

Antibacterial Activity of Bacteriocin from *Pediococcus pentosaceus* Against *Propionibacterium acnes*: Molecular Docking, In Vitro, and 16S rRNA Genetic Identification

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Abstract

This study aimed to evaluate the antibacterial potential of bacteriocin produced by lactic acid bacteria against *Propionibacterium acnes* through an integrative approach involving molecular docking, in vitro assays, and genetic identification using 16S rRNA sequencing. The target protein PDB 7LBU, representing the cell wall of *P. acnes*, was docked with Small Bacteriocin obtained from PubChem. The best binding affinity was recorded at -7.5 kcal/mol, indicating a stable interaction, supported by cavity analysis and ligand-protein interaction mapping using PyMOL. The results of the in vitro study, as determined by antibacterial activity testing against *P. acnes*, showed an average inhibition zone of 20.6 mm. In addition, genetic identification through BLAST confirmed the isolate as *Pediococcus pentosaceus* strain DSM 20336 with a similarity of 99.64%. These findings indicate that the bacteriocin derived from *P. pentosaceus* has promising potential as a natural agent in the development of alternative therapies for acne.

Keywords: 16S rRNA; antibacterial; *Pediococcus pentosaceus*; *Propionibacterium acnes*; small bacteriocin.

INTRODUCTION

Indonesia has a wide variety of fermented foods, ranging from Sabang to Merauke. The seasonings used in fermented foods are often passed down through generations. There are several types of fermented foods, such as *Bekasam*, *Tempoyak*, *Dadih*, and others (Masdarini, 2011). *Dadih* is a fermented food originating from West Sumatra, made from buffalo milk. The process of making *dadih* begins with pouring buffalo milk into bamboo containers, then letting it sit for approximately 24–48 hours at room temperature. This process leads to fermentation, a process that involves beneficial bacteria, specifically lactic acid bacteria. These bacteria have many benefits, are very safe for consumption, non-pathogenic, and allow for a long product shelf life. Lactic acid bacteria are known as probiotic bacteria (Yuliana & Minda, 2022).

Lactic acid bacteria are capable of producing lactic acid as a result of the fermentation of sugars or carbohydrates. Lactic acid bacteria are characterized as Gram-positive, non-spore-forming, and catalase-negative in catalase testing. The addition or presence of these bacteria does not alter the quality of food. The classification of lactic acid bacteria includes *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*,

Lactococcus, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, and *Vagococcus*. These bacteria are found in processed food products derived from meat, fish, and milk, as well as in vegetables and fruits. They are also found in the organs of living organisms, such as the genital tract, intestinal tract, and respiratory tract of humans and animals (Pribadhi et al., 2021).

Acne vulgaris is a skin disease that causes inflammation in the pilosebaceous unit. This condition can become chronic, but it can also resolve on its own. *Cutibacterium acnes*, formerly known as *Propionibacterium acnes*, is the causative agent of *Acne vulgaris*. Adolescents commonly experience this disease; however, individuals of all ages can also be affected. *Acne vulgaris*, commonly referred to as acne, is caused by the accumulation of sebum, which eventually clogs the skin pores, triggering bacterial activity and inflammation in the skin (Sifatullah & Zulkarnain, 2021). *P. acnes* is characterized as Gram-positive and belongs to the *Corynebacteria* group. This bacterium is part of the normal flora of the human body. The infection mechanism of this bacterium involves breaking down triglycerides originating from the sebaceous glands into free fatty acids, which leads to an increase in *P. acnes* and subsequently triggers inflammation (Clatici et al., 2015). The results of a study conducted by Asbullah et

al. in 2018 showed that 109 out of 122 students (89.3%) at SMAN 1 Pelangiran, Indragiri Hilir Regency, suffered from acne. A total of 47 students aged 17 years were affected by this condition (Asbullah et al., 2021).

Acne treatment often involves the use of antibiotics, including tetracycline, erythromycin, doxycycline, and clindamycin. Other treatments that can be used include benzoyl peroxide, azelaic acid, and retinoids. These medications have several side effects during use. The use of antibiotics can lead to resistance and relapse. Research conducted in France revealed that 75.1% of patients with acne infected with *P. acnes* were resistant to erythromycin, and 9.5% were resistant to tetracycline. In other countries, 82% of acne patients were resistant to azithromycin. At Hasan Sadikin Hospital in Bandung, 12.9% of acne cases were resistant to tetracycline, 45.2% were resistant to erythromycin, and 61.3% were resistant to clindamycin (Pariury et al., 2021). Therefore, alternative treatments, such as the use of lactic acid bacteria, are needed to counteract *P. acnes*.

