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Characteristics and activity of chitosan from mud crab shells on acne bacteria: *Staphylococcus aureus, S. epidermidis* and *Propionibacterium acnes*

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Abstract. *Luthfiyana N, Bija S, Anwar E, Laksmitawati DE, Risalinda GL. 2022. Characteristics and activity of chitosan from mud crab shells on acne bacteria:* Staphylococcus aureus, S. epidermidis *and* Propionibacterium acnes. *Biodiversitas 23: 6645-6651.* Chitosan is a product of chitin deacetylation, which has the potential to act as acne bacteria. The study aimed to determine the characteristics and activity of chitosan from mud crab shells on acne bacteria, i.e., *Staphylococcus aureus* FNCC 0047, *Staphylococcus epidermidis* FNCC 0048 and *Propionibacterium acnes* ATCC 11827. The research used a descriptive method for analyzing the quality of chitosan and antibacterial activity using analysis of variants at a 95% confidence interval with one factor, while the addition of chitosan as much as 0.25%; 0.5%; 0.75%; and 1% with three replications. The results showed that the value of deacetylation degree (DD) was 76%. The morphology of chitosan shows irregular, wavy, porous shapes and contains several elements: C, O, Ca, and Al. The antibacterial activity of chitosan at concentrations of 0.25%, 0.5%, 0.75%, and 1% showed an inhibition zone of *S. aureus* (9.5±0.71 mm, 10±0 mm, 11±0 mm, 13.5±0.71 mm), *S. epidermidis* (9±0 mm, 10±0 mm, 11±0 mm, 12.5±0.71 mm), and *P. acnes* (28.5±0.7 mm, 28.5±0.7 mm, 31±5.56 mm, 34.5±6.36 mm). The concentration of 1% gives the effectiveness in inhibiting the growth of acne bacteria. This study concludes that chitosan from mud crab shells could be the potential as an anti-acne.

Keywords: Anti-acne, chitosan, morphology, mud crab shells, SEM

INTRODUCTION

Acne is not a life-threatening skin disease, but the presence of acne can have a psychological effect that will bring down a person's confidence and influence their quality of life (Ramos-e-Silva et al. 2015). Acne affects almost 85% of the world's population, with an age range from 11-30 years (Okoro et al. 2016). Women's skin problems are higher than men, with a percentage of 65% and 35%, respectively. The prevalence of acne sufferers was 72.5% in Indonesia, dominated by adolescents aged 18-25 years and adults 26-35 years, as much as 27.5% (McCarty 2016). The main factors involving pimples are increased sebum production, sloughing of keratinocytes, inflammation, and bacterial growth (Sparavigna et al. 2015). The bacteria that cause acne on the skin are *Staphylococcus epidermidis, Staphylococcus aureus* and *Propionibacterium acnes*.

Acne bacteria (*S. epidermidis, S. aureus,* and *P. acnes*) cause opportunistic infections of acne, especially during puberty, due to increased androgen activity which triggers the growth of sebaceous glands and increased sebum production (Foster et al. 2014). One of the ways to treat acne is by using antibiotics such as erythromycin, doxycycline, and clindamycin. However, the use of antibiotics continuously can cause irritation, resistance, and immuno-hypersensitivity (Dinant and Boulous 2016). Seeing the impact of antibiotics, it is necessary to look for other alternatives using natural ingredients that have an antibacterial function. One of them was sourced from fisheries, a product that is chitosan.

Chitosan is a process of deacetylation from fisheries waste such as mud crab shells (Sivanesan et al. 2021). Chitosan is a biopolymer from chitin deacetylation, which is biocompatible, biodegradable, non-toxic and has antibacterial properties (Bellich et al. 2016), that inhibit the growth of pathogenic bacteria and spoilage microorganisms, including Gram-positive and Gram-negative bacteria (Tan et al. 2013). The antimicrobial activity of chitosan is influenced by pH, concentration, molecular weight, degree of polymerization, and cross-linking (Elsabee and Abdou 2013). Chitosan, as a long chain polycationic molecule, can adhere to gram-positive bacteria better than Gram-negative because it does not has an outer membrane. Therefore, the inhibition of chitosan was more effective towards Gram-positive bacteria (Khan et al. 2015).

