

DENTIN
JURNAL KEDOKTERAN GIGI
Vol VII. No 2. AGUSTUS 2023

**COMPARISON OF ANTIBACTERIAL CHITOSAN OF HARUAN FISH
 SCALES (*Channa striata*) WITH Chlorhexidine gluconate AGAINST
*Lactobacillus acidophilus***

Tomy Wira Wahyuda¹⁾, Deby Kania Tri Putri²⁾, Muhammad Yanuar Ichrom Nahzi³⁾

¹⁾Department of Oral Biology, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

²⁾Biomedical Department, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

³⁾Department of Dental Conservation, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

ABSTRACT

Background: *Lactobacillus acidophilus* plays an important role in the development of caries, especially advanced caries. Mouthwash such as chlorhexidine is the main choice to prevent the colonization of caries-causing bacteria, but in long-term use it causes side effects, so that new, more biocompatible materials are needed, namely chitosan of Haruan fish scales (*Channa striata*), which contains an amine group (NH₂).

Purpose: To analyze and compare the antibacterial effectiveness of chitosan haruan fish scales at concentrations of 2.5%, 5%, 7.5%, and 10% with chlorhexidine gluconate 0.2% on the growth of *Lactobacillus acidophilus* bacteria. **Method:** True Experimental Laboratory Research with a procedure consisting of making chitosan from Haruan fish scales through the processes of deproteinization, demineralization, and deacetylation followed by an antibacterial test with 5 treatment groups on the growth of *Lactobacillus acidophilus* bacteria using the diffusion method with inhibition zone parameters on the test material. **Result:** Haruan fish scale chitosan has the lowest antibacterial effectiveness, namely a concentration of 2.5% with an average inhibition zone value of 8.6 mm, and the highest is a 10% concentration with an inhibition zone average value of 21.3 mm, and a concentration of Haruan fish scale chitosan. 10% had antibacterial effectiveness comparable to 0.2% chlorhexidine gluconate against the growth of *Lactobacillus acidophilus* bacteria. **Conclusion:** Haruan fish scale chitosan concentrations of 2.5%, 5%, 7.5%, and 10% had antibacterial effectiveness against the growth of *Lactobacillus acidophilus* bacteria, and a concentration of 10% was comparable to 0.2% chlorhexidine gluconate in inhibiting the growth of *Lactobacillus acidophilus* bacteria.

Keywords : Caries, chitosan haruan fish scales, *Lactobacillus acidophilus*.

Correspondence: Tomy Wira Wahyuda, Program Studi Kedokteran Gigi Fakultas Kedokteran Gigi Universitas Lambung Mangkurat University, Jalan Veteran No. 12B, Banjarmasin, Kalimantan Selatan, E-mail: 181111210043@mhs.ulm.ac.id.

INTRODUCTION

Dental and oral health are very important because they affect the health of the body as a whole¹. Caries is a chronic infectious disease of the teeth that often affects the people of Indonesia. This is evidenced by the 2018 Riskesdas regarding the prevalence of caries in Indonesia, reaching 88.8% and in South Kalimantan by 46.90%.² Caries begins with the metabolic process of microorganisms forming biofilms attached to tooth surface receptors. Microorganisms contained in the biofilm will ferment carbohydrates and produce lactic acid. This will cause demineralization and degradation of the organic matrix of type I collagen fibers at tooth surface receptors, resulting in a decrease in tooth surface pH. Demineralization that occurs

continuously will lead to the formation of cavities in the teeth.³⁻⁵ Microorganisms that play a role in caries include *Streptococcus mutans*, *Actinomyces*, *Enterococci*, *Streptococcus aureus*, *Streptococcus sanguis*, and *Lactobacillus acidophilus*.⁶⁻⁸