In silico studies are also necessary before conducting *in vitro* tests. The purpose of conducting *in silico* testing is to demonstrate the inhibitory potential of the Small Bacteriocin produced by lactic acid bacteria against the cell wall of *P. acnes*. Therefore, further research is needed to explore the potential of lactic acid bacteria in counteracting pathogenic bacteria that cause acne. The lactic acid bacteria used in this study, derived from the fermented food *cincalok*, namely *Weissella confusa*, have

been shown to inhibit the growth of acne-causing bacteria (Pribadhi et al., 2023)

MATERIALS AND METHODS

Materials and Equipment

This study used materials including lactic acid bacteria isolates, *Propionibacterium acnes*, MRSA medium, CaCO_3 , 70% alcohol, BHI and MHI media, blank disks, tetracycline antibiotic, immersion oil, and tissue. The equipment used in this study included test tubes, test tube racks, micropipettes and tips, measuring cylinders, Erlenmeyer flasks, inoculating loops, petri dishes, autoclave, microwave, Bunsen burner, incubator, analytical balance, microscope slides and cover slips, matches, wrapping materials, refrigerator, centrifuge, laptop, MegaX software, <http://clab.labshare.cn/cb-dock/>, software, PyMOL software, and microscope.

Procedures

Protein and Ligand Preparation

The protein structure used in this study was obtained from RCSB.org with PDB ID 7LBU, which represents the cell wall of *Propionibacterium acnes*. The Small Bacteriocin ligand was obtained in SDF format from PubChem. The protein and ligand were then prepared using tools provided by CB-Dock for appropriate format conversion and energy optimization.

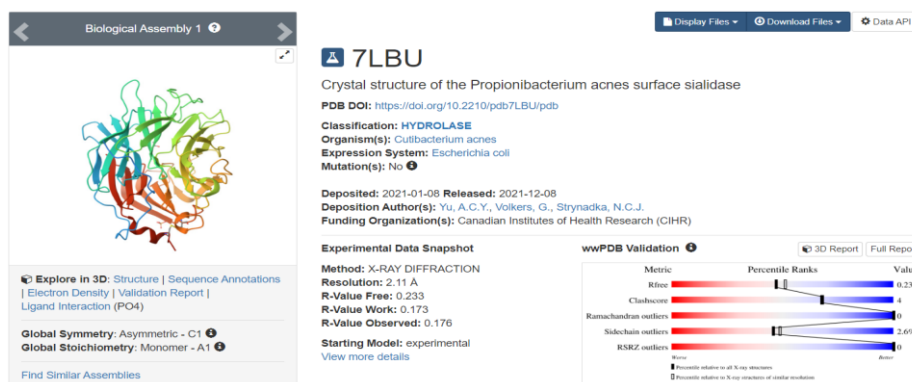


Figure 1. Structure of *P. acnes* surface sialidase.



Figure 2. Small Bacteriocin.

Docking Procedure

The docking process was carried out using a blind docking approach through the tool available at <http://clab.labshare.cn/cb-dock/>. The blind docking approach allows exploration of the entire protein surface to identify potential binding sites without the need to specify a predefined binding site.

- Grid box center: XYZ (-16, -7, 3)
- Grid box size: 27 x 27 x 27 Å
- Method: AutoDock Vina via CB-Dock

Docking Validation

Before performing docking of the Small Bacteriocin, the docking method was tested through redocking validation to ensure the accuracy of binding site prediction. This process confirmed that the docking protocol was capable of predicting the binding site with adequate accuracy.

MRSA Medium Preparation

This study used 6.5 g of MRSA medium and 0.75 g of CaCO₃ per 100 mL of distilled water.

Preparation

All equipment and media used in this study were sterilized using an autoclave at 121°C for 20 minutes to avoid contamination from undesired bacteria.