There are huge numbers of finding on the ability of chitosan as an antibacterial. Kusnadi (2022) stated the chitosan extract from a white shrimp shell (*Penaeus indicus*) had a deacetylation degree of 73.46%, the largest inhibition zone found at 1000g/mL on *S. aureus* (14.40±0.03mm). Mohan et al. (2020) confirmed that chitin and chitosan from shrimp and crab shells possess

significant antibacterial activity. Shrimp and crab shell chitosan showed better action to Gram-negative bacteria. Feng et al. (2021) stated chitosan is a polymeric biomaterial with antibacterial and hemostatic activity and the ability to boost skin regeneration.

Two common cutaneous pathogens, *P. acnes* and *S. aureus*, have resistance to frontline antibiotics increasingly (Song et al. 2011). Chitosan has the potential to act as an effective antibacterial agent against *S. aureus* and *P. acnes*, which has been identified as a factor in the pathogenesis of *Acne vulgaris* (Champer et al. 2013). Chitosan has been shown that effective against *P. acnes* and *S. aureus* under specific conditions in vitro (Champer et al. 2013). Moreover, chitosan has been reported to be a moisturizer, has wound curing, anti-inflammatory properties and removes oil and sebum, it might enhance its antibacterial activity for acne treatment (Friedman et al. 2013). The difference between this study and the previous one is that the chitosan used is from mud crab shells and uses three bacteria that can cause acne. The purpose of this study was to determine the characteristics and activity of chitosan from mud crab shells on acne bacteria, i.e., *Staphylococcus aureus* FNCC 0047, *Staphylococcus epidermidis* FNCC 0048 and *Propionibacterium acnes* ATCC 11827.

MATERIALS AND METHODS

Sample collection

Samples of mud crab shells were obtained from the Soka crab cultivation in Tarakan City, North Kalimantan, Indonesia. The samples were cleaned using clean water and dried using an oven at a temperature of 80°C and then mashed to 50 mesh. The reagents and chemicals used in this study were supplied by Brataco Chemika, Indonesia. The research equipment was provided by the Nutrition Laboratory and Water Quality Laboratory of Fisheries and Marine Sciences Faculty at Borneo Tarakan University, the Q-Lab Laboratory of Pharmacy Faculty at Pancasila University, and PT. Cipta Mikro Material Bogor, West Java, Indonesia.

Preparation of chitin

These mud crab shells were then treated using the procedure of Luthfiyana et al. (2022). In the deproteination stage, 200 g of mud crab shell powder was soaked in a 3N NaOH solution with a ratio (1:10 w/v) at 80˚C for 60 min. The sample was neutralized with aquadest until its pH was 7 (neutral) and dried in an oven at 80˚C. The next stage is demineralization. The sample was immersed in 1N HCl solution in a ratio (1:15 w/v) for 60 min at room temperature and stirred. Before the deacetylation stage, the sample is neutralized again.

Deacetylation of chitin

The production of chitin into chitosan through the deacetylation step refers to the procedure of El Knidri et al. (2018) with modifications. Samples were immersed in 60% NaOH solution with a ratio of 1:10 w/v at a temperature of 140˚C for 60 min to derivative chitin into chitosan. The sample was neutralized with aquadest until the pH reached 7 (neutral). Then, it was filtered and dried using an oven at 80˚C. The sample was dried for 24 hrs at 100˚C in an oven, chilled in a desiccator, and weighed until it reached a constant weight. Then, it was analyzed by Fourier transform infrared spectrophotometer (FTIR). A total of 0.002 g chitosan was placed into the sample holder on the FTIR-QATRS (Shimadzu, Japan) to read the results. The known peaks will be compared using the IR table with a wavelength of 4000-400 cm^{-1}. FTIR will identify the functional groups of these compounds so that they can be distinguished and quantified. To determine the deacetylation degree (DD%), Domszy and Roberts (1985) formulated the baseline method. The DD% was calculated from the ratio between the absorbance at 1655 cm^{-1} and the absorbance at 3450 cm^{-1} with the formula:

$$DD\% = 100\ [(A_{1655}/A_{3450}) \times 100/1.33]$$

Characterization of chitosan from mud crab shells was followed by morphological analysis using scanning electron microscopy, and energy-dispersive X-ray spectroscopy (SEM-EDX) (Quanta 650, UK). The chitosan powder was affixed to the carbon tape in the form of a rectangle with a size of ±0.5 cm. Chitosan that adheres to the stub perfectly is attached to the holder and inserted into the chamber. This analysis was conducted with a low vacuum because the sample is a non-conductor. The analyzed chitosan can be viewed on a monitor, and the magnification was adjusted.

Acne bacterial activity of chitosan

The test for detecting acne bacterial activity of chitosan from mud crab shell refers to Al-Zahrani (2021) modified. This test includes preparing chitosan solutions, liquid media (nutrient broth and NaCl solution), solid media (Nutrient agar, blood agar, and Mueller Hinton agar), bacterial refreshment, bacterial rejuvenation, bacterial culture, and anti-acne activity test. The type of bacteria used were *S. aureus* FNCC 0047, *S. epidermidis* FNCC 0048 and *P. acnes* ATCC 11827, that was taken from the Center for Food and Nutrition Studies (PSPG) Gadjah Mada University, Yogyakarta, Indonesia. The antibacterial activity test occurred by the diffusion method using the paper disk.

Preparation of chitosan solution, positive and negative control

The preparation of chitosan solution refers to Benhabiles et al. (2012). The concentrations of chitosan used were 0.25%, 0.5%, 0.75%, and 1%, and then dissolved in 1% acetic acid 25 mL. The positive control antibiotic used is ampicillin. A total of 10 mg of ampicillin powder was weighed and then dissolved with 1 mL of distilled water. The negative control used the same solvent as chitosan, 1% acetic acid.

Bacterial rejuvenation

Bacterial rejuvenation of *S. aureus* FNCC 0047 and *S. epidermidis* FNCC 0048 refers to Abdel-Rahman et al.

(2015). A total of 2 g nutrient agar (NA) was dissolved in 100 mL of distilled water and heated at 120˚C until all dissolved. The NA solution was sterilized in an autoclave at 121˚C for 15 min. The sterile NA solution was poured into a 10 mL test tube and waited for it to solidify in an inclined at 45˚angle position. Bacterial cultures of *S. aureus* and *S. epidermidis* from the primary strain were taken using a needle and then streaked into solidified NA and incubated at 35˚C for 24 hrs. Bacterial rejuvenation of *P. acnes* ATCC 11827 refers to the study of Nand et al. (2012). The blood agar (BA) media 3.7g was dissolved in 100 mL of distilled water and heated at 100˚C. The BA solution was sterilized by autoclaving at 121˚ for 15 min. It was poured into a 20 mL Petri dish and allowed to solidify. The stock of *P. acnes* bacteria from the primary strain was taken as much as two oses, then scratched on BA media and incubated for 24 hrs.

Bacterial culture

The production of liquid bacterial cultures of *S. aureus* FNCC 0047 and *S. epidermidis* FNCC 0048 refers to Christensen et al. (2016). A total of 0.4 g nutrient broth (NB) was dissolved with 50 mL of aquadest and then put into an Erlenmeyer. The NB solution was heated at 120˚C and sterilized using an autoclave at 121˚C for 15 min and then allowed to cool. The regenerated bacteria were taken in as many as one colony using a needle and put into the NB solution, then incubated at 35˚C for 24 hrs.