Lactobacillus acidophilus plays an important role in the development of caries, especially advanced caries. This is evidenced by the increase in the colonization of *Lactobacillus acidophilus* bacteria, namely 3% in non-caries cases to 3–24% in carious cases and also 9.6% in plaque.^{6,9} These bacteria are also found in saliva, the dorsum of the tongue, mucous membranes, and the hard palate.¹⁰ *Lactobacillus acidophilus* has the ability to live in an acidic environment and metabolize sugars and food waste rapidly in the

form of organic acids such as lactic acid.⁶ Mouthwashes such as chlorhexidine are the main choice to prevent colonization of caries-causing bacteria.¹¹ Chlorhexidine as an antimicrobial agent is effective as a broad-spectrum synthetic disinfectant against gram-negative and gram-positive bacteria, fungal spores, and dermatophytes, including HBV and HIV viruses. Apart from its effectiveness, chlorhexidine has drawbacks, namely causing allergies if used continuously, continuous sensation, erosion of the mucous membranes of teeth, tongue, and dental restorations.^{6,12,13} Therefore, new materials are needed that are more biocompatible and have properties that do not cause negative effects on mucosal tissue and have antibacterial properties, such as haruan fish scale chitosan, which is proven to have antibacterial power against gram negative and positive bacteria.¹⁴⁻¹⁶ Chitosan can inhibit bacterial growth due to the amine group (NH₂), which contains a positive charge so that it can bind to negative compounds of bacterial cells such as protein-forming microbes, polysaccharides, and nucleic acids, thereby inhibiting their growth.^{16,17}

Based on the research of Widyaningrum et al (2019), haruan fish scale chitosan is effective in inhibiting the growth of *Staphylococcus aureus* bacteria with a minimum inhibitory concentration of 1.5% and a minimum killing concentration of 3.5%, with a concentration of 3.5% having an antibacterial effectiveness comparable to chlorhexidine gluconate 0.25% in inhibiting the growth of *Staphylococcus aureus* bacteria. Chitosan also has a strong bactericidal effect against gram-positive bacteria.¹⁵ Another study conducted by Hutami et al (2020) who examined the antibacterial activity of haruan fish scale chitosan against *Streptococcus sanguis* showed a concentration of 1.25% was the minimum inhibitory level and 2.5% the minimum killing rate.¹⁶ Research by Rachfa et al (2021) proved that chitosan of Haruan fish scales (*Channa striata*) at concentrations of 1.5%, 2.5%, 3.5%, and 4% had antibacterial effectiveness equivalent to chlorhexidine gluconate 0.2% in inhibiting the activity of the glucosyltransferase enzyme *Streptococcus mutans*.¹⁸

Based on the research evidence above, it was found that the effectiveness of haruan fish scales chitosan (*Channa striata*) against several dominant bacteria causing caries, but its effectiveness against *Lactobacillus acidophilus* bacteria that play a role in advanced caries is not yet known, so researchers conducted a study to determine the antibacterial effectiveness of haruan fish scales chitosan on the growth of *Lactobacillus acidophilus* bacteria with the parameter of the diameter of the inhibition zone on the test material.

RESEARCH METHODS

This research was started after obtaining the letter of ethics no. 019 KEPKG-FKGULM/EC/IV/2022 issued by the ethics committee of FKG ULM. Antibacterial test research was carried out in the Biomedical Laboratory of FKG ULM and the manufacture of chitosan from Haruan fish scales (*Channa striata*) was carried out at the Biochemistry Laboratory, Faculty of Medicine, Banjarbaru.

This research is purely experimental with a post-test only control group design. The number of replications in each group was 3, which was obtained based on the unpaired numerical comparative analysis formula. The samples used consisted of isolates of *Lactobacillus acidophilus* ATCC 4357, chitosan of Haruan fish scales with concentrations of 2.5%, 5%, 7.5%, and 10%, and chlorhexidine gluconate 0.2% as positive controls.

Research Tools and Materials

Research Materials

The ingredients for the manufacture of haruan fish scale chitosan consist of 1 kg of haruan fish scales, 1% lactic acid, 50% NaOH, 1% HCl, 4% NaOH, aquades.

