against *Streptococcus Mutans* bacteria.

1.4 Research Bevefits

The benefits of this writing are:

1.4.1 Theoretical Benefits

1. Developing natural science in the field of modern dentistry regarding Glass Ionomer Cement Core Build-Up.
2. Developing the theory of Glass Ionomer Cement Core Build-Up for the benefit of prosthodontics.

1.4.2 Practical Benefits

Adding knowledge in reviewing several journals regarding the Effect of Glass Ionomer Type VI Powder Composition From Diatom Nanosilica, Chitosan and Hydroxyapatite From Crabs Sheels on the Toxicity and Antibacterial Properties of Core Build-Up.

1.4.3 Benefits to Society

To provide the latest information on the Effect of Glass Ionomer Type VI Powder Composition From Diatom Nanosilica, Chitosan and Hydroxyapatite From Crabs Sheels on the Toxicity and Antibacterial Properties of Core Build-Up.

CHAPTER II
RESEARCH METHOD

2.1 Research type and design

This research is Experimental Laboratories research, with a post test only control group design.

2.2 Time and Place of Research

2.2.1 Research Time

This research was conducted in August - October 2024.

2.2.2 Research Place

This research was conducted at the Physics Material and Energy Laboratory, Faculty of Natural Sciences, Hasanuddin University, the anti-bacterial test was conducted at the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University and the toxicity test was conducted at the Marine Microbiology Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University.

2.3 Research Samples

The samples of this study were GIC formulations with the addition of diatomaceous nanosilica and hydroxyapatite and chitosan from mangrove crab shells. The samples in this study were divided into 8 treatment groups, namely:

Table 2. 1 Formulasi *Glass Ionomer Cement*

Bahan	Formulasi (%)							
	F1	F2	F3	F4	F5	F6	F7	F8
SiO_2 Diatom	41%							
Al_2O_3	27%	91%	99%	98%	97%	90%	89%	88%
CaF_2	21%							
$AlPO_4$	11%							
Hydroxyapatite	-	9%	-	-	-	9%	9%	9%
Chitosan	-	-	1%	2%	3%	1%	2%	3%

The materials in GIC in general are SiO_2, Al_2O_3, CaF_2, $AlPO_4$, NaF, and AlF_3. However, in the formation of GIC, the main ingredients used are SiO_2, Al_2O_3, CaF_2,

and $AlPO_4$ because NaF and AlF_3 compounds only have a small percentage in the preparation of GIC and the function of these compounds is already contained in the main GIC constituent compounds. The composition of GIC powder varies depending on the classification of GIC based on its application or function (Fatmawati *et al*., 2023).

In this study, the number of samples was determined using the Federer formula, as follows:

$$(t - 1)(n - 1) \geq 15$$

$$(8 - 1)(n - 1) \geq 15$$

$$7(n - 1) \geq 15$$

$$7n - 7 \geq 15$$

$$7n \geq 15+7$$

$$7n \geq 22$$

$$n \geq 3{,}14$$

$$n \geq 3$$

So in this study a sample of 3 samples was used for each group. In this study, 8 treatment groups were used so that the number of samples used was 24 samples.

2.4 Research Variables

1. Free Variable: Variation in the concentration of diatomaceous nanosilica as well as hydroxyapatite and crab shell chitosan.
2. Consequence Variables: Anti-bacterial properties and toxicity of GIC.
3. Control Variables: Sample storage and observation of test biota.
4. Confounding Variables: Bacterial contamination.

2.5 Operational Definition

1. Glass Ionomer Cement (GIC) Core Build-Up consists of two main components namely powder and liquid. The powder in GIC is calcium fluoroaluminosilicate glass consisting of Silica (SiO_2), Alumina (Al_2O_3), Aluminum Fluoride (AlF_3), Calcium Fluoride (CaF_2), Sodium Fluoride (NaF), and Aluminum Phosphate ($AlPO_4$) which dissolve in acidic liquids. GIC liquid is a liquid of polyacrylic acid with a concentration of 40 - 50%.
2. Nanosilica taken from diatoms (*thalassiosira sp*) using the sol-gel method and obtained nanosilica powder which will be modified with GIC.