The liquid for the bacterial culture of *P. acnes* ATCC 11827 refers to the research by Kim et al. (2017). The liquid saline medium used was 0.85% physiological NaCl. Five milliliters of 0.85% physiological NaCl solution was put into a test tube and then sterilized using an autoclave at 121˚ for 15 min and cooled. Bacteria that have been rejuvenated are taken as much as two oses and then put into a sterile 0.85% physiological NaCl solution and homogenized.

Anti-acne activity test

The analysis of anti-acne activity for chitosan refers to Arancibia et al. (2014), which has been modified. A total of 3.8 g Mueller Hinton agar (MHA) was dissolved in 100 mL of distilled water and heated at a temperature of 120˚C. The MHA solution was sterilized in an autoclave at 121˚C for 15 min. The sterilized MHA solution was poured into a 15 mL Petri dish and allowed to solidify. A total of 10 mg/mL of liquid bacterial culture was taken using a micropipette and then flattened using a sterile cotton bud. Disc paper is immersed in 0.25%; 0.5%; 0.75%; and 1% chitosan solution. The disc paper was then placed into MHA, which had been given bacteria and incubated at 35˚C for 24 hrs. Testing the inhibition of chitosan against *S. aureus*, *S. epidermidis*, and *P. acnes* bacteria can be determined by measuring the clear zone formed.

Data analysis

The data analysis of chitosan characteristics was presented descriptively. The antibacterial activity of chitosan obtained in this study used the analysis of variant test with one factor, it was the addition of chitosan with 0.25%; 0.5%; 0.75%; and 1% concentration. Acetic acid 1% and ampicillin 10 mg/mL were used as a negative and positive control, respectively. Each experiment was done in triplicates. The data were analyzed using variance (ANOVA) at a 95% confidence interval. If the analysis results of variance are significantly different, then further tests are carried out using the BNT test.

RESULTS AND DISCUSSION

Deacetylation degree of chitosan

Chitosan produced from mud crab shells was characterized by infrared spectroscopy to identify functional groups and determine the deacetylation degree of chitosan (Figure 1).