Materials for bacterial culture and antibacterial effectiveness tests consisted of *Lactobacillus acidophilus* ATCC 4357 isolate, Trypton Yeast Cystein (TYC) media, Brain Heart Infusion Broth (BHI-B) media, Mueller-Hinton Agar (MHA) media, 70% alcohol, aqueous solution Mc Farland standard 0.5, sterile paper disk, and 0.2% chlorhexidine gluconate.

Research Tools

The tools for making haruan fish scale chitosan consist of an analytical balance, an Erlenmeyer flask, beaker glass, blender, an oven, an icebox, a centrifuge, and a universal pH.

The tools for the antibacterial effectiveness test consist of an anaerobic incubator, autoclave, paper disk, petri dish, dropper pipette, ose, measuring cup, rack, and test tube, micropipette, mask, handsoon, laminar air flow, markers, Bunsen lamp, matches, a millimeter scale calliper, and sterile cotton swab.

Haruan Fish Scale Collection

Haruan fish scales were obtained from haruan fish cracker producers in the Kuin Cerucuk area of South Kalimantan. The Haruan fish scales were then washed with running water until clean, and dried at 50°C for 24 hours using an oven. After drying, the Haruan fish scales were ground into powder using a blender.^{14,19}

Deproteination

The smoothed haruan fish scales were then washed and placed in a 1000 ml glass, then soaked in boiling sodium hydroxide for one hour with a ratio of powder and 1:4 w/v solution to dissolve sugar and protein. The sample was cooled at room temperature for 30 minutes and then filtered to remove the sample residue. Samples were neutralized by repeated washing using distilled water. The sample residue was filtered again and dried for 24 hours at a temperature of 50°C using an oven.^{14,19}

Demineralization

The results of the deproteination of haruan fish scale powder were put in 1% HCl solution with a ratio of powder to 1:4 w/v solution and soaked for 24 hours to remove mineral content (especially calcium carbonate). The sample residue was filtered and washed repeatedly until the pH was neutral. The sample was dried again for 24 hours using an oven at a temperature of 50°C.¹⁴

Chitin Test

To test the presence of chitin content, you can use the Van Wesslink color reaction. By reacting the sample with a 1% 12-KI solution, which will produce a brownish yellow color and will turn purplish red if 1 M H₂SO₄ is added. The color change indicates a positive reaction for the presence of chitin.¹⁴

Deacetylation

The deacetylation process used 50% NaOH with the ratio of powder to solution of 1:4 w/v. The sample was boiled for 2 hours at 100°C on an electric stove. After 2 hours, the sample was cooled at room temperature for 30 minutes. Furthermore, pH neutralization by repeated washing using distilled water. The sample was filtered and dried for 24 hours at 50°C using an oven. After 24 hours, 27 grams of chitosan were obtained in the form of a white powder, and 2.5 grams were taken to be made into several concentrations.¹⁴

The Making Of Chitosan Concentration

Chitosan was dissolved in 1% lactic acid to form solutions with concentrations of 2.5%, 5%, 7.5%, and 10% (w/v).¹⁴

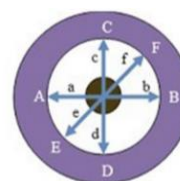
Cultivation Of Bacteria

Lactobacillus acidophilus ATCC 4357 was taken from the laboratory of Brawijaya University and then inoculated into 5 ml of liquid BHI. It was then incubated using an anaerobic incubator for 48 hours at 37°C. After 48 hours, the bacterial suspension was diluted in liquid BHI media until the turbidity was equivalent to the standard Mc

Farland 0.5 or the bacteria equivalent to 1.5x10⁸CFU.²⁰

Antibacterial Test

Applying *Lactobacillus acidophilus* ATCC 4357 with a sterile cotton swab on Mueller-Hinton agar. Soak the paper disk in each test material for 3 hours. Attach a paper disk containing the test material to Mueller-Hinton Agar media containing *Lactobacillus acidophilus* ATCC 4357 using tweezers and then incubate for 24 hours at 37°C using an anaerobic incubator. After 24 hours, the inhibition zone was measured using a calliper in mm.²⁰