3. Chitosan from mangrove crab shell (*Scylla serrata*) obtained through alkaline deacetylation process of chitin and obtained chitosan powder.
4. Hydroxyapatite from mud crab shell (*Scylla serrata*) was obtained through calcination process, then sintering process was carried out and hydroxyapatite powder was obtained.
5. Anti-bacterial properties to prevent the growth and surface colonization of Streptococcus Mutans bacteria that can cause secondary caries formation by measuring the diameter of the inhibition zone.
6. Toxicity properties indicate the concentration of exposure to toxic substances through the *Brine Shrimp Lethality Test* (BSLT) test method, using *Artemia salina Leach* as the test biota.

2.6 Tools and materials

2.6.1 Tools

2.6.1.1 Extraction and Preparation of GIC Core Build-Up Tools

1. Beakers (250 ml, 500ml, 1000ml)
2. Measuring cup
3. Measuring flask
4. Stirring rod
5. Large and small drop pipettes
6. Magnetic Stiter + magnetic bar
7. Furnace machine
8. Oven
9. Digital scales
10. Universal Testing Machine
11. Mini compressor
12. Shaker

2.6.1.2 Anti-bacterial Test Tools

1. Incubator
2. Petri dish
3. Sterile cotton applicator
4. Sterile well mold
5. Plastic instrument
6. Sterile ose needle
7. Cement spatula
8. Glass mixing slab
9. Water bath
10. Hallway

2.6.1.3 Toxicity Test Tools

1. Digital scales
2. Artemia salina hatching tank
3. Aerator
4. Lamp
5. Drip pipette
6. Spatula
7. Beaker cups of 1000 ml and 50 ml volume
8. Pipettes of 0.5 ml, 1 ml and 5 ml volume
9. Vial bottle

2.6.2 Materials

2.6.2.1 Materials for Extraction and Preparation of GIC Core Build-Up specimens

1. Al_2O_3
2. CaF_2
3. $AlPO_4$
4. Mangrove crab shell
5. Diatom Thalassiosira Sp
6. Whatman GF/C paper
7. 1 M NaOH solution
8. HCl 1 M solution
9. Aquades
10. Paper pH meter
11. Aluminum foil
12. Plastic wrap
13. Aquadest
14. NaOH 50g

2.6.2.2 Anti-bacterial Test Materials

1. GIC F1, F2, F3, F4, F5, F6, F7 and F8
2. Liquid GIC
3. Müeller Hinton Agar (MHA) (Oxoid)
4. Nutrient Agar (NA) (Himedia)
5. Nutrient Broth (Merck)
6. Aquadest (One Lab Water One)
7. *Streptococcus mutans* bacterial isolate obtained from the Lab. Microbiology Faculty of Medicine UNHAS
8. NaCl 0.9%

2.6.2.3 Toxicity Test Materials

1. Artemia salina eggs
2. Water + salt (Seawater)
3. Aquadest
4. GIC F8
5. DMSO 1% (Dimethyl sulfoxide)

2.7 Working procedure

2.7.1 Preparation of nanosilica from diatoms

Diatom powder preparation. A total of 1500 g of diatoms were cleaned and then dried in the sun. Next, it was soaked with 1 M HCl solution for 1 hour, then rinsed using distilled water and dried again in the sun to dry. Then, the bamboo leaves were fired in a furnace at 600^{o}C for 7 hours.
Silica extraction. A total of 20 g of diatoms was added to 500 ml of 1 M NaOH stirred at 60-80^{o}C for 3 hours, then allowed to stand after which it was filtered to produce a sodium silicate solution filtrate. Then, as much as 100 ml of sodium silicate solution measured the initial pH then added HCl 1 M drop by drop while stirring until pH 7 was obtained, and allowed to stand overnight so that a gel (hydrogel) was obtained. The gel obtained was washed using distilled water until the washing water was neutral. After that, the gel was heated in the oven at 80^{o}C until dry, resulting in dry silica gel (serogel). Then the silica serogel was crushed and sieved.
Preparation of nano silica. Silica was pulverized using ultrasonic miling method (20KHz-10MHz) and diatomaceous nanosilica powder was obtained (Taqwim, 2023).