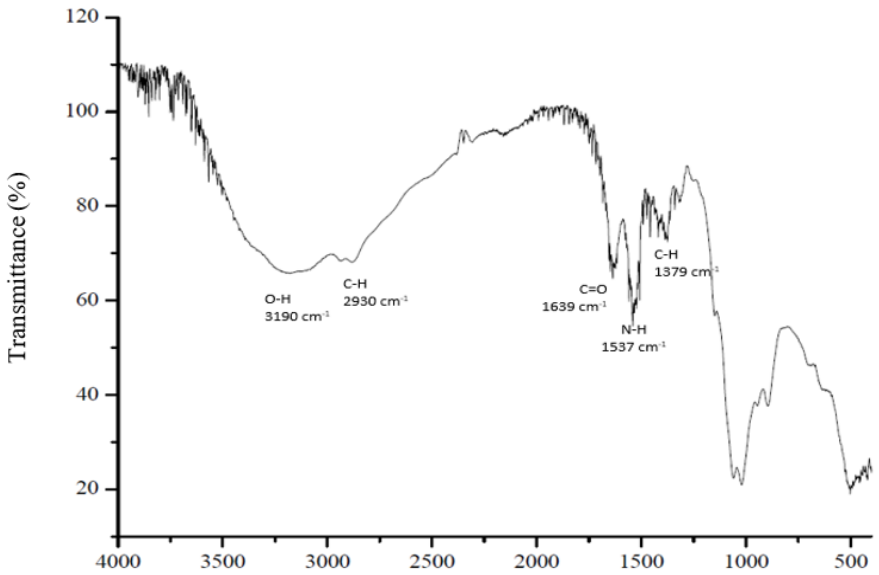


Figure 1. FTIR spectrum of chitosan from mud crab shell

The FTIR spectrum with the OH absorption peak was observed at a wavelength of 3190 cm^{-1}. The wavelengths of 2930 cm^{-1} and 1379 cm^{-1} have C-H stretching vibrations. The absorption peak of the amide group is at a wavelength of 1634 cm^{-1} while the absorption peak of the N-H bending vibration is seen at a wave number of 1537 cm^{-1} (Figure 1). Wanule et al. (2014), reported that the amide group still present in chitosan indicated that the group had not completely deacetylated to become an amine group. The peak of amide was seen in the absorption wavelength of 1639 cm^{-1}. The appearance of amide peaks due to the fact that the chitosan produced had not been completely deacetylated, thus affecting the quality of the chitosan.

The quality of chitosan is determined by the percentage of the deacetylation degree. Jiang et al. (2017) reported that the deacetylation degree affects chitosan's physical, chemical, and biological properties, such as acid-base and electrostatic properties, biodegradability, self-aggregation, absorption properties, and the ability to chelate metal ions. The deacetylation degree of mud crab shell chitosan in this study was 76% based on the calculation of the Domzy and Robets baseline method. It meets the standards of Protan Laboratory (≥70%) and SNI No. 7949 of 2013 (≥75%) but not in accordance with the European Food Standards Authority (EFSA) 2010 (≥90%). Zhang et al. (2012) reported that the higher the deacetylation degree value of chitosan, the better the quality.

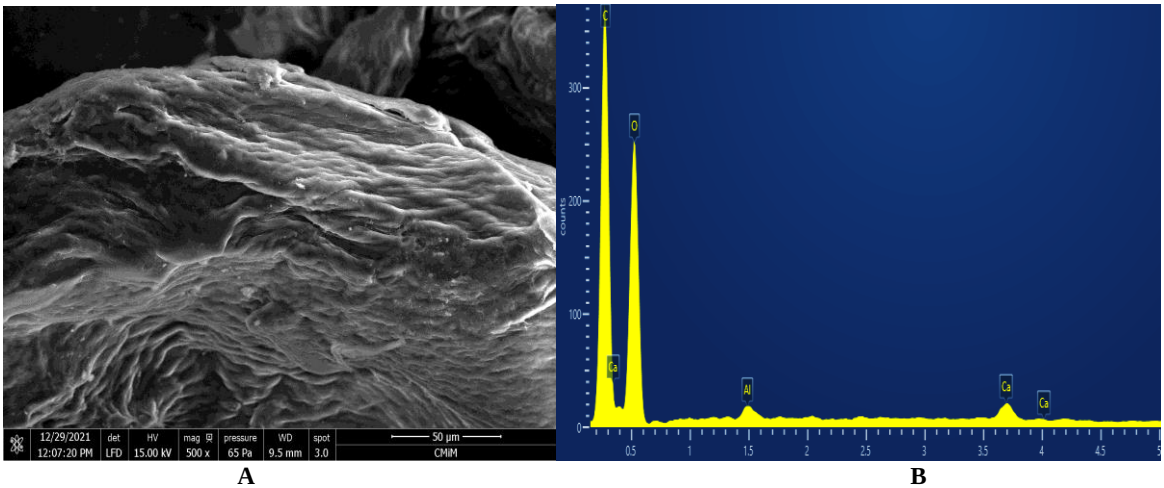
The high and low deacetylation degree is influenced by the concentration and ratio between the sample and the strong alkaline solution used, so it will produce different DD%. This statement is supported by several studies including Mathaba and Daramola (2020), chitosan is obtained from chitin through deacetylation, treating chitin with a strongly alkaline solution. The most important parameter that characterizes chitosan samples is the deacetylation degree. It affects the synthesized chitosan's physical, biological, and chemical properties. The deacetylation degree determines which free amino groups expose due to the removal of acetyl groups from the chitin molecular chain. Musmade and Mahatma (2021) stated the deacetylation process involves the removal of an acetyl group from the chitin molecular chain, leaving a complete amino group ($-NH_2$), and the versatility of chitosan depends mainly on the reactive amino group in this process.

