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Characteristics and antibacterial activity of chitosan nanoparticles from mangrove crab shell (*Scylla* sp.) in Tarakan Waters, North Kalimantan, Indonesia

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Abstract. *Luthfiyana N, Bija S, Nugraeni CD, Lembang MS, Anwar E, Laksmitawati DR, Nusaibah, Ratrinia PW, Mukmainna. 2022. Characteristics and antibacterial activity of chitosan nanoparticles from mangrove crab shell (*Scylla *sp.) in Tarakan Waters, North Kalimantan, Indonesia. Biodiversitas 23: 4018-4025.* The aim of this study was to determine the characteristics and antibacterial activity of chitosan nanoparticles from mangrove crab shells (*Scylla* sp.) in Tarakan Waters, North Kalimantan, Indonesia. Chitosan was produced from deproteination, demineralization, and deacetylation processes. Nanoparticles of chitosan were produced by the ionic gelation method. The quality of produced chitosan was tested, and characterized the obtained nanoparticles of chitosan. The results showed that the yield of chitosan was 5.82 ± 0.02%, moisture 1.66 ± 0.05%, ash 1.59 ± 0.21%, nitrogen 1.32 ± 0.06%, and degree of deacetylation (DD) 76 ± 0.00%. All results were in accordance with the Protan Laboratory and Food Safety Authority (EFSA) 2010 standard. Next, the characteristics of chitosan nanoparticles showed that the highest intensity was 15.69 nm, with a polydispersity index of 0.346 and a zeta potential of -26.1 mV. The morphology of chitosan nanoparticles was observed by scanning electron microscopy, energy-dispersive x-ray spectroscopy (SEM-EDX) at 7,500 times magnification. Several elements, such as C and O, were found as constituent elements of chitosan, whereas Mg, Al, P, and Ca, were metal impurities of the shell. Na was derivate from NaTTP, a reactant from mangrove crab nano chitosan. The results of antimicrobial activity revealed that 1% (P4) chitosan extract showed highest zone of inhibition i.e. 13.55 mm and 13.43 mm against *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively.

Keywords: Chitosan, ionic gelation, SEM-EDX, *Staphylococcus aureus*, *Staphylococcus epidermidis*, zeta potential

INTRODUCTION

Mangrove crab (*Scylla* sp.) has become one of the pioneers of macroeconomics (Khotimah et al. 2018) and the mainstay of non-oil and gas export commodities in Tarakan City, North Kalimantan, Indonesia. The data obtained by the Ministry of Maritime Affairs and Fisheries revealed that the export volume increased by 13.25% from 2019 to 2020 (Amalia et al. 2021). In fact, the high consumption of crab meat leads to increased shell waste. Mangrove crab shell waste reached 40%-60% of the total weight of crabs (Azizi et al. 2020). Mangrove crab shell waste that accumulates can cause environmental pollution. A strategy is needed to deal with the pile of mud crab shell waste by making chitosan. Chitosan is a by-product of mud crab shell waste, which is economically useful. These products solve various issue, including environmental and waste management can use products such as these (El Knidri et al. 2018). Chitin from crab shell waste is a profitable asset. This raw material is a cost-effective and renewable resource because it is extracted from crustacean waste generated from the seafood industry (Sivanesan et al. 2021).

The biopolymer extracted from chitin is usually obtained from the exoskeleton of crustaceans, especially crabs, is chitosan. Chitosan is polyglucosamine or scientifically known as 1, 4-2 acetamido-2-deoxy-d-glucose. This compound is produced from chitin by deacetylation process in high temperature and high alkali concentration (Gupta and Diwan 2016). Chitosan has been studied for the development for various applications due to the availability of abundant raw materials available in nature, biocompatibility, non-toxic, biodegradability mucoadhesive properties, and no side-effects produced by bio-decomposition in the environment (Bellich et al. 2016). Wastewater purification can use chitosan which is also applied in various industrial fields, such as food, pharmaceutical, health, and the environment industries (Chattopadhyay et al. 2019). Chitosan has antibacterial activity against gram-positive and gram-negative bacterial species. Damage to microbial cell walls caused by the binding of chitosan cationic sites to anionic surfaces,

causing cell death (Ramezani et al. 2015). Modified chitosan has bactericidal properties against several types of bacteria (Tan et al. 2013). Research with the aim of increasing the ability of chitosan as an active substance continues to be carried out, and one way is to modify the size of chitosan by making chitosan nanoparticles.