Isolation of Lactic Acid Bacteria

A total of 1 g of *dadih* sample was taken and transferred into a container containing 9 mL of distilled water (10⁻¹ dilution), followed by serial dilutions from 10⁻² to 10⁻⁸. The media containing bacterial colonies were incubated in an incubator at 37°C for 48 hours. The growing bacterial colonies were transferred using a round-loop inoculating needle and streaked onto fresh media. Colony purification was continuously carried out until a single bacterial colony was obtained. The medium used was MRSA supplemented with 0.75% calcium carbonate (CaCO₃).

Characterization of Lactic Acid Bacteria Isolates

The Lactic Acid Bacteria isolates that had become pure cultures were characterized macroscopically by observing the shape, color, edge, and elevation of the colonies, as well as by conducting a catalase test. Meanwhile, microscopic observation involved examining the cell morphology and performing Gram staining.

- Catalase Test
The catalase test was performed by dropping 3% H₂O₂ onto a glass slide, and the bacteria to be tested were placed onto the solution. If no bubbles formed on the isolate, it indicated a negative reaction.
- Gram Staining
Gram staining was also performed, starting with the placement of the bacterial sample on a glass slide, followed by fixation. Gram stain A was applied and left for 1 minute. Next, Gram stain B was added and left for 1 minute. Gram stain C was then applied for

30 seconds, followed by Gram stain D, which was left for 2 minutes. The bacterial specimen was observed under a microscope at 1000× magnification using immersion oil. Gram-positive bacteria appear purple, while Gram-negative bacteria appear red.

Preparation of Lactic Acid Bacteria Starter

The rejuvenated isolate was cultured in MRSA medium and then transferred to BHI medium for 24 hours at 37°C. The liquid culture was subsequently centrifuged at a speed of 10,000 rpm. The resulting supernatant was filtered using a 0.22 µm bacterial filter into a sterile tube (Sari et al., 2018).

Antimicrobial Test Using the Agar Diffusion Method

The test bacterium, *Propionibacterium acnes*, from BHI medium was spread onto MHA medium. Then, 20 microliters of the probiotic bacterial supernatant was dropped onto a blank paper disk. The tetracycline antibiotic paper disk (positive control), the probiotic bacterial supernatant paper disk, and the blank disk (negative control) were then placed systematically on the MHA medium containing the test microorganism and incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured to determine the antibacterial activity of the test substance (Kursia et al., 2021).

Identification of Lactic Acid Bacteria Using 16S rRNA

The lactic acid bacteria with the highest antibacterial activity against *Propionibacterium acnes* were sent to Genetika Science for molecular sequencing using 16S rRNA analysis.

Sequencing Result Interpretation

The nucleotide sequence of the lactic acid bacteria obtained from Genetika Science was first processed using BioEdit software and then analyzed using the Basic Local Alignment Search Tools (BLAST) on the NCBI website. This program identified regions of local similarity between the examined sequence and the database-stored sequence information to calculate the statistical significance of the match. The result from NCBI indicated the strain/species of the lactic acid bacteria. The identified strain was then used to construct a phylogenetic tree to determine its evolutionary relationship with other bacterial species.

Data analysis

Data dari penelitian ini akan dianalisis secara deskriptif dan disajikan dalam bentuk tabel dan gambar.

RESULTS AND DISCUSSION

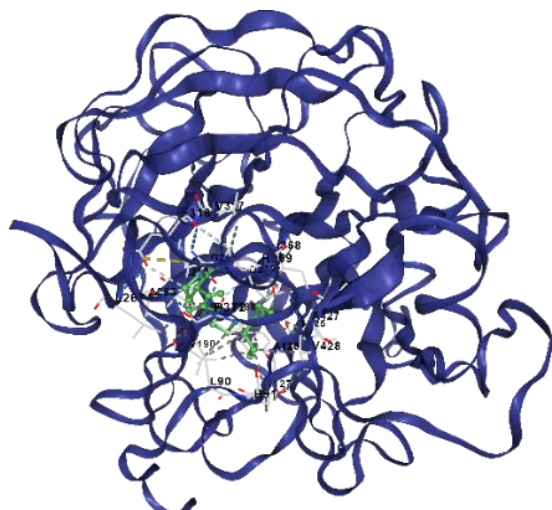
Binding Affinity

The docking process generated several binding poses ranked based on the Vina score. The results obtained are presented in Table 1:

Table 1. Binding Affinity.