The DD value is influenced by several factors, such as the solvent used in the deacetylation process, the stirring time, and the temperature used. In this study, it was used 60% NaOH solvent at a temperature of 140ºC with a stirring time of 60 minutes. Hossain and Iqbal (2014) stated that the DD of chitosan was influenced by the amount of alkaline solution used, time, and temperature. Khan et al. (2013) explained that the higher of NaOH concentration, the higher of chitosan's deacetylation degree. However, the NaOH concentration, which is too high, can result in a depolymerization process and the breakdown of the acetyl amide group in chitosan, causing a decrease in the deacetylation degree's value. Rasweefali et al. (2021) stated that the higher of temperature used, the deacetylation reaction will take place faster, but the higher temperature can damage the structure of chitosan.

SEM-EDX of chitosan

Mud crab shell chitosan was analyzed for morphology and its constituent elements through scanning electron microscope energy dispersive X-ray (SEM-EDX) analysis (Figure 2).

The morphology of mud crab shell chitosan with a magnification of 500x and obtained irregular and rough (Figure 2a). SEM is one of the most widely used analyzers to observe surface morphology, particle size, and microstructure of crystalline or amorphous polymer materials (El-Hami et al. 2020). These results are in accordance with Tolesa et al. (2019), the SEM analysis results show an irregularity in chitosan structure and its surface morphology: Smooth surface with some rough, irregular membrane fissures and patterns. The image of the chitosan reveals that the surface is rough and disordered.



A **B**

Figure 2. Scanning Electron Microscope Energy Dispersive X-Ray (A) morphology and (B) elements of chitosan from mud crab shells (*Scylla* sp.)

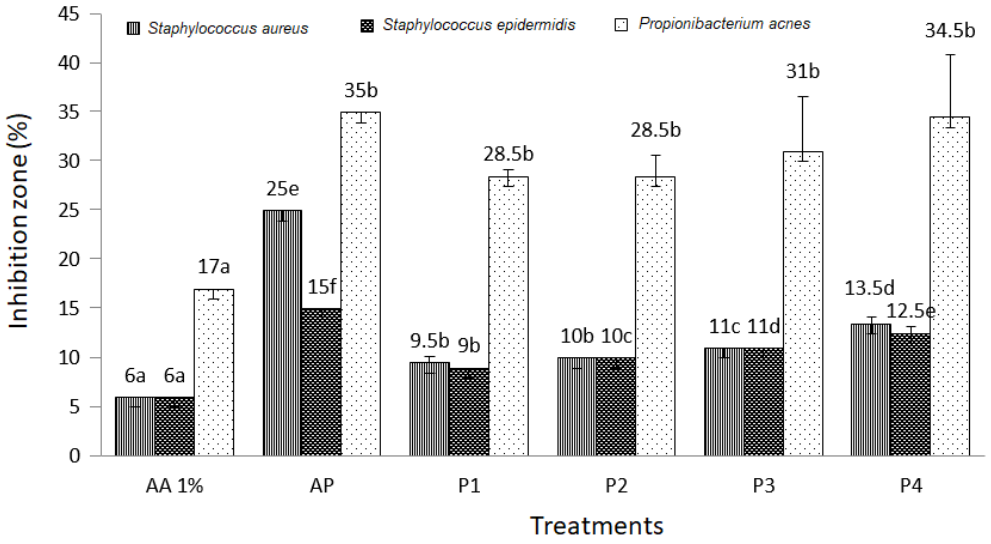


Figure 3. Inhibition zones of mud crab shell chitosan towards acne bacteria (*S. aureus, S. epidermidis,* and *P. acnes*), with AA 1% (acetic acid/negative control), AP (ampicillin/positive control) and chitosan P1 (0.25%), P2 (0.50%), P3 (0.75%), P4 (1%). Note: letters from the same alphabet indicate non-significant at $p < 0.05$. The data were expressed as mean ± standard deviation of three replications