Picture 1. Measurement of the Inhibitory Zone.¹⁸

Information :

- : Bacterial growth zone
- : Bacterial inhibition zone
- : test material
- : How to measure the zone of inhibition

The first measurement of the distance between A and B is subtracted from the distance between a and b. The second measurement is the distance between C and D subtracted from the distance between c and d. The third measurement of the distance between E and F is subtracted by the distance between e and f. The results of the three measurements are added up and then divided by 3 to get the average measurement or results from the inhibition zone.²⁰

Table 1: Daniel M. Inhibition Zone Diameter Category.²⁰

Diameter	Barrier zone classification
<5 mm	weak
6-10 mm	Medium
11-20 mm	Strong
>21 mm	Very strong

RESULTS

The diameter of the inhibition zone was obtained from the measurement of the clear area around the paper disk using a calliper in mm. The results of these measurements are then statistically calculated using SPSS, as shown in table 2.

Table 2. Table of the results of the mean and standard deviation of the comparison of the antibacterial effectiveness of haruan fish scale chitosan with 0.2% chlorhexidine gluconate on the growth of *Lactobacillus acidophilus* bacteria.

Treatment	N	Mean (mm)	Std. Deviation
KSIH 2,5%	3	8,6	±0,57
KSIH 5%	3	11,6	±0,57
KSIH 7,5%	3	14,5	±0,50
KSIH 10%	3	21,3	±0,57
CHX 0,2%	3	22,1	±0,28

Information :

KSIH 2,5% : Chitosan Haruan Fish Scales 2,5%

KSIH 5% : Chitosan Haruan Fish Scales 5%

KSIH 7,5% : Chitosan Haruan Fish Scales 7,5%

KSIH 10% : Chitosan Haruan Fish Scales 10%

CHX 0,2% : 0,2% chlorhexidine gluconate

The data was then tested for normality using the Shapiro Wilk test with the conditions $p > 0.05$ and the results obtained were four treatment groups that had a significance value of 0.05, namely the chitosan of Haruan fish scales with a concentration of 2.5%, 5%, 10% and chlorhexidine gluconate 0.2%, which means the data is not normally distributed. Based on this, the non-parametric Kruskal-Wallis test was carried out with a 95% confidence level.

Based on the non-parametric test of Kruskal-Wallis, the value of sig. = 0.009 ($p > 0.05$), which means that there is a significant difference in the mean value of the inhibition zone diameter of the chitosan concentration of Haruan fish scales. Based on this, the Mann Whitney test was carried out to determine the concentration of chitosan in Haruan fish scales, which gave a significant difference, which can be seen in table 3.

Table 3. Mann Whitney test table comparing the antibacterial effectiveness of haruan fish scale chitosan with 0.2% chlorhexidine gluconate on the growth of *Lactobacillus acidophilus* bacteria..

Treat ment	KSIH 2,5%	KSIH 5%	KSIH 7,5%	KSIH 10%	CHX 0,2%
KSIH 2,5%		0,043*	0,046*	0,043*	0,043*
KSIH 5%			0,046*	0,043*	0,043*
KSIH 7,5%				0,046*	0,046*
KSIH 10%					0,099

*They have significant differences ($p < 0,05$)

Based on the results of the above test, it can be seen that the antibacterial effectiveness of chitosan haruan fish scales at concentrations of 2.5%, 5%, and 7.5% has a significant difference when compared to 0.2% chlorhexidine gluconate, and the antibacterial effectiveness of chitosan haruan fish scales at a concentration of 10% does not have a significant difference when compared with 0.2% chlorhexidine gluconate. Based on this, it can be concluded that the antibacterial effectiveness of chitosan haruan fish scales at concentrations of 2.5%, 5%, and 7.5% is not comparable to chlorhexidine gluconate 0.2%, while chitosan haruan fish scales concentration of 10% is comparable to chlorhexidine gluconate 0.2% in inhibiting the growth of *Lactobacillus acidophilus* bacteria.