2.7.2 Extraction of Chitosan from crab shells

Crab Shell Powder Preparation. Crab shells were washed with water and brushed thoroughly, dried in the sun until dry. The dried crab shells were then pulverized using a mortar and pestle, blender and sieved using a 100 mesh sieve. The resulting powder is stored in a closed place.
Chitin Extraction. Deproteination using 3N NaOH in a ratio of (1:10 gr/mL) at 80°C for 60 minutes. Then neutralized with distilled water until the pH showed 7 and oven at 80°C for 2 hours. Then, demineralized using 1 N HCl solution, with different ratios of 1:10 and 1:15 for 60 minutes at room temperature. Then filtered and neutralized to neutral pH. The sample was then oven at 80°C for 1 hour and chitin powder was obtained.
Chitosan extraction. Through the deacetylation stage, namely by soaking chitin using 60% NaOH solvent in a ratio of 1:10 at 140°C for 60 minutes. The sample is then filtered to be neutralized again using distilled water until pH 7. The sample is

dried using an oven at 80°C for 1 hour and chitosan powder is obtained (Luthfiyana *et al*, 2022).

2.7.3 Hydroxyapatite Synthesis from crab shells

Crab Shell Powder Preparation. Crab shell powder was sieved using a 200 mesh sieve. The resulting powder was stored in a closed place.

Crab Shell Calcination. Crab shell powder was weighed as much as 8 g and calcined in a furnace at 850, 900, 950 and 1000°C for 5 hours 1000°C for 5 hours. The calcined powder was transferred to a desiccator and weighed until its mass was constant.

Hydroxyapati Synthesis. The calcined powder was then precipitated by mixing various concentrations of KH2PO4 (0.25, 0.5, 0.75, and 1M) into the calcined 0.3 M CaO solution. The CaO solution mixture was then stirred using a magnetic stirrer at 37°C for 30 minutes. The precipitated solution was then kept at room temperature for 24 hours to obtain a white hydroxyapatite precipitate. The precipitate is then dried, then the sintering process is carried out to form hydroxyapatite at 800 ° C for 4 hours (Romadhona *et al.,* 2023).

2.7.4 Specimen Preparation

GIC powder was prepared by mixing diatomaceous nanosilica powder, alumina, calcium flouride, aluminum phosphate, hydroxyapatite and crab shell chitosan. The weight percentage of GIC according to groups F1, F2, F3, F4, F5, F6, F7 and F8 based on (**Table 2.1)**, was weighed using digital scales. Mixed in an erlenmeyer tube using a shaker for 15 seconds. So that the nanosilica modified GIC powder, hydroxyapatite and chitosan are obtained (Taqwim, 2023).

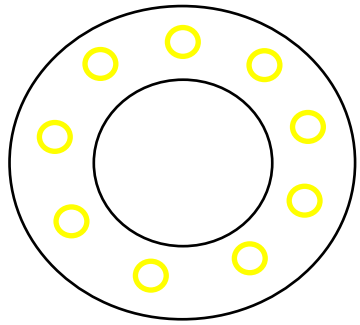
2.7.5 GIC Core Build-Up anti-bacterial test

Bacterial Rejuvenation. *Streptococcus mutans* bacteria are taken using a sterile ose needle, then implanted on a slanted Nutrient Agar (NA) medium by scratching. Bacteria that have been scratched on NA media are incubated at 37°C for 1x24 hours (Hasanuddin and salnus, 2020).

Preparation of Suspension. One ose of bacterial culture that has been rejuvenated on NA media is suspended into a tube containing 5 ml of Nutrient Broth (NB) media and incubated for 1x24 hours at 37^{o}C. Then, the bacterial suspension was diluted using sterile 0.9% NaCl until the turbidity was equivalent to a standard solution of 0.5 (approximately 1.5 × 10 8 bacteria/ml) Mc. Farland I (Hasanuddin and salnus, 2020).

Well Molding. A total of nine 3 mm diameter micropipette tips were cut off and glued on top of a circular iron using glue. The finished mold was then sterilized**.**

Figure 2. 1 Illustration of a GIC Core Build-up well mold



Antibacterial Activity by Disk Diffusion Method. A total of 5.7 g of MHA medium was mixed with 150 ml of distilled water, then homogenized and dissolved using a water bath. Then, insert the well mold into the Petri dish and pour the MHA medium in the first layer. Then for the second layer, 200 µl of bacterial suspension was mixed into 20 ml of Mueller Hinton Agar (MHA) media, then poured into a Petri dish again and waited for the media to freeze. After freezing, the well mold was removed and 9 wells were obtained.

Manipulation of GIC core build-up. Modified GIC powder and polyacrylic acid liquid in the ratio of 1:1 were mixed manually using a cement spatula on a glass mixing slab. The mixing was done gradually bit by bit with a folding motion to obtain a homogeneous mixture. Once homogeneous, the sample was inserted into each well using a plastic instrument until it was filled almost completely.