The SEM-EDX analysis of mud crab shell chitosan contains several elements, including C and O, which are the constituents of chitosan and other impurities such as Ca and Al (Figure 2b). The results of this study are in line with Al Hoqani et al. (2021), who reported SEM-EDX results of shrimp shell chitosan detecting the presence of carbon (C), oxygen (O), nitrogen (N), sodium (Na), calcium (Ca), and magnesium (Mg). In this study, it was also found that there were impurities in the chitosan pad, namely Ca and Al, which were caused by the suboptimal demineralization process during the preparation of chitosan. Fathoni et al. (2018) reported that chitosan is a long-chain polymer of glucosamine with the molecular formula ($C_6H_{11}NO_4$) consisting of 47% C, 6% H, 7% N, and 40% O, also has a similar structure to cellulose.

Acne bacterial activity of chitosan

The bacterial acne activity of chitosan from mud crab shells was obtained by measuring the inhibition zone formed. The results of measuring the diameter of the inhibition zone of chitosan towards *S. aureus, S. epidermidis* and *P. acnes* were recorded (Figure 3).

The results using ANOVA with a 95% confidence level (α: 0.05%) showed that chitosan could inhibit the growth of *S. aureus* bacteria and the concentration of chitosan was significantly different from the resulting inhibition zone (Figure 3). The higher chitosan concentration indicates greater inhibition towards *S. aureus* bacteria growth. Kusnadi and Purgiyanti (2021) stated that the antibacterial activity of chitosan was enhanced with increasing concentration. The further BNT test showed that the treatments P1 (0.25%) and P2 (0.50%) were not different, but these were significantly different from P3 (0.75%) and P4 (1%). The best antibacterial activity of chitosan to *S. aureus* is a concentration of 1% with an inhibition zone of 13.5±0.71 mm (Figure 3). The results of this study were much lower when it compared to Kusnadi et al. (2022), who reported that commercial chitosan with a concentration of 1000 ppm had an inhibition zone of 13.62±0.05 mm.

Chitosan can inhibit the growth of *S. epidermidis* bacteria. The greater of chitosan concentration used, the greater the inhibition zone produced. According to Chang et al. (2019), the higher of chitosan concentration, its antibacterial activity also increases. In the advanced test, BNT showed that the treatments P1 (0.25%), P2 (0.5%), P3 (0.75%), P4 (1%) were significantly different. The best antibacterial activity of mud crab shell chitosan to *S. epidermidis* bacteria was a concentration of 1% with an inhibition zone of 12.5±0.71 mm (Figure 3). Goy et al. (2016) reported that chitosan could inhibit the growth of Gram-positive bacteria, where the minimum Inhibitory Concentration was observed at a concentration of 1%. The ANOVA results with a 95% confidence level (α: 0.05%) showed that chitosan could inhibit the growth of *P. acnes* bacteria. In the advanced test, BNT showed that the treatment and the concentration of chitosan were not significantly different from AP (ampicillin), P1 (0.25), P2 (0.50), P3 (0.75), and P4 (0.10) but significantly different from AA 1% (acetic acid). Liu et al. (2006) reported that chitosan with large molecular weights forms a film on the bacteria membrane, preventing nutrient adsorption, disturbing physiological activities, and leading to bacterial death. Chitosan influenced the antibacterial activity markedly, especially in *P. acnes* and *S. epidermidis*. Supreedee et al. (2015) reported that the electron microscopy test of *P. acnes* under chitosan treatment was similar to previous results on Gram-positive. Some bacteria, such as *P. acnes, S epidermidis*, and *S. aureus*, are pathogenic skin bacteria that cause acne. These bacteria cause inflammation and abnormal keratinization of follicles in the skin. The 1% acetic acid (negative control) can inhibit the growth of *S. aureus, S. epidermidis,* and *P. acnes* bacteria by 6 mm (Figure 3). Acetic acid can inhibit

the growth of bacteria because it can damage the protein from the bacterial cell wall. Magani et al. (2020) reported that the use of 1% acetic acid could inhibit bacterial growth by 8.89 mm. Hafsa et al. (2016) stated that acetic acid was able to damage bacterial cell walls caused by the denaturation of proteins in cell walls. It can cause the death of bacteria. Chitosan and acetic acid work synergistically so that they can inhibit bacterial growth maximally.