Pose	Vina Score (kcal/mol)	Cavity Size (Å ³)	Cavity Center (XYZ)	Grid Box Size (XYZ)
Pose 1	-7.5	432	(-16,-7.3)	(27, 27, 27)
Pose 2	-6.6	290	(-21, 12,17)	(27, 27, 27)
Pose 3	-5.3	424	(-12, 15, 17)	(27, 27, 27)
Pose 4	-5.0	285	(-6, 16, 2)	(27, 27, 27)
Pose 5	-4.7	96	(-28, -19, 14)	(27, 27, 27)

Binding Site Analysis

**Figure 3.** Docking Complex of 7LBU with Small Bacteriocin -7.5.

Isolation of Lactic Acid Bacteria

**Figure 4.** Clear Zone Formed by Lactic Acid Bacteria Isolated from Dadih.

Characterization of Lactic Acid Bacteria Isolates

The results of the characterization of lactic acid bacteria isolates from *Dadih* are presented in Table 2.

Table 2. Characterization of Lactic Acid Bacteria Isolates Isolate.

Characterization	Lactic Acid Bacteria Isolates DIH-1
Colony shape	Circular
Colony color	Cream
Elevation	Convex
Margin	Smooth
Cell morphology	Coccus
Gram staining	(+)
Catalase test	(-)

Antimicrobial Activity

Table 3. Antimicrobial Activity of Bacteriocin from Lactic Acid Bacteria against *P. acnes*.

Treatment No	Inhibition Zone (mm)		
	Control (-)	<i>P. acnes</i>	Control (+)
1	0	21	30
2	0	21	30
3	0	20	30
Average	0	20.6	30

16S rRNA Sequencing Results

Table 4. Homologous Sequences from Alignment with 16S rRNA of Lactic Acid Bacteria Isolate.

Deskripsi	Max Score	Total Score	Query Cover	E. value	Per. Ident
<i>Pediococcus pentosaceus</i> strain DSM 20336	2560	2560	100%	0.0	99.64%
<i>Pediococcus stilesii</i> strain FAIR-E 180	2447	2447	100%	0.0	98.15%
<i>Pediococcus acidilactici</i> DSM 20284	2442	2422	100%	0.0	97.86%
<i>Pediococcus argentinicus</i> CRL 776	2383	2383	97%	0.0	98.17%
<i>Pediococcus clausenii</i> ATCC BAA 344	2368	2344	97%	0.0	97.82%
<i>Pediococcus clausenii</i> P06	2344	2344	99%	0.0	97.06%
<i>Pediococcus acidilactici</i> NGRI 0510Q	2333	2333	100%	0.0	96.74%
<i>Pediococcus parvulus</i> NBRC 100673	2265	2265	99%	0.0	95.93%

Discussion

In Table 1, a conformation with a Vina Score of -7.5 was identified within a relatively large cavity with a volume of $432 \text{ \AA}^3$, allowing the ligand to interact with various key residues surrounding the cavity. Further analysis of ligand–protein interactions using visualization software such as PyMOL revealed the presence of hydrogen bonds and hydrophobic interactions, which contributed to the binding stability. These results indicate that Small Bacteriocin has potential as an inhibitor of the *P. acnes* cell wall, which is relevant to the development of bacterium-based therapies produced by *Pediococcus*. These findings provide a foundation for further experiments exploring the inhibitory mechanism through *in vitro* studies (Yang, 2022).

During the isolation process of lactic acid bacteria, only one pure isolate was obtained from the fermented food *Dadih*. In the purification process using the MRSA + CaCO_3 medium, lactic acid bacteria on the medium formed a clear zone. According to Putri et al. (2018), the clear zone formed on MRSA + CaCO_3 medium is the result of acid secretion into the medium.

The lactic acid bacteria obtained showed colony characteristics that included a circular shape, whitish-yellow color, convex elevation, and smooth margins. Gram staining revealed a round cell morphology and a Gram-positive (purple) reaction. Lactic acid bacteria typically have round or rod-shaped cell morphology, and under Gram staining, they appear purple (Pribadhi et al., 2021). Bacteria can be distinguished based on the composition of their cell walls, which can be identified through the Gram staining method. Gram-positive bacteria, which appear purple, contain approximately 90% peptidoglycan in their cell walls. The purple color results from the formation of a crystal violet–iodine complex. Bakteri Gram negatif atau berwarna merah memiliki dinding sel dengan kandungan peptidoglikan 5–20%. The red color in bacteria results from the addition of 95% alcohol, which removes the purple stain and leaves the cells colorless. The red color appears following the addition of safranin (Madigan et al., 2011). The catalase test showed a negative result, indicating that the bacteria were unable to break down hydrogen peroxide into water and oxygen. The catalase test is used to determine the presence of the catalase enzyme in bacteria (Putri et al., 2014).

