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The antibacterial effects of chitosan nanoparticles from *Scylla* sp. on acne-related bacteria: *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Abstract. One of the many advantages of chitosan obtained from the shells of mud crabs is that it acts as an antibiotic against acne. This study set out to determine chitosan's characteristic properties that were taken from *Scylla* sp shells, the nanoparticle's dimension, and the optimal chitosan nanoparticle concentration to stop the growth of acne-causing *Staphylococcus aureus* and *Staphylococcus epidermidis*. A descriptive approach is used in this research methodology to evaluate the chitosan's quality and antibacterial efficacy. Chitosan nanoparticles at varying concentrations (0.20%, 0.25%, 0.50%, 1%, and 1.25%) are included as the only independent factor, and changes are examined within a 95% confidence interval. The positive control is ampicillin, while the negative control is acetic acid. A Particle Size Analyzer (PSA) was used to analyze the chitosan nanoparticles' dimensions, and the results showed that they were 47.04 nm. At different doses, chitosan nanoparticles' inhibitory effect on the growth of *S. aureus* and *S. epidermidis* was assessed. Both *S. aureus* and *S. epidermidis* growth were effectively inhibited at a concentration of 0.50 percent. The chitosan nanoparticle activity in this investigation fell within the moderate to strong range.

1 Introduction

Due to its tropical climate, Indonesia facilitates the growth of germs, parasites, and even fungi, which can lead to a number of skin conditions, including acne. Both men (95–100%) and women (83–85%) can get acne, which can have psychological implications by lowering self-esteem and affecting one's quality of life [1]. Increased keratinocytes, inflammation,

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bacterial growth, and sebum production are the primary elements that contribute to the development of acne [2]. *Staphylococcus aureus* and *Staphylococcus epidermidis* are two bacteria that can cause acne on the skin. This specific gram-positive bacterial strain has the ability to induce inflammation of the oily gland lining and infection of the skin, as well as keratin accumulation and blockage. [3]. Antimicrobial activity of bacterial species *S. epidermidis* is often found in acne vulgaris patients however, only a few studies have been reported. *S. aureus* and *S. epidermidis* are types of normal bacteria that inhabit the skin and easily acquire and transmit various antimicrobial resistance genes [4]

Acne risk rises as a result of increased sebum production and sebaceous gland expansion brought on by the rise in testosterone activity throughout adolescence [5]. Antibiotics such as tetracycline, erythromycin, doxycycline, and clindamycin are used to treat acne. However, long-term antibiotic use might result in immunological hypersensitivity, resistance, and discomfort [6]. Alternative solutions utilizing natural substances with antibacterial qualities ought to be taken into consideration, considering the adverse consequences linked to antibiotics. Chitosan is one of the natural compounds found in fisheries products.

Crab and shrimp shells are examples of fish waste materials that undergo the deacetylation process to produce chitosan. It is possible to prevent hazardous gram-positive and gram-negative germs by chitosan's antibacterial qualities [7]. Degree of polymerization, pH, concentration, molecular weight, and crosslinking all affect chitosan's antimicrobial effectiveness [8]. Electrostatic interactions on the bacterial cell wall, cell membrane, and other vital components are the mechanism by which chitosan exhibits antibacterial activity. There is no outer membrane on gram-positive bacteria like *S. aureus* and *S. epidermidis*. Consequently, since chitosan is a polycationic molecular chain, compared to gram-negative bacteria, it can attach to gram-positive microbes more readily. [9].

Chitosan nanoparticles are chitosan measuring 10-1000 nm. Due to their specific surface and smaller size, chitosan nanoparticles have a better adsorptive ability [10]. The bacterial cell membrane can be swiftly penetrated by tiny chitosan nanoparticles. Natural or synthetic antimicrobials, antioxidants, enzymes, or functional materials like plant extracts, probiotics, minerals, or vitamins can all be added to chitosan nanoparticles. Throughout the storage period, chitosan nanoparticles had greater antibacterial activity than chitosan itself. [11]. Several studies discuss the ongoing and widespread use of chitosan nanoparticles as an antibacterial. According to [12] by lowering the amount of protein in the bacterial cell wall, chitosan nanoparticles given in vitro can stop the growth of gram-positive bacterial colonies. The infections *Streptococcus mutans* and The fungus *Candida albicans* could be managed with nanoparticles that have a size of 20 to 30 nm [13]. At an amount of 0.50 percent, chitosan nanoparticles can stop bacteria such as *S. aureus* and *E. coli* bacteria from growing by 12.31 mm [14].

Chitosan from mud crab shells was first made into chitosan nanoparticles through ionic gelation. This research wants to change the particle size of chitosan from mud crab shells to nano size to increase its effectiveness as an antibacterial. Different amounts of nanochitosan will be employed to stop the growth of the bacteria *S. aureus* and *S. epidermidis*, which cause acne on the skin. This study sought to ascertain the chitosan quality in mud crab shells, as well as the size of the nanoparticles and the ideal chitosan nanoparticle concentration for inhibiting the growth of *S. aureus* and *S. epidermidis* bacteria, which cause acne.