DISCUSSION

Based on the results of research, chitosan of Haruan fish scales at concentrations of 2.5%, 5%, 7.5%, and 10% was proven to have antibacterial activity against the growth of *Lactobacillus acidophilus* bacteria. Based on the results of the study, it can also be seen that the higher the concentration of chitosan of Haruan fish scales, the higher the antibacterial properties. This study is in line with the research of Hutami et al (2020) and Widyaningrum et al (2019), which stated that chitosan haruan scales (*Channa striata*) had more effective antibacterial properties as the concentration of chitosan used increased.^{15,16} The antibacterial properties possessed by chitosan depend on the positively charged amine group (NH_2) contained in the concentration of chitosan. The higher the concentration of chitosan, the higher the content of the positively charged amine group (NH_2). This causes differences in antibacterial power resulting from each concentration of chitosan of haruan fish scales. The amine group (NH_2) contained in the chitosan of haruan fish scales is directly proportional to the degree of deacetylation (DD). Based on the research of Putri et al (2020), the degree of deacetylation (DD) of haruan fish scale chitosan is 85.25% higher than the SNI of chitosan, which is 75%. The higher the degree of deacetylation, the more acetyl groups are removed and replaced by amine groups. The positive charge in the chitosan solution comes from hydrogen bonds with amine groups, so the more amine groups, the more positive charges that can increase the antimicrobial properties of chitosan.^{16,21,22} This statement is evidenced by the concentration of 5%, 7.5%, and 10% chitosan scales showing higher antibacterial power when compared to the haruan fish scale chitosan with a concentration of 2.5%. This is in line with the research of Hutami et al (2020), which states that the higher the amine

group, the more effective the antibacterial effectiveness for inhibiting bacterial growth.¹⁶

Based on the classification of the diameter of the inhibitory zone, Daniel M. The antibacterial power of haruan fish scale chitosan with a concentration of 2.5% was weak, 5% and 7.5% were strong, and 10% and 0.2% chlorhexidine gluconate were very strong. Based on this classification, it can be concluded that the concentration of 5% and 7.5% of Haruan fish scale chitosan has higher antibacterial power than the concentration of 2.5% but has lower antibacterial power when compared to the concentration of 10% and chlorhexidine gluconate, 0.2%. Chitosan concentration of 10% has higher antibacterial activity compared to concentrations of 2.5%, 5%, and 7.5% and has the same category of antibacterial activity as 0.2% chlorhexidine gluconate.²⁰

Concentrations of 2.5%, 5%, and 7.5% had lower antibacterial activity compared to 0.2% chlorhexidine gluconate. This lower antibacterial power is due to the positive charge contained in that concentration being smaller than the positive charge contained in chlorhexidine gluconate 0.2%.²³ A concentration of 10% showed antibacterial power comparable to 0.2% chlorhexidine gluconate in inhibiting the growth of *Lactobacillus acidophilus*. This equality indicates that 10% concentration of Haruan fish scale chitosan has antibacterial properties comparable to 0.2% chlorhexidine gluconate in inhibiting the growth of *Lactobacillus acidophilus* bacteria. The equivalence of the antibacterial power of a 10% concentration of chitosan is similar to the research of Rafcha et al (2021), which showed chitosan of Haruan fish scales at concentrations of 1.5%, 2.5%, 3.5%, and 4% equivalent to 0.2% chlorhexidine gluconate in inhibiting the activity of the glucosyltransferase enzyme *Streptococcus mutans*.