The probiotic bacteria isolated from *Dadih* that were used to inhibit acne-causing bacteria, most commonly *P. acnes*, had not yet been identified at the species level. Based on research conducted by Pribadhi, the bacterium *W. confusa* was considered capable of inhibiting *P. acnes* (Pribadhi et al., 2023).

The results of the antimicrobial test using the agar diffusion method showed that this probiotic bacterium possesses antimicrobial compounds, as evidenced by its ability to inhibit acne-causing pathogenic bacteria. The presence of a clear zone around the paper disk confirmed this. The antimicrobial test was repeated three times for each *P. acnes* sample. The positive control in this test used the antibiotic tetracycline, while the negative control used a blank paper disk.

The average result of the negative control for both acne-causing bacteria was 0 mm, due to the absence of antimicrobial compounds. The average result of the positive control against *P. acnes* was 30 mm. The average result of the probiotic antimicrobial test against *P. acnes* was 20.6 mm. A study conducted by Kursia et al. (2021) reported that lactic acid bacteria can inhibit the growth of *P. acnes*, with an inhibition zone ranging from 8.2 to 11.1 mm. This indicates that *Weissella confusa* has a greater ability to inhibit *P. acnes* and *S. epidermidis*.

The antimicrobial compound commonly produced by probiotic bacteria is bacteriocin. This compound has a broad-spectrum activity in inhibiting the growth of various pathogenic bacteria. The presence of a clear zone observed on the antimicrobial test medium can be attributed to the bacteriocin's activity, which is capable of killing the pathogenic bacterial cells being tested.

The sequencing of the DDH 1 DNA amplification product aimed to obtain information on the nucleotide sequence of the PCR-amplified target DNA with a specific length. The sequencing data obtained were aligned using the Basic Local Alignment Search Tools (BLAST) on the NCBI website. This program identifies regions of local similarity between the analyzed sequence and the database-stored sequence information to calculate the statistical significance of the match.

The BLAST results showed that isolate CIN-1 had the highest similarity with *Pediococcus pentosaceus* strain DSM 20336, with a similarity of 99.64% (Table 2). According to Drancourt et al. (2004), a similarity of 97–99% in bacterial 16S rRNA sequences indicates similarity at the genus level, while a similarity of >99% sequence gen is the criterion used for identification at the species level. The alignment results also showed an identical max score and total score, with DDH 1 scoring 2560. An E-value of 0.0 indicates a significant alignment. The phylogenetic relationship among species was analyzed by constructing a phylogenetic tree using the MEGA X program. The phylogenetic tree of isolate DDH-1 was analyzed using the Neighbor-Joining (NJ) method.