2. Materials and Methods

2.1. Materials

Among the materials used is chitosan made from mud crab shells, Sodium Tripholi Phosphate (NaTPP), aquadest (water one), tween 80, chitosan, and 1% acetic acid (Emsure). The analytical materials used were The strains Staphylococcus aureus FNCC 0047 and Staphylococcus epidermidis, strain FNCC 0048 were acquired from Universitas Gajah Mada Yogyakarta, Indonesia's Center for Food and Nutrition Studies (PSPG). Brataco Chemika, Indonesia, provided the chemicals and reagents used in this investigation. The research The Q-Lab Laboratory of Pharmacy Faculty at Pancasila University, the nutritional Laboratory and the Quality of Water Laboratories of Fisheries and Marine Sciences Faculty at Universitas Borneo Tarakan, and PT. Cipta Mikro Material Bogor, West Java, Indonesia, also contributed equipment.

2.2. Methods

2.2.1. Procedure

Initially, the Fourier transform infrared (FTIR) test was employed to ascertain the amount of deacetylation in the chitosan made from *Scylla* sp shells; the higher the DD, the purer the chitosan. The resultant chitosan is subsequently converted into chitosan nanoparticles using ionic gelation. The next step is to evaluate the chitosan nanoparticles and determine their ultimate size using a Particle Size Analyzer. Testing the chitosan nanoparticles' capacity to stop *S. aureus* and *S. epidermidis* bacteria from growing at concentrations of 0.2%, 0.25%, 0.5%, 1%, and 1.25% was the last step. A negative control was 1% acetic acid, while a positive control was ampicillin 10 g/mL.

2.2.2. Chitosan nanoparticle production using the ionic gelation method

The process of creating chitosan nanoparticles by ionic gelation relates to the study of [15] with modification. After weighing, 100 mL of a mixture comprising 1% acetic acid were used to dissolve 0.2 grams of chitosan. For eight hours, the solution of chitosan was agitated at a speed of 3000 rpm using a magnetic stirrer. Emulsification was used to create chitosan nanoparticles. To do this, 25 mL of emulsifier (Tween 80) 0.2% was added gradually, up to a volume of 25 mL, and stirred constantly for two hours using a magnetic stirrer. After adding 7mL of 0.1% NaTPP progressively, the liquid was stirred for two hours using a magnetic stirrer. The chitosan nanoparticle solution's dimension derived from *Scylla* sp shells was subsequently ascertained using the Particle Size Analyzer.

2.2.3. Chitosan nanoparticle solution preparation, including positive and negative controls

The procedure for preparing a nanochitosan involves researching [16] with modifications. To reach the desired concentration, powdered chitosan nanoparticles were dissolved in 1% acetic acid. Nanochitosan was employed in the following concentrations: 0.2%, 0.25%, 0.5%, 1%, and 1.25%. The research's positive control was the antibiotic ampicillin. Within a volumetric bottle of 100 mL, 10 g/mL of ampicillin powder was dissolved with distilled water until the 15-mark was reached. The same solvent used for chitosan, the negative control was 1% acetic acid.

2.2.4. Regeneration of bacteria

Regeneration of bacteria refers to [17] with modification. An erlenmeyer was used to 100 mL of distilled water was used to dissolve 2 g of NA, and the liquid was then heated until it dissolved fully and cooked. For fifteen minutes, to sanitize the NA solution, it was sterilized at 121 °C in autoclave. A 10 mL test tube was filled with the sterilized NA solution, and it was let to harden at a temperature between 30 and 45 °C. The bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* bacterial cultures from the main strain were transferred into solidified NA using an ose needle, and After that, they underwent incubation for 24 hours at 35°C.

2.2.5. Bacterial culture-4

The manufacture of liquid bacterial cultures refers to [18] which has been modified. After dissolving 0.4 g of sodium broth (NB) in 50L of aquadest, the mixture was put in an Erlenmeyer. The NB solution was brought to a boil, sterilized for 40 minutes at 121°C in an autoclave, and then allowed to cool.

2.2.6. Fourier Transform InfraRed (FTIR)-1 and Degree of Deacetylation

FTIR testing refers to [19], to interpret the results, 0.002 g of chitosan was put into a container and put on FTIR-QATRS. The resulting chromatogram is compared to the infrared table at a wavelength of 4000-400 cm−1. Domszy's baseline approach was utilized to determine the degree of deacetylation (DD%). Using the formula, The absorbance at 1655 cm-1 is divided by the absorbance at 3450 cm-1 to calculate DD.

$$DD = 100\left[\left(\frac{A1655}{A3450}\right) \times \frac{100}{1.33}\right]$$

$$\text{with (A1655) amida} = \text{Log}_{10}\left(\frac{DF1}{DE}\right) \ (A_{3450}) : \text{hydroxyl} = \text{Log}_{10}\left(\frac{AC}{AB}\right)$$

2.2.7. Particle Size Analyzer (PSA)-2

PSA analysis refers to [20], To make sure there were no air bubbles, a 1 mL sample was poured to the cell or cuvette. After that, a holder is used to position the cuvette on the Particle Size Analyser (PSA). The monitor screen will show the measurement results.