Dispersal of particulates or solid particles with sizes in the range of 10-1000 nm is the definition of chitosan nanoparticles (Kreuter 2001). The nanoparticles can penetrate the intercellular space impenetrable by the size of colloidal particles which is one particular characteristic of nanoparticles (Martien et al. 2012). The advantages of chitosan nanoparticles are larger surface area, and enhance stable chitosan adsorption power and can improve delivery capability (Harahap 2012). Due to the ratio factor and high surface area, nano-sized chitosan (nanoparticles, nanomaterials) is more effective in penetrating and disrupting bacterial cell membranes (Vellingiri et al. 2013). Chitosan nanoparticles can combine natural or chemical compounds, antimicrobial agents, antioxidants, enzymes, or active substances such as plant extracts, probiotics, minerals, or vitamins. Chitosan nanoparticles show higher antimicrobial activity than chitosan (Ramezani et al. 2015). The ionic gelation method is the most ordinary method applied by chitosan nanoparticles. The gelation method utilizes the positively charged -OH group of chitosan to form ionic interactions with negatively charged polyanions or crosslinkers (Kunjachan et al. 2010). In ionic gelation method, chitosan dissolved in an acid solution to obtain cationic chitosan (Hu et al. 2013). Next, anionic tripolyphosphate (TPP) solution is added to the solution to produce nano-sized particles (Bhattarai et al. 2006). Tripolyphosphate is considered as the best crosslinking agent (Mohanraj and Chen 2006).

There have been many research studies related to the manufacture of chitosan nanoparticles and their methods. Versatile nutraceutical obtained from the preparation and characterization of nanochitosan derived from exoskeleton waste (Sivanesan et al. 2021), the beads-milling method can be used with crab shell waste (Rochima et al. 2017), the aqueous phase process is used to remove Fe (II) and Mn (II) in shrimp shells (Ali et al. 2018), nanochitosan from (*Xylotrupes gideon*) can reduce the surface roughness value of GIC at critical saliva pH (Pratiwi et al. 2021). Therefore, the aim of this study was to investigate the characteristics and antibacterial activity of chitosan nanoparticles from mangrove crab shell (*Scylla* sp.) in Tarakan Waters, North Kalimantan, Indonesia.

MATERIALS AND METHODS

Raw material

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

Preparation of chitosan

The manufacture of chitosan through the stages of deproteination, demineralization, and deacetylation was performed according to the modified method of Younes et al. (2014). 200 g of mud crab shell powder was neutralized in distilled water (pH 7) and then dried at 80°C. The deproteination process was performed using 3N NaOH solvent (1:10 w/v) at 80°C using a hot plate under continuous stirring for 60 minutes. Chitin was formed after going through the process of deproteination and demineralization. Then, chitin was processed into chitosan through a deacetylation step using 60% NaOH in a ratio of 1:10 at 140°C for 60 minutes with stirring. The sample was neutralized again using aquadest (pH 7) and dried at 80°C. Samples were neutralized with distilled water (pH 7). The samples were filtered and dried in an oven at 80°C. Based on the method described by Cahyono (2018), the result was calculated by weighing the resulting chitosan and then divided by the weight of processed dry raw material.

$$\text{yield \%} = \frac{\text{Dry chitosan mass (g)}}{\text{Dry raw material mass (g)}} \text{ x } 100$$