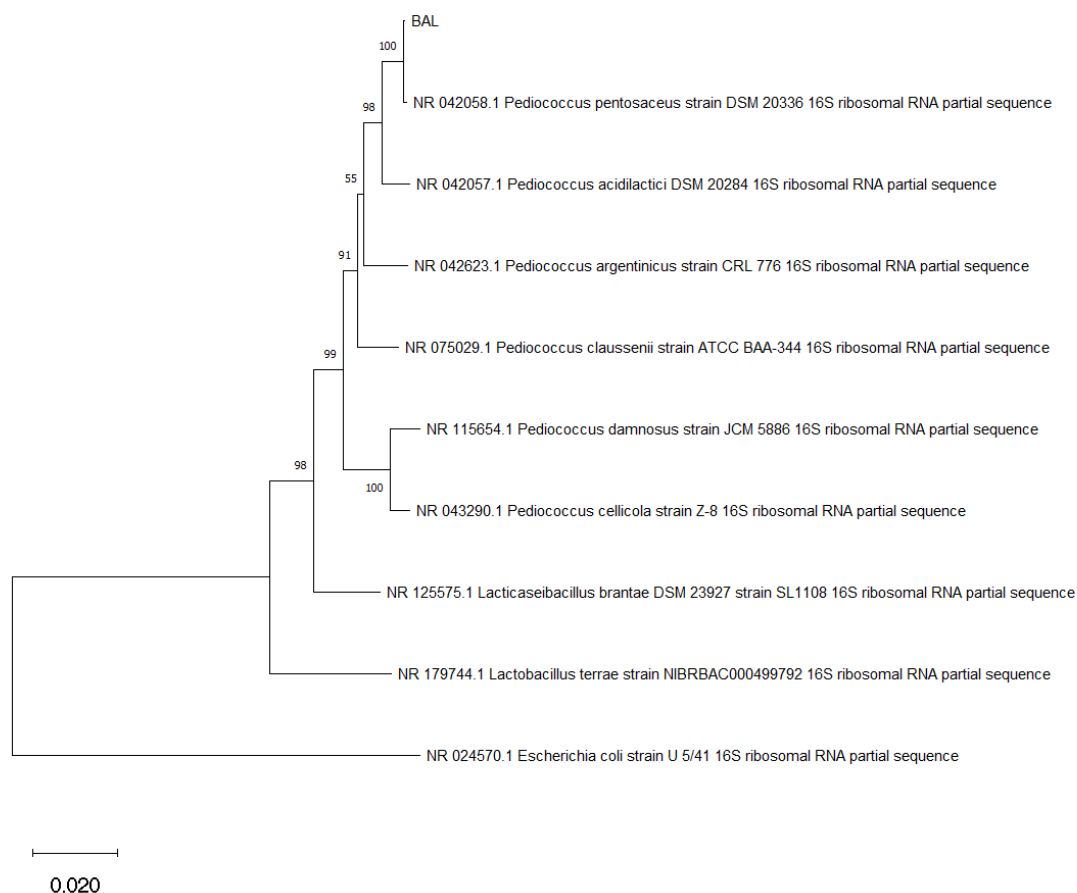


Figure 5. Phylogenetic Tree of *P. pentosaceus* Strain DSM 20336.

The phylogenetic tree based on the 16S rRNA gene of isolate DDH 1 was constructed using Neighbor-Joining analysis and a bootstrap phylogeny test with 1000 replicates. The results showed that isolate DDH 1 had a bootstrap value of 100 with *Pediococcus pentosaceus* strain DSM 20336. A bootstrap value of 100 indicates that in 1000 iterations, the same tree topology was formed 1000 times. According to Dharmayanti (2011), bootstrap analysis is a method used to test how well the dataset supports the model, and the values are usually placed on the branches of the phylogenetic tree. Bootstrap values are categorized as high (>85%), moderate (70–85%), weak (50–69%), or very weak (Lestari et al., 2018).

The phylogenetic tree with a scale bar of 0.02 indicates a genetic distance equivalent to two nucleotide changes per 100 base pairs. Based on observations, the DDH-1 isolate exhibited a round cell morphology, was Gram-positive upon Gram staining, and showed a negative result in the catalase test. *Pediococcus pentosaceus* belongs to the Gram-positive, anaerobic Firmicutes group. This bacterium is a coccus-shaped microbe characterized by being non-motile and non-spore-forming. It is classified as a “lactic acid bacterium” because the end product of its metabolism is lactic acid (Lin et al., 2019).

CONCLUSIONS

This study showed that the bacteriocin activity produced by lactic acid bacteria has potential as an antibacterial agent against *Propionibacterium acnes*. The results of molecular docking showed a strong binding affinity with a score of -7.5 kcal/mol. At the same time, the in vitro antibacterial activity test yielded an average inhibition zone of approximately 20.6 mm. The analysis of the 16S rRNA results identified the isolate as *Pediococcus pentosaceus* strain DSM 20336 with a similarity level of 99.64%. This indicates that the bacterium has potential as an alternative treatment for acne. It also presents an opportunity for the development of probiotic products or bacteriocin-based formulations in the future.

Authors' Contributions: Adhitya Naufal Pribadhi and Penggalih Mahardika Herlambang designed the study, analyzed the data, and wrote the manuscript. Ellyka carried out the laboratory work. All authors read and approved the final version of the manuscript

Competing Interests: The authors declare that there are no competing interests.

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