2.2.8. Chitosan Nanoparticles' Inhibitory Action Against Bacteria-3

The analysis of the inhibitory power of nanochitosan refers to [21] which has been altered. A 100 mL of aquadest were used to dissolve 3.8 grams of Mueller-Hinton agar (MHA), and the resulting mixture was then put in an Erlenmeyer and heated to a boil. An autoclave was used to sterilize the MHA solution for 15 minutes at 121ºC. A 15L petri dish containing the sterilizing MHA solution was left to solidify. A sterile cotton butt was used to flatten 10 L of liquid bacterial culture that had been obtained using a micropipette. The paper discs were treated with chitosan nanoparticle solutions at concentrations of P1(0.2%), P2(0.25%), P3(0.5%), P4(1%), and P5(1.25%). After that, the paper discs were put into MHA, which had been contaminated and incubated at 35° C for 24 hours. The clear zone that emerged was used to measure how well chitosan nanoparticles inhibited *S. aureus* and *S. epidermidis* bacteria.

2.2.9. Data analysis

A descriptive presentation of the chitosan quality (FTIR) and chitosan nanoparticle size (PSA) data analysis was made. The antibacterial inhibition of the chitosan nanoparticles created in this work was assessed using the analysis of variation tests with one factor—the addition of chitosan nanoparticles at the levels of P1(0.2%), P2(0.25%), P3(0.5%), P4(1%), and P5(1.25%). A 1% acetic acid was used as a negative control and 10 g/mL of ampicillin as a positive control. This study included three replications. The data was assessed at a 95% confidence level using ANOVA. If the variance analysis revealed a significant difference, further testing was conducted using the BNT test.

3. Result and Discussion

3.1. Fourier Transform Infrared Spectrophotometer (FTIR)

The interplay of matter and energy is the fundamental working principle of FTIR [19]. An infrared spectrometer was used to measure the degree of chitosan deacetylation and describe the chitosan that was isolated from mud crab shells. Figure 1 shows the range of chitosan found in mangrove crab shells. The interplay of matter and energy is the fundamental working principle of FTIR.

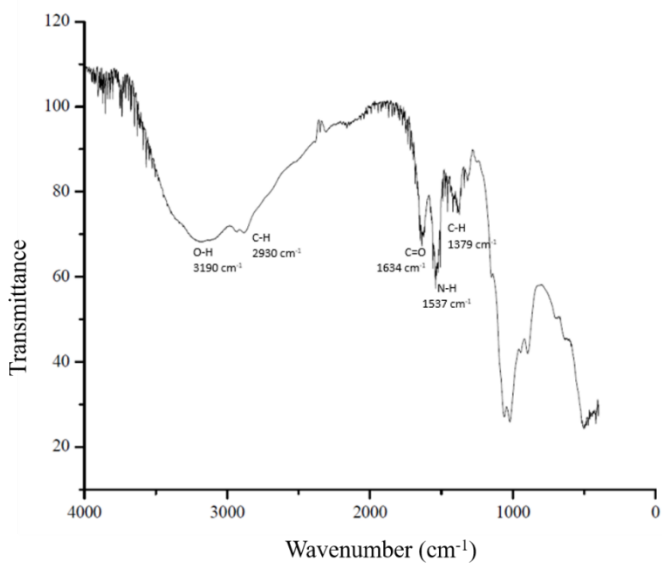

Fig. 1. FTIR spectrum of chitosan from *Scylla* sp

After that, the image is deciphered and contrasted with Table 1's standard chitosan infrared spectrum provided by the Institute for Research and Industrial Standards.

Table 1. FTIR Absorption peak of chitosan from *Scylla* sp

Functional groups	Wave Number (cm^{-1})	
	Standard*	Research result
OH stretching	3342.60	3190

Functional groups	Wave Number (cm^{-1})	
	Standard*	**Research result**
CH(CH3) bending	2922.80	2930
C=O (-NHCOCH3) stretching amide I	1660.55	1634
NH(-NHCOCH3) bending amide II	1587.94	1537
CH (-CH2) bending sym	1377.11	1379

Table 1: The spectra display the peak of OH absorption, a broadening peak at a wave number of 3190 cm-1. The C-H vibration of stretching exhibits absorption maxima at wave numbers between 1379 cm-1 and 2930 cm-1. The amide and bending vibrations are represented by the wavelengths 1634 cm-1 and 1537 cm-1, respectively, that are present in amides, respectively, was verified by the absorption peaks. The Research and Industrial Standards Institute's chitosan standard and the findings of this investigation do not differ substantially. The OH functional group is represented by wave number 3342.60, the stretching vibrations by waves 1377.11 and 2922.80, and the amide and bending vibrations by waves 1587.94 and 1660.55.