The positively charged amine group (NH₂) interacts with the OH-carboxylate group of the negatively charged bacterial cell membrane. This interaction forms electrostatics, which results in instability and increased permeability of the bacterial cell membrane, resulting in leakage of K⁺ ions, proteins, nucleic acids, and glucose. The leakage resulted in the death of bacterial cells, so that bacterial growth was inhibited. Chitosan also has the ability to bind to essential metals such as Ca²⁺ and Mg²⁺ because chitosan contains NH₂, hydroxyl, and H₂O amine groups. This bond results in teichoic acid in the bacterial cell wall not getting essential metals, which causes disruption of the molecular transfer process into cells and the production of adhesins. Molecular adhesion acts as a receptor to produce adhesion to oral epithelial cells. If the production of adhesins is inhibited, it will cause the attachment of epithelial cells to be

disrupted by bacteria so that bacteria cannot attach to the epithelial cells of the oral cavity. Chitosan can also inhibit the processes of DNA transcription, RNA and protein synthesis. Chitosan penetrates the bacterial nucleus by permeation, which then binds to bacterial cell DNA.^{16,24}

Based on this study, it was proven that haruan fish scale chitosan has the potential as a mouthwash formulation that is biocompatible and has antibacterial power comparable to 0.2% chlorhexidine gluconate in inhibiting the growth of *Lactobacillus acidophilus* bacteria that causes advanced caries. Based on the results of the study, it can be decided that the concentration of chitosan of Haruan fish scales is 2.5%, 5%, 7.5%, and 10% effectively inhibits the growth of *Lactobacillus acidophilus* bacteria and haruan fish scale chitosan concentration of 10% has an antibacterial effect comparable to 0.2% chlorhexidine gluconate in inhibiting the growth of *Lactobacillus acidophilus* bacteria.

REFERENCES

1. Puspitasari AM, Ratnawati DE, Widodo AW. Klasifikasi Penyakit Gigi Dan Mulut Menggunakan Metode Support Vector Machine. J-Ptiik. 2018; 2(2): 802–810.
2. Kementerian Kesehatan Badan Penelitian dan Pengembangan Kesehatan. Hasil Utama Risetdas. Kementerian Kesehatan Republik Indonesia. 2018. Hal: 133.
3. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, & Ismail, A. Dental caries. Nature Review Disease Primers. 2017;30:1-34.
4. Pyar H, Peh KK. Characterization And Identification Of *Lactobacillus Acidophilus* Using Biolog Rapid Identification System. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(1):189–193.
5. Zhao IS, Gao SS, Hiraishi N, Burrow MF, Duangthip D, Mei ML, Duangthip D, Mei ML, Lo EC, Chu CH. Mechanisms of silver diamine fluoride on arresting caries: a literature review. Int Dent J. 2018; 68:67–76.
6. Bilqis NM, Erlita I, Putri DKT. Daya Hambat Ekstrak Bawang Dayak (*Eleutherine palmifolia* (L.) Merr) Terhadap Pertumbuhan Bakteri *Lactobacillus acidophilus*. J Kedokt Gigi. 2018; 2(1):26–31.
7. Endriani R, Rafni E, Siregar FM, Setiawan RA, Rasyid F. Pola Bakteri Pada Karies Gigi Pasien Diabetes Melitus. J Kedokt Gigi Univ Padjadjaran. 2020; 32(1):34-40.
8. Ahirwar SS, Gupta MK, Snehi SK. Dental Caries and *Lactobacillus*: Role and Ecology in the Oral Cavity. International Journal of Pharmaceutical Sciences and Research. 2019;10(11):4818–4829.
9. Li Y, Argimon S, Schon CN, Saraithong P, Caufield PW. Characterizing Diversity of *Lactobacillus* Associated With Severe Early