The antibacterial activity of mud crab shell chitosan against *S. aureus*, *S. epidermidis*, and *P. acnes* bacteria in this study was in the moderate to strong category. The higher of chitosan concentration used, the stronger of antibacterial activity. Benhabiles et al. (2012) reported that if the inhibition zone formed was less than 5 mm, the inhibition was weak, the 5-10 mm inhibition zone was categorized as moderate, the 10-19 mm inhibition zone was categorized as strong, and the inhibition zone of more than 20 mm was categorized as very strong. Increasing of the concentration used can accumulate amine groups (NH_2) in chitosan, so it is effective in killing bacteria. The purity of chitosan affects its ability as an antibacterial and can be determined based on the deacetylation degree. The higher of DD chitosan produced, the purer it is and stronger in inhibiting bacterial growth. In this study, the DD value was 76%. Hosseinnejad et al. (2016), stated that the greater of chitosan's DD, so the higher of homogeneity, so the chitosan reacts more quickly in damaging bacterial cell walls. The deacetylation degree (DD 69.67%) with a concentration of 2% was only able to inhibit the growth of *S. aureus* bacteria by 11.30%.

The bacteria used in the present study were *S. aureus*, *S. epidermidis*, and *P. acnes* bacteria which are Gram-positive bacteria. The mechanism of chitosan as an antibacterial is more effective for Gram-positive bacteria than Gram-negative. It is because Gram-positive bacteria do not have an outer membrane, so chitosan is easily attached to the bacteria. Goy et al. (2016) reported that the mechanism of the antibacterial activity of chitosan to Gram-positive bacteria was through 2 stages. The first stage, chitosan with cationic ions is absorbed on the surface of Gram-positive bacteria. Second, the direct diffusion of chitosan into Gram-positive bacteria and inhibition of bacterial cell growth occurs through a series of processes starting with chitosan that binds to the plasma membrane, inactivates enzymes and proteins, DNA becomes damaged, which causes disruption of cell function and metabolic processes, also ultimately ends with cell death. Khan et al. (2015), reported that the outer membrane of Gram-negative bacteria consists of an asymmetric lipid-protein bilayer (lipopolysaccharide). Divalent cations (Ca^{2+} and Mg^{2+}), which exist in the outer membrane, have an important role in stabilizing the anionic nuclear charge of lipopolysaccharide molecules. Chitosan, through electrostatic interactions, causes disruption of the membrane gives rise to lysis. Chao et al. (2019) stated that chitosan, as an ionic long-chain polymolecule, can adhere to gram-positive bacteria, so the inhibition of chitosan is more effective.

In conclusion, the quality of mud crab shell chitosan has a deacetylation degree of 76% according to the Protan Laboratory standard (≥70%) and SNI No.7949 of 2013 (≥75%) but does not meet with the European Food Standards Authority (EFSA) 2010 (≥90%). The morphology of mud crab shell chitosan has an irregular, wavy and porous surface shape, C and O elements were found, which confirmed the constituents of chitosan. The best concentration of mud crab shell chitosan at 1% has proven to be effective in inhibiting the antibacterial activity that causes acne. This study concluded that chitosan from mud crab shells has the potential as an anti-acne. Chitosan with a concentration of 1% inhibited the activity of Gram-positive and Gram-negative bacteria, i.e., *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes*. The chitosan from mud crab shells could be the potential as an anti-acne.

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