According to [15] At 1.655-1.310 cm-1, the O-H resonance with a discernible maximum in the amide bonding group and NH buckling separately with moderate intensity are represented by the absorption wave number of 3,350-3,300 cm-1 of commercial chitosan. Strengthened by [22] The presence of *Portunus pelagicus* in chitosan causes its vibrational pattern to exhibit carbonyl (C-O) stretching, stretching (O-H), and amine group (N-H) bending. The FTIR spectrum data from the investigation showed that the synthesis of chitosan was successfully completed.

3.2. Degree of Deacetylation

The percentage of DD generated can be used to assess the quality of chitosan. The chitin molecule chain (-COCH3) releases an extremely reactive amine group (-NH2) as a result of the deacetylation process. Because it can influence its physical characteristics and help decide the best usage for the finished chitosan product, one of the most important aspects of its manufacture is the degree of deacetylation. The mass of the molecular structure decreases as the degree of deacetylation rises because more acetyl groups are eliminated [23].

According to the baseline method estimate, 76% of the mangrove crab shell chitosan in this investigation was deacetylated [24]. Table 2 shows the level of deacetylation of the chitosan derived from *Scylla* sp. SNI No. 7949 and Protan Laboratory standards are already met, but the European Food Authority (EFSA) standards have not been met. Chitin has been transformed into chitosan for use in accordance with its designation, as confirmed by the resulting DD value of 76%.

Table 2. Level of mud crab shell chitosan deacetylation (DD)

DD*	Research result	Protan Laboratory (1987)	SNI: 7949 (2013)	EFSA (2010)
This research	76%	(≥70%)	(≥75%)	(≥90%)

According to [25] if deacetylation <70%, the polymer is called chitin; if it is >70%, it is called chitosan. According to [26] understanding the degree of deacetylation in chitosan is important since it will impact its utility. The degree of chitosan deacetylation increases the quantity of chitin that is successfully converted to chitosan.

The main factors influencing the DD of chitosan are the temperature utilized, the type of solvent utilized throughout the deacetylation process, and the time required to complete the process. [27] stated that Because the amount of alkali utilized, time, and temperature all affected the chitosan's DD, the chitosan deacetylation rate also rose as the concentration of NaOH increased. Nevertheless, the acetyl amide group's termination in chitosan and the depolymerization process might be brought on by an excessive concentration of NaOH. As a result, the DD decreases in value. The DD is influenced by temperature, time, particle size, and concentration. [28]. The higher the temperature, the more acetyl groups are released from chitin. Consequently, the value of the degree of deacetylation falls. Temperature, time, size of particles, and intensity all affect the degree of deacetylation. so the deacetylation observed in chitosan increases. The reaction will run slowly at low temperatures, while high temperatures can damage the structure of the base material.

3.3. Particle Size Analyzer (PSA)-2

The size of the resulting particles and a description of the chitosan nanoparticle quality were obtained using a Particle Size Analyzer (PSA). Utilizing a particle size analyzer (PSA), Figure 2 displays the chitosan particle absorption spectrum from *Scylla* sp shells.

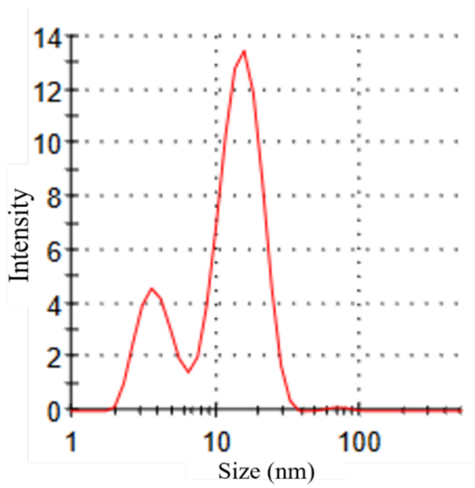

Fig. 2. PSA Spectrum of *Scylla* sp Shell Chitosan

With an average value of 47.04 nm and the highest intensity of 28.21 nm, Figure 2 displays the findings of the ionic gelation method used to analyze the size of the chitosan nanoparticles of mangrove crab shells. The study's findings verified that the chitosan generated was already nanoscale. The diameters of nanoparticles range from 1 to 100 nm. In this study, chitosan nanoparticles were made utilizing the ionic gelation process, which involves reducing their size using a high-speed magnetic stirrer. [29] indicated that a more uniform, stable particle size can be achieved by size reduction with a magnetic stirrer operating at a high speed. Stable nanoparticles are created throughout the drying process since there is no agglomeration between particles.

To prevent clumping in the chitosan solution, NaTTP and Tween 80 were added gradually. Each of these substances serves to fortify the chitosan particles, making them more difficult to break. [15] explained how Tween 80 and NaTTP may be added to chitosan to strengthen its mechanical capabilities. This allows the chitosan molecules to break ionic bonds with one another, control the size of the particles they contact with, and prevent them from happening.