- Childhood Caries: A Study Protocol. *Adv Microbiol.* 2015; 5(1): 9-20.
10. Ahirwar SS, Gupta MK, Snehi SK. Dental Caries and Lactobacillus: Role and Ecology in the Oral Cavity. *International Journal of Pharmaceutical Sciences and Research.* 2019;10(11):4818–4829.
 11. Misrulloh A, Rosiani E, Liawati I, Astutik AKF, Uji Daya Hambat Ekstrak Daun Jambu Biji Putih Dan Merah Terhadap Pertumbuhan Bakteri Karies Gigi (*Lactobacillus Acidophilus*). *F. Teknik Universitas Wahid Hasyim Semarang.* 2017;8:12-16.
 12. Archana V, Prabhuji MLV, Karthikeyan BV, Selvan A. Control Of Streptococcus Sanguinis Oral Biofilm By Novel Chlorhexidine-Chitosan Mouthwash: An In Vitro Study. *J Exp Integr Med.* 2013;3(2):165-169.
 13. Fiorillo L. Chlorhexidine gel use in the oral district: A systematic review. *Gels.* 2019;5(31):1–16.
 14. Rahma Dania AJ, Tri Putri DK, Taufiqurrahman I. Antibacterial Activity Of Chitosan From Haruan (*Channa Striata*) Fish Scales Against The Growth Of *Porphyromonas Gingivalis*. *Dentino J Kedokt Gigi.* 2020;5(1):53-56.
 15. Widyaningrum DRW, Kania D, Putri T, Taufiqurrahman I. Antibacterial Activities Of Chitosan In Haruan Fish Scales (*Channa Striata*) To The Growth Of *Staphylococcus Aureus*. *Jurnal Kedokteran Gigi.* 2019;4(2):162–167.
 16. Hutami WDW, Tri Putri DK, Taufiqurrahman I. The Antibacterial Activity Of Chitosan From Haruan (*Channa Striata*) Fish Scales On The Growth Of *Streptococcus Sanguinis*. *Dentino J Kedokt Gigi.* 2020;5(1):109-114.
 17. Suherman B, Latif M, Rosmala Dewi STR. Potensi Kitosan Kulit Udang Vannemei (*Litopenaeus Vannamei*) Sebagai Antibakteri Terhadap *Staphylococcus Epidermidis*, *Pseudomonas Aeruginosa*, *Propionibacterium Agnes*, Dan *Escherichia Coli* Dengan Metode Difusi Cakram Kertas. *Media Farm.* 2018;14(1):116-125.
 18. Rachfa MAF, Tri Putri DK, Dewi RK. Uji Kitosan Sisik Ikan Haruan (*Channa Striata*) Terhadap Aktivitas Enzim Glukosiltransferase *Streptococcus Mutans*. *Dentino J Kedokt Gigi.* 2021;5(2):87–91.
 19. Ifa L, Artiningsih A, Julniar, Suhaldin. Pembuatan Kitosan Dari Sisik Ikan Kakap Merah. *Journal Of Chemical Process Engineering.* 2018;3(1):47-49.
 20. Mozartha M, Silvia P, Sujatmiko B. Perbandingan Aktivitas Antibakteri Ekstrak Curcuma zedoaria dan Bahan Irigasi Natrium Hipoklorit 2.5% terhadap *Enterococcus faecalis*. *J Material Kedokteran Gigi.* 2019;8(1):22-29.
 21. Se Know Kim. Chitin and Chitosan Derivatives. New York. CRC Press Taylor and Francis Group. 2014
 22. Putri DKT. Synthesis And Characteristic Of Kitosan From Haruan (*Channa Striata*) Fish Scales. *Sys Rev Pharm.* 2020; 11(4): 15-20
 23. Balagopal S, Arjunkumar R. Chlorhexidine: The Gold Standard Antiplatelet Agent. *J. Pharm. Sci. & Res.* 2013; 5(12): 270 – 274
 24. Djunaidy VP. Pengaruh Kitosan Sisik Ikan Haruan (*Channa striata*) Terhadap Jumlah Koloni Interaksi *Streptococcus sanguis* Dan *Streptococcus mutans* Secara In Vitro. *Dentino.* 2020; 4(3): 100-110.