The quality of chitosan was assessed by analyzing the water, ash, and nitrogen content based on the AOAC (2005). FTIR spectra was used to calculate the yield of chitosan using wavelengths ranging from 4,000-400 cm^{-1}. The DD% was calculated from the ratio between the absorbance at 1,655 cm^{-1} and 3,450 cm^{-1}:

$$DD\% = 100 \left[\left(\frac{A_{1,655}}{A_{3,450}}\right) \text{ x } \frac{100}{1.33}\right]$$

Preparation of nanochitosan

Nanochitosan was manufactured by ionic gelation method according to Dong et al. (2022) with modifications. 0.2 g chitosan was dissolved in 100 ml of 1% acetic acid solution. The chitosan solution was stirred at 3,000 rpm with a magnetic stirrer for 8 hours. The formulation of nanochitosan was carried out through emulsification by slowing adding (Tween 80) 0.1% of 50 µL and stirring for 2 hours with a magnetic stirrer. The stabilization step was carried out by adding 7 ml of 0.1% NaTPP surfactant slowly and stirring for 2 hours with a magnetic stirrer.

Particle size analyzer (PSA) was used to determine the chitosan nanoparticles size and zeta potential. The nanochitosan sample was dissolved with distilled water and then put into a cuvette for measurement (Muller et al. 2000). Characterization of nanochitosan from mud crab shells was followed by morphological analysis using scanning electron microscopy, energy-dispersive X-ray spectroscopy (SEM-EDX). Next, the chitosan nanoparticle solution was ground into powder using freeze-drying. The

nanochitosan powder was affixed on the carbon tax in the form of a rectangle (±0.5 cm). This analysis was performed with a low vacuum because the sample was non-conductor. The analyzed nanochitosan can be viewed on a monitor, and the magnification was adjusted (Girao et al. 2017).

Antimicrobial activity of nanochitosan

Antibacterial activity test of nanochitosan from mud crab shell was performed with modification according to Al-Zahrani (2021). This test included the preparation of nanochitosan test solutions, liquid media (nutrient broth), solid media (Nutrient Agar and Mueller Hinton Agar), bacterial refreshment, and bacterial rejuvenation antibacterial activity test. The test bacteria used were *Staphylococcus aureus* FNCC 0047 and *Staphylococcus epidermidis* FNCC 0048, obtained from the Center for Food and Nutrition Studies (PSPG) Gadjah Mada University, Yogyakarta, Indonesia. The antibacterial activity test was carried out by disc diffusion method.

The nanochitosan test solution was made according to Rahman (2012) with the modification of concentration. The concentrations of chitosan used were 0.25%, 0.5%, 0.75%, and 1%. 10 mg/ml of ampicillin antibiotic was used as a positive control and 1% acetic acid was used as a negative control. A total of 3.8 g of Mueller Hinton Agar (MHA) was dissolved in 100 ml of distilled water and then put into an Erlenmeyer and heated at a temperature of 120˚C. The MHA solution was then sterilized in an autoclave at 121˚C for 40 minutes. The sterilized MHA media 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 spreader. Paper disc containing test samples, positive control, and negative control were placed in the MHA media. Antibacterial activity was measured by observing the inhibition zone formed around the paper disc and experiment was performed twice.

RESULTS AND DISCUSSION

Quality of chitosan

In this study chitosan from the shells of mangrove crab was analyzed for quality based on water, ash, nitrogen, and degree of deacetylation (DD). This study compared the quality of chitosan based on Protan Laboratory and Food Safety Authority (EFSA) 2010. The quality of chitosan from mangrove crab shells is present in Table 1.