The degree of particle size homogeneity with respect to a component is indicated by the Polydispersity Index (PI) value, which is a measure of particle dispersion. The Polydispersity Index (PI) result for this investigation was 0.258. These findings show that the final particle size is uniform. [30] said that a component has a uniform size level if its Polydispersity Index (PI) value is less. Particles between 100 and 300 nm are classified as excellent if their PDI value is less than 0.3.

3.4. Chitosan Nanoparticle Antibacterial Activity-3

The antibacterial capability of chitosan nanoparticles made from *Scylla* sp shells was assessed by looking at the inhibitory zone that was formed. The outcomes of determining the chitosan's inhibition zone diameter towards *S. aureus* and *S. epidermidis* are shown in Figure 3.

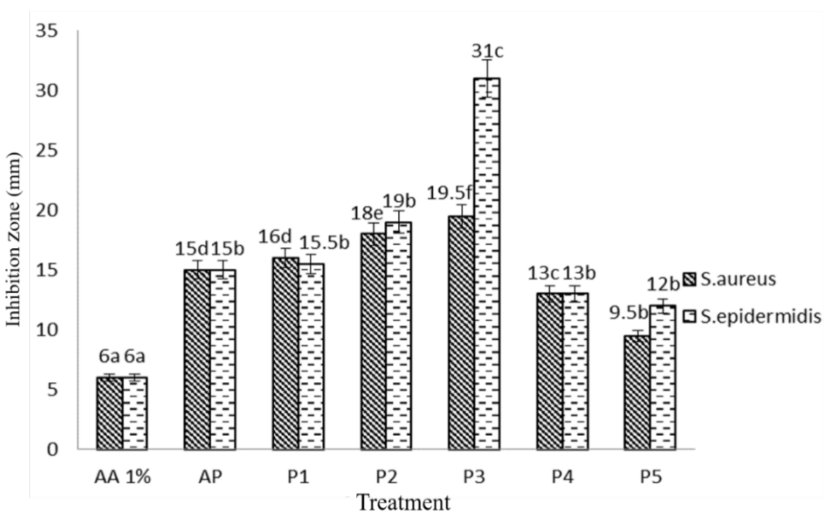

Fig. 3. The zones of inhibition of *Scylla* sp shell-derived chitosan nanoparticles against acne-causing bacteria (*S. aureus* and *S. epidermidis*) There is no discernible difference between letters on the graph that belong to the same alphabet (P>0.05).

With a 95% confidence level (α=0.05%), an ANOVA calculation demonstrated that chitosan nanoparticles might stop S. aureus bacteria from growing. The resulting inhibitory zone differed significantly from the chitosan nanoparticle concentration (Figure 3). According to this study, P3 chitosan nanoparticles (0.50%) were the most effective at inhibiting the development of *S. aureus* bacteria. Because P5's viscosity influences the diffusion process, P5 chitosan nanoparticle concentration (1.25%) had the lowest inhibitory power. [14] found that 1.25% chitosan nanoparticles exhibited mild inhibition and 0.50% chitosan nanoparticles had solid inhibition. The diffusion process at a concentration of 0.5% was superior to that at a concentration of 1.25%, resulting in weak inhibition because the concentration of 1.25% has a thicker texture. Therefore, the speed of antibacterial activity affected the ability to suppress the development of the test bacteria.

The further BNT test revealed that P1 (0.20%) was not substantially different from that of AP (ampicillin), while P3 (0.50%) was different from all treatments. According to Figure 3, the optimal inhibition zone for chitosan nanoparticles' antibacterial activity against *S. aureus* was 19.5±0.70 mm at P3 (0.50The area of inhibition produced by this study was noticeably larger than [14]. According to the latter, the inhibition zone of commercial nanoparticles of chitosan at a 0.50 concentration percent was 12.31 mm.

According to ANOVA calculations with a 95% confidence level (α=0.05%), Figure 3 demonstrated that chitosan nanoparticles may suppress the growth of *S. epidermidis* bacteria and that the amounts generated were significantly different. The AA treatment was significantly different from the concentrations of P1 (0.20%), P2 (0.25), P4 (1%), and P5 (1, 25%), according to the additional BNT test, whereas P3 (0.50%) was significantly different from all treatments. With an inhibition zone of 31±1.41 mm, P3 (0.50%) exhibited the greatest antibacterial activity of *Scylla* sp shell chitosan nanoparticles against *S. epidermidis* bacteria. The latter discovered that chitosan nanoparticles formed from fresh shrimp shelled components at a level of 0.5% had an inhibiting zone of 5.20 mm. This is due to chitosan nanoparticles' ability to collect amine groups (NH), which increases their potency in breaking down bacterial cell walls [31].