Sample storage. The plates were incubated at 37°C in a bacteriological incubator for 24 hours. The clear area formed around the GIC was observed, then the diameter of the clear zone around the test material formed was measured using a caliper. The test was performed in three repetitions (Ferreira *et al*., 2011; Rudyardjo *et al*., 2020).

Statistical Analysis. The reading of the results of the bacterial inhibition test with the disk diffusion method is to observe the formation of the inhibition zone around the GIC. After determining the average inhibition zone, the inhibition ability is classified based on (**Table 2.2**). Then statistically analyzed by conducting a data normality test first followed by the One Way ANOVA (analysis of variance) test. Furthermore, the LSD (Least Significant Difference) test was conducted (Hasanuddin and salnus, 2020).

Table 2. 2 Classification of bacterial growth inhibition response according by Davis and Stout (1971)

Bright Zone Diameter	Growth Inhibition Response
≥ 20 mm	Very Strong

10 – 20 mm	Strong
5 – 10 mm	Medium
≤ 5 mm	Weak

Source: Minarni and Rosmalia, 2022

2.7.6 Toxicity test of GIC Core Build-Up

Hatching of Artemia salina eggs. Artemia salina eggs used in this study are orange with small size. A total of 2 g of Artemia salina eggs were put in a hatching tank that had been filled with water with salt content (sea water) so that in this study sea salt was used which was added to 1000 ml of water and had been supplied with oxygen using an aerator and lamp light. Egg hatching time is 48 hours and continues to be controlled. After hatching into larvae, Artemia salina can be directly used as test animals (Suprayogi *et al*., 2021).

BSLT test sample preparation. A total of 0.1 g of GIC F8 powder was dissolved in 0.5 ml 1% DMSO solvent and seawater was added until it reached 50 ml. Then, it was homogenized and the mother liquor was obtained. A total of 5 ml of the mother liquor was put into a vial bottle containing 5 ml of seawater and then homogenized and obtained a concentration of 1000 ppm. Furthermore, the parent solution of the solvent was diluted to obtain a concentration of 500 ppm, 250 ppm, 125 ppm, 62.5 ppm and 31.25 ppm in 5 ml of sample solution. In the control included 1% DMSO as much as 0.25 ml and then added seawater to reach 5 ml. After that, 10 Artemia Salina were each put into a vial bottle (6 samples and 1 control) using a drop pipette (Andini *et al*., 2021; Suprayogi *et al*., 2021). The table of test animal treatment groups can be seen in (**Table 2.3**).

Table 2. 3 Treatments group in the *Artemia Salina* test

No.	Sample	Group Treatment	Treatment
1.	GIC F8	Control	10 shrimp larvae without GIC extract
2.		Treatment 1	10 shrimp larvae with GIC extact at a concentration of 1000 ppm in the media
3.		Treatment 2	10 shrimp larvae with GIC extact at a concentration of 500 ppm in the media
4.		Treatment 3	10 shrimp larvae with GIC extact at a concentration of 250 in the media
5.		Treatment 4	10 shrimp larvae with GIC extact at a concentration of 125 ppm in the media
6.		Treatment 5	10 shrimp larvae with GIC extact at a concentration of 62,5 ppm in the media
7.		Treatment 6	10 shrimp larvae with GIC extact at a

concentration of 31,25 ppm in the media

BSLT test. All samples and controls were incubated for 24 hours. Observations were made by counting the number of surviving larvae and calculating the LC50 using excel to determine the probit value of Artemia salina. This test was replicated three times for each group (Suprayogi *et al*., 2021).

Analysis of results. The cytotoxicity effect can be done by calculating the % mortality of Artemia salina larvae death with the following formula:

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of dead larvae}} \times 100\%$$

The mortality rate (%) is obtained by comparing the number of dead larvae divided by the total larvae. The LC_{50} value is obtained from the determination of the probit value, which converts the value of the percentage of death with the probit table. The toxicity category of each sample can be determined based on (**Table 2.4**). Furthermore, plotting the data between the probit value and the log concentration will obtain the regression equation as follows.

$y = ax + b$

Description:
y = Probit value.
a = Regression concentration.
$x = \log^{10}$ test concentration.
b = Regression slope.

Table 2. 4 LC_{50} categories according to Meyer *et al*, 1982

Category	**LC_{50} (ppm)**
Very toxic	< 30
Toxic	30 - 1000
Not toxic	> 1000

Source: Andini *et al,* 2021.

2.8 Research flow