Yield chitosan

The yield of chitosan was obtained from the percentage ratio between the weight of chitosan produced and the weight of sample from crab shells before processing. Based on the results, yield of chitosan was 5.82 ± 0.02% (Table 1). The chitosan yield obtained in this study is similar to that of Bolad et al. (2010), who reported that the chitosan yield of crab shell was as 4.65%. The yield of chitosan may be affected by the use of NaOH. The higher concentration of NaOH used, the lower the yield produced. The high concentration of NaOH causes the depolymerization process of chitosan molecular chain, eventually leading to a decrease in chitosan molecular weight. This is in accordance with the statement of Hossain et al. (2014), who stated that yield variable of chitosan might be due to depolymerization of the chitosan polymer, loss of sample mass/weight from excessive removal of acetyl groups from the polymer during deacetylation, and loss of chitosan particles during washing. Cahyono (2018) reported that several factors affect the percentage of chitosan yield, including particle size, reagents, temperature, and the type of raw materials used. According to Bolat et al. (2010), the differences of chitin percentages in crabs shells was influenced by species and surrounded environment, such as temperature and season.

Moisture content

Moisture is one of the crucial parameters in determining the quality of chitosan. The moisture content was 1.66±0.05% which complied with chitosan quality standard of the Protan Laboratory and EFSA (2010) (Table 1). Hossain and Uddin (2020) stated that water absorption during the storage process, relative humidity and light can affect the moisture content of chitosan produced, and it is hygroscopic. According to Mulia et al. (2020), water content in chitosan is suspected to be low because chitosan contains acetyl groups that are hydrophobic or dislike or water-repellent. The hydrophobic nature causes the inability to bind water.

Ash content

Chitosan ash content was 1.59±0.21% according to the quality requirements of Protan Laboratory and EFSA (2010) (Table 1). The successful outcome parameter in the demineralization process in the chitin isolation was the ash content. The ash content of chitosan products determines the purity level of the chitosan produced. According to Kania et al. (2020), testing the ash content of chitosan showed that inorganic compounds are contained in the samples of raw materials used. Hao et al. (2021) reported that Ca, Mg, Na, K, and Fe are the most frequent chemical compounds found in crustacean shells. The concentration of HCl, the solvent used, the demineralization temperature, the length of time and stirring, and the washing process were also measured in this experiment. The lower the ash content produced, the higher the quality and level of chitosan purity.

Table 1. Quality of chitosan from mangrove crab shells

Quality parameters	Results (%)	Standards (%)	
		Protan laboratory	EFSA 2010
Yield	5.82 ± 0.02	-	-
Moisture	1.66 ± 0.05	≤ 10	≤ 10
Ash	1.59 ± 0.21	≤ 2	≤ 3
Nitrogen	1.32 ± 0.06	-	≤ 6
Degree of deacetylation (DD)	76 ± 0.00	≥ 70	≥ 90

Note: The results were expressed as mean ± standard deviation of three replications

Nitrogen content

Based on the study results, nitrogen content was 1.32 ± 0.06% which was in accordance with the chitosan quality standard of EFSA (2010) (Table 1). Poeloengasih et al. (2008) also noted that the nitrogen content of chitosan was in the range of 3.56%-7.59%. Demineralized chitosan exhibits high nitrogen content. In addition, nitrogen levels can also be affected by the presence of an amino group (NH2). Chitosan exhibits high chemical reactivity due to the presence of an amine group (NH2), so that chitosan can dissolve in the acetic acid solvent and bind to water. Modaso et al. (2013) stated that N element in each chitosan monomer expressed as an active group because the element is associated with high nitrogen content in the polymer chain.

Degree of deacetylation (DD)

The results showed that the degree of deacetylation was obtained by 76%. The ratio of sample HCl in the demineralization process affected the degree of deacetylation of chitosan produced. A higher amount of HCl demonstrates a higher degree of deacetylation. The degree of deacetylation (DD) is one of the crucial parameters that affect the characteristics of chitosan such as biological, physicochemical, and mechanical properties (Metin et al. 2019). Several researches reported that DD% is influenced by several factors such as base concentration, reaction time, and reaction temperature during deacetylation (Fatima 2020). However, DD% cannot be reached up to 100% because a copolymer exists between N-acetylglucosamine and glucosamine, which supports the creation of biocompatible, biodegradable, and adsorption properties (Cheng 2020).