Because it can harm the protein found in the bacterial cell wall, acetic acid can stop bacteria from growing. The development of *S. aureus* and *S. epidermidis* bacteria can be inhibited by 6 mm by 1% acetic acid (negative control) (Figure 3). [14] found that 1% acetic acid may stop the growth of bacteria by 8.89 mm. Bacterial cell walls may sustain damage from 1% acetic acid due to the denaturation of cell wall proteins. Bacteria will die if their cell walls are damaged. When combined, chitosan and acetic acid can effectively prevent bacterial growth. [32]. In this study, mangrove crab shell chitosan nanoparticles shown moderate to strong antibacterial activity against *S. aureus* and *S. epidermidis* bacteria. The antibacterial activity of chitosan nanoparticles increases with concentration. If the inhibition zone is less than 5 mm, it is considered weak; if it is between 5 and 10 mm, it is classified as moderate; if it is between 10 and 19 mm, it is vital; and if it is more than 20 mm, it is classified as very strong. [33]. The degree of deacetylation (DD) in chitosan indicates its purity, which influences its antibacterial properties. The purer and more effective the DD chitosan is at inhibiting bacterial growth, the more of it is generated. The resulting DD in this study is 76%. Chitosan becomes more reactive due to the high number of amine groups that replace acetyl groups in its molecular chain, which increases with chitosan's DD [34].

Gram-positive *S. aureus* and *S. epidermidis* microorganisms were employed in this investigation. Gram-positive bacteria are more susceptible to the antibacterial effects of chitosan than gram-negative ones. This is due to the fact that chitosan readily adheres to bacteria as gram-positive bacteria lack an outer membrane. [35] described the two stages of chitosan's antibacterial activity against Gram-positive bacteria. The first stage entails cationic ionization chitosan adhering to Gram-positive bacteria's surface. Next, through a number of mechanisms, chitosan diffuses into Gram-positive bacteria and stops their cell development. In the first, chitosan attaches itself to the plasma membrane, renders proteins and enzymes inactive, damages DNA, and causes cellular function and metabolic processes that ultimately lead to cell death. [36] revealed that lipopolysaccharides make up the outer membrane of gram-negative bacteria. By penetrating the porin, the outermost layer of gram-negative bacteria, and arriving at the peptidoglycan layer, chitosan nanoparticles inhibit these bacteria. Additionally, chitosan nanoparticles will lyse cells by binding to proteins. [35] demonstrated that Gram-positive bacteria could be more receptive to chitosan in the form of long-chain ionic polymolecules. Therefore, chitosan's ability to inhibit Gram-positive bacteria was more potent.

4 Conclusion

This study concludes that according to the SNI and Protan Laboratory standard, the grade of *Scylla* sp shell chitosan nanoparticles has a degree of deacetylation. The chitosan nanoparticles were the typical size for nanoparticles. Acne-causing bacteria (*S. aureus* and *S. epidermidis*) were found to be inhibited by mangrove crab shell chitosan nanoparticles at the optimal concentration of 0.50 percent.

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References

1. M. McCarty, Evaluation and management of refractory acne vulgaris in adolescent and adult men Dermatol. Clin. **34**, 203 (2016). https://doi.org/10.1016/j.det.2015.11.007
2. S.O.N Fissy, R. Sari, L. Pratiwi, Effectivebess of Anti Acne Gel Containing Ginger Ethanol Extract (Zingiber officinale Rosc.Var. Rubrum) against Propionibacterium acnes dan Staphylococcus epidermidis J. Ilmu Kefarmasian Indones. **12**, 193–201 (2014). http://jifi.farmasi.univpancasila.ac.id/index.php/jifi/article/view/148
3. S.W. Huang, Y.J. Chen,S.T.Wang, L.W. Ho, J.K. Kao, M. Narita, M. Takahashi, C.Y. Wu, H.Y. Chengand, J.J. Shieh, Azithromycin impairs TLR7 signaling in dendritic cells and improves the severity of imiquimod-induced psoriasis-like skin inflammation in mice. J. Dermatol. Sci. **84**, 59–70 (2016). https://doi.org/10.1016/j.jdermsci.2016.07.007
4. S. Kardeh, N. Saki, F. Jowkar, B.Kardeh, S.A. Moein, M.H. Khorraminejad-Shirazi, Efficacy of Azithromycin in Treatment of Acne Vulgaris: A Mini Review World J. Plast. Surg. **8**, 127–34. (2019). https://doi.org/10.29252/wjps.8.2.127
5. T.J. Foster, J.A. Geoghegan,V.K. Ganesh, M. Höök, Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus Nat. Rev. Microbiol. **12**, 49–62 (2014). https://www.nature.com/articles/nrmicro3161
6. K. Nakase, H. Nakaminami, Y. Takenaka, N. Hayashi, M. Kawashima, N. Noguchi., Relationship between the severity of acne vulgaris and antimicrobial resistance of bacteria isolated from acne lesions in a hospital in Japan J. Med. Microbiol. **63**, 721–8 (2014). https://doi.org/10.1099/jmm.0.067611-0
7. J. Hafsa,M.A. Smach, B. Charfeddine, K. Limem, H. Majdouband S. Rouatbi, Antioxidant and antimicrobial proprieties of chitin and chitosan extracted from Parapenaeus Longirostris shrimp shell waste. Ann. Pharm. Fr. **74** 27–33 (2016). https://doi.org/10.1016/j.pharma.2015.07.005
8. M.Z. Elsabee, E.S Abdou , Chitosan based edible films and coatings: a review. Mater. Sci. Eng. C. Mater. Biol. Appl. **33**, 1819–41 (2013). https://doi.org/10.1016/j.msec.2013.01.010
9. A. Khan, K.D. Vu, B. Riedl, M. Lacroix, Optimization of the antimicrobial activity of nisin, Na-EDTA and pH against gram-negative and gram-positive bacteria LWT - Food Sci. Technol. **61**, 124–9 (2015). https://doi.org/10.1016/j.lwt.2014.11.035

10. M.S. Sivakami, T. Gomathi, J. Venkatesan, H.S. Jeong, S.K. Kim, P.N. Sudha, Preparation and characterization of nano chitosan for treatment wastewaters Int. J. Biol. Macromol. **57**, 204–12 (2013). https://doi.org/10.1016/j.ijbiomac.2013.03.005
11. K. Divya, S. Vijayan, T.K. George, M.S. Jisha, Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity Fibers Polym. **18**, 221–30 (2017). https://link.springer.com/article/10.1007/s12221-017-6690-1
12. I. Francolini, G. Donelli, C. Crisante, V. Tarescoand A. Piozzi, Antimicrobial polymers for anti-biofilm medical devices: state-of-art and perspectives BT - biofilm-based healthcare-associated infections: volume II *Biofilm-based Healthcare-associated Infections* ed G Donelli (Cham: Springer International Publishing) pp 93–117 (2015). https://doi.org/10.1007/978-3-319-09782-4_7
13. R. Ikono A. Vibriani, I. Wibowo, K.E. Saputro W. Muliawan, B.M. Bachtiar, E. Mardliyati, E.W. Bachtiar, N.T. Rochman, H. Kagami, L Xianqi, T. Nagamura-Inoue, A. Tojo, Nanochitosan antimicrobial activity against Streptococcus mutans and Candida albicans dual-species biofilms. BMC Res. Notes. **12**, 383 (2010). https://doi.org/10.1186/s13104-019-4422-x
14. A.K Magani, T.E. Tallei, B.J. Kolondam, Uji Antibakteri Nanopartikel Kitosan terhadap Pertumbuhan Bakteri Staphylococcus aureus dan Escherichia coli. J. Bios Logos. **10**, 7 (2020). https://ejournal.unsrat.ac.id/v3/index.php/bioslogos/article/view/27978
15. N. Luthfiyana, S. Bija, C.D. Nugraeni, M.S. Lembang, E. Anwar, D.R. Laksmitawati, Nusaibah, P.W. Ratrinia, Mukmainna, Characteristics and antibacterial activity of chitosan nanoparticles from mangrove crab shell (Scylla sp.) in Tarakan Waters, North Kalimantan, Indonesia Biodiversitas. **23**, 4018–25 (2022). https://doi.org/10.13057/biodiv/d230820
16. M.S. Benhabiles, R. Salah, H. Lounici, N. Drouiche, M.F.A. Goosen, N. Mameri, .Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste Food Hydrocoll. **29**, 48–56 (2012). https://doi.org/10.1016/j.foodhyd.2012.02.013
17. R.M. Abdel-Rahman, R. Hrdina, A.M. Abdel-Mohsen, M.M.G. Fouda, A.Y. Soliman, F.K. Mohamed, K. Mohsin, T.D. Pinto, Chitin and chitosan from Brazilian Atlantic Coast: Isolation, characterization and antibacterial activity Int. J. Biol. Macromol. **80**, 107–20 (2015). https://doi.org/10.1016/j.ijbiomac.2015.06.027
18. N.Patibha, D. Sushmaand G. Rajinder, Screening for antioxidant and antibacterial potential of common medicinal plants in the treatment of acne. Int. J. Drug Dev. Res. **4**, 65–71 (2012). http://www.ijddr.in
19. H. El Knidri, R. Belaabed, A. Addaou, A. Laajeb, A. Lahsini, Extraction, chemical modification and characterization of chitin and chitosan. Int. J. Biol. Macromol. **120**, 1181–9 (2018). https://doi.org/10.1016/j.ijbiomac.2018.08.139
20. T.K. Sau, A. Pal, N.R. Jana, Z.L. Wang, T. Pal, Size controlled synthesis of gold nanoparticles using photochemically prepared seed particles. J. Nanoparticle Res. **3**, 257–61 (2001). https://link.springer.com/article/10.1023/A:1017567225071
21. G.J.M. Christensen, C.F.P. Scholz, J. Enghild, H. Rohde, M. Kilian, A.Thürmer, E. Brzuszkiewicz, H.B. Lomholt, H. Brüggemann H, Antagonism between Staphylococcus epidermidis and Propionibacterium acnes and its genomic basis. BMC Genomics. **17**, 152 (2016). https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-016-2489-5
22. N.M. Ahyat, F. Mohamad, A. Ahmad, A.A. Azmi, Chitin and chitosan extraction from Portunus pelagicus Malaysian. J. Anal. Sci. **21**, 770–7 (2017). https://doi.org/10.17576/mjas-2017-2104-02
23. M. Mathaba, M.O. Daramola, Effect of chitosan's degree of deacetylation on the