The FTIR spectra of chitosan showed the band at 3,190 cm-1 corresponded to O-H vibration, whereas 2,930 and 1,379 cm^{-1} corresponded to C-H stretching. The C=O stretching and N-H bending in amides groups were separately at 1,634 cm^{-1} and 1,537 cm^{-1}, respectively (Figure 1). Similarly, observed absorption peak in commercial chitosan was 3,350-3,300 cm^{-1} corresponding to O-H vibration with strongly broad peak and NH-bending in amides groups was separately at 1,655-1,310 cm^{-1} with medium intensity (Samimi Gharaie et al. 2018). FTIR vibration patterns from crabs *Portunus pelagicus* also showed stretching of hydroxyl (O-H), amine (N-H) and carbonyl (C-O) groups indicated the presence of chitosan (Ahyat 2017). The FTIR spectra data in the present study indicated that the chitosan synthesized was successfully formed.

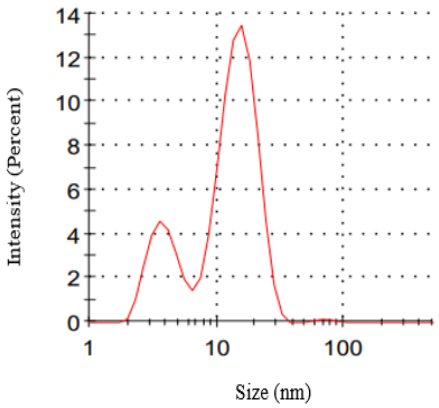

Figure 2. Size distribution of nanochitosan

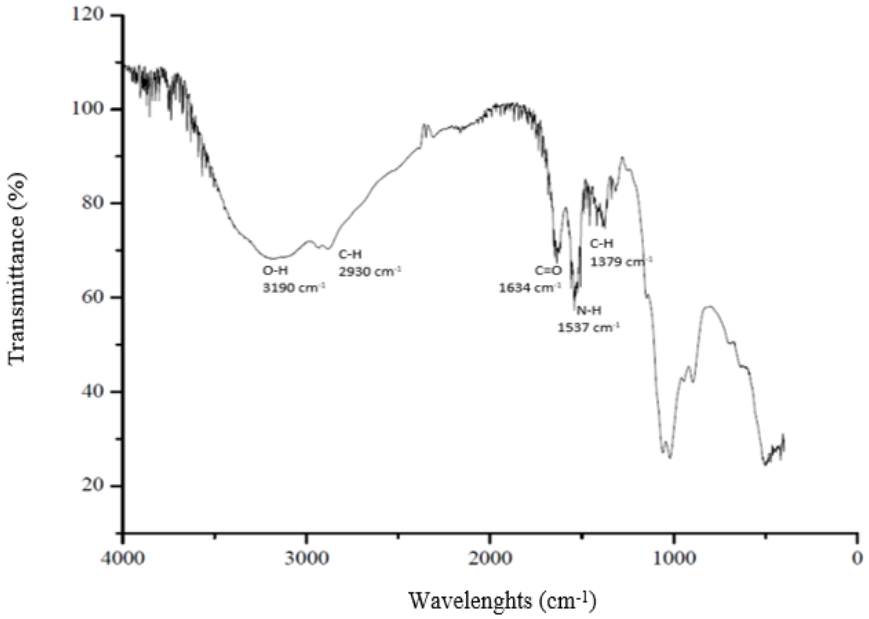

Figure 1. FTIR spectrum of the mangrove crab shell (*Scylla* sp.)

Characterization of nanochitosan

Particle size analyzer

Particle size analyzer (PSA) can be used to analyze the particles of sample (Sau et al. 2001). The nanochitosan from mangrove crab shells characterization is presented in Figure 2.

Based on particle size analyzer (PSA) measurements, the nanochitosan size was obtained based on the highest intensity of 15.69 nm with a PDI value of 0.346. This size of nanochitosan indicates that the nanoparticles were formed. According to Hosokawa et al. (2007), nanoparticles are particles measuring 1-100 nm. Qonitannisa et al. (2020) reported the polydispersity index (PDI) value describing the particle size distribution. A good PDI value indicates good long-term stability and small PDI value indicates the stability of nanoparticle size.