performance of PES membrane infused with chitosan during AMD treatment Membranes (Basel). **10**, (2020). https://doi.org/10.3390/membranes10030052
24. J.G. Domszy, G.A.F. Roberts, Evaluation of infrared spectroscopic techniques for analysing chitosan Die Makromol. Chemie. **186**, 1671–7 (1985). https://doi.org/10.1002/macp.1985.021860815
25. D. Wanule, D.J. Balkhande, P. Ratnakar, A. Kulkarni, C. Bhowate , Extraction and FTIR analysis of chitosan from american cockroach, Periplaneta americana. Int. J. Eng. Sci. Innov. Technol. **3**, 299–304 (2014). https://doi.org/10.7324/JAPS.2022.120321
26. G. Hao, Y. Hu, L. Shi, J. Chen, A. Cui, W. Weng, K. Osako, Physicochemical characteristics of chitosan from swimming crab (Portunus trituberculatus) shells prepared by subcritical water pretreatment. Sci. Rep. **11**, 1646 (2021). https://www.nature.com/articles/s41598-021-81318-0
27. N.A. Musmade, L. Mahatma, Extraction and characterization of chitosan by simple technique from mud crabs. Int. J. Curr. Microbiol. Appl. Sci. **10**, 513–8 (2021). https://doi.org/10.20546/ijcmas.2021.1006.055
28. J. Cheng, H. Zhu, J. Huang, J. Zhao, B. Yan, S. Ma, H. Zhang, D. Fan, The physicochemical properties of chitosan prepared by microwave heating. Food Sci. Nutr. **8**, 1987–94 (2020). https://doi.org/10.1002/fsn3.1486
29. S. Sungkharak, N. Supasit, S. Choopan, Antibacterial activity against acne involved bacteria of chitosan in a soluble state and as nanoparticles. Chiang Mai J. Sci. **43**, 1150–9 (2016). http://epg.science.cmu.ac.th/ejournal/
30. Y. Luo, T. Wang, P. Chen, J. Sun, Q. Wang, Encapsulation of indole-3-carbinol and 3, 3'-diindolylmethane in zein/carboxymethyl chitosan nanoparticles with controlled release property and improved stability Food Chem. **139**, 224–30 (2013). https://doi.org/10.1016/j.foodchem.2013.01.113
31. J. Li, S. Zhuang, Antibacterial activity of chitosan and its derivatives and their interaction mechanism with bacteria: current state and perspectives. Eur. Polym. J. **138**, 109984 (2020). https://doi.org/10.1016/j.eurpolymj.2020.109984
32. I.J. Winayu, N. Ekantari, I. Puspita, Ustadi, W. Budhijanto, P. Nugraheni, The effect of reduced acetic acid concentration on nano-chitosan formulation as fish preservative. International Conference on Food Science and Engineering (IOP Publishing) pp 0–6 (2019). https://doi.org/10.1088/1757-899X/633/1/012040
33. C.R. Alfath, V. Yulina, Sunnati, Antibacterial effect of Granati fructus cortex extract on Streptococcus mutans In Vitro. J. Dent. Indones. **20**, 5–8 (2013). https://doi.org/10.14693/jdi.v20i1.126
34. M.K. Rasweefali, S. Sabu, K.V. Sunooj, A. Sasidharan, K.A.M. Xavier, Consequences of chemical deacetylation on physicochemical, structural and functional characteristics of chitosan extracted from deep-sea mud shrimp Carbohydr. Polym. Technol. Appl. **2**, 100032 (2021). https://doi.org/10.1016/j.carpta.2020.100032
35. C. Duan, X. Meng, J. Meng, M.I.H. Khan, L. Dai, A. Khan, X. An, J. Zhang, T. Huq, Y. Ni, Chitosan as A preservative for fruits and vegetables: A review on chemistry and antimicrobial properties. J. Bioresour. Bioprod. **4**, 11–21(2019). https://doi.org/10.21967/jbb.v4i1.189
36. M. Zahid, M. Ashraf, M. Arshad, G. Muhammad, A. Yasmin, H.M.A. Hameed, Antimicrobial activity of bacteriocins isolated from lactic acid bacteria against resistant pathogenic strains. Int. J. Nutr. Food Sci. **4**, 326–31 (2015). https://doi.org/10.11648/j.ijnfs.20150403.20