Zeta potential

The quality of nanoparticles yield is defined by zeta potential. Zeta potential is a parameter of electric charge between colloidal particles. The greater the zeta potential value, the more it inhibits flocculation (the event of merging colloids from small to large). The zeta potential measurement on mud crab shell nanochitosan is presented in Figure 3.

Based on the results, zeta potential value was -26.1 mV. The zeta potential value indicates the stability of nanoparticle dispersion system formed. According to Honary et al. (2013), particles with a significant positive or negative zeta potential value cause a repulsive force between particles. In contrast, if the zeta potential value is low, both positive and negative, it causes an attractive force between particles, causing the particles to join and become unstable.

The high solubility in acetic acid causes the potential value of acetic acid to be high and stable. The obtained value was inclined towards negative due to the influence of acetic acid, which exhibits a negative charge. This large charge causes a repulsive force between the nanoparticles formed to prevent aggregation into large sizes. According to Teguh (2013), chitosan is more soluble in acetic acid.

SEM-EDX

The obtained chitosan nanoparticles were seen for their morphology and compared with chitosan using scanning electron microscopy, energy-dispersive X-ray spectroscopy (SEM-EDX) presented in Figure 4.

Scanning electron microscopy (SEM) with 1,000 times magnification revealed that the chitosan of mud crab shells exhibited a large flake shape with an irregular surface (Figure 4A1). Chitosan nanoparticles produced by ionic gelation showed homogeneous (uniform) morphology at 7,500 times magnification (Figure 4B1). In this study, nanochitosan was formed using the ionic gelation method, and the resulting size was 184.9-381.8 nm. According to Veronika (2015), nanoparticles are particles measuring 10-1000 nm. The high speed magnetic stirrer can equalize the energy received by the solution used for size reduction, producing more homogeneous particle size. Putri et al. (2018) reported that the addition of NaTTP can increase the strength of the chitosan matrix, thus making the nanoparticles stronger and harder to split. The addition of surfactant Tween 80 could reduce the size of chitosan nanoparticles and prevent agglomeration between particles.

Nano energy dispersive X-ray spectroscopy EDX is a non-destructive X-ray analysis used to identify material composition (Kweinor et al. 2021; Sultan 2022; Zhou 2022). The result of EDX showed the elemental structures that formed the peaks (Figure 4). Chitosan contains several elements, namely carbon, oxygen, magnesium, aluminum, phosphorus, and calcium (Figure 4A2). The presence of carbon and oxygen were the constituent elements of chitosan, while magnesium, aluminum, phosphorus, and calcium were metal impurities from the initial component of chitosan, crab shells. Figure 4B2 shows an additional sodium element from NaTTP, a reactant to produce nanochitosan. Chitosan and nanochitosan exhibited different weight percentages. The difference in weight percentage was due to the addition of elements NaTTP, sodium, oxygen, and phosphorus, resulting in nanochitosan demonstrating a higher rate of sodium, oxygen, and phosphorus than chitosan.

Antibacterial activity of nanochitosan

The results of the diameter of inhibition zone of chitosan against *Staphylococcus aureus* and *Staphylococcus epidermidis* is presented in Figure 5.

Statistical analysis showed that nanochitosan could inhibit the growth of *S. aureus*, and the concentration of nanochitosan exhibited a significant effect on the inhibition zone (Figure 5a). The higher the given concentration of nanochitosan, the larger the zone of inhibition formed to inhibit *S. aureus*. According to Chandrasekaran et al. (2020), concentration of nanochitosan also increases the antibacterial activity. The LSD follow-up test showed that the treatment was not significantly different from 0.25% (P1) to 0.75% (P3), but the three treatments were significant at 1% (P4). The best antibacterial activity of nanochitosan against *S. aureus* was achieved at a concentration of 1%, with the largest zone of inhibition of 13.55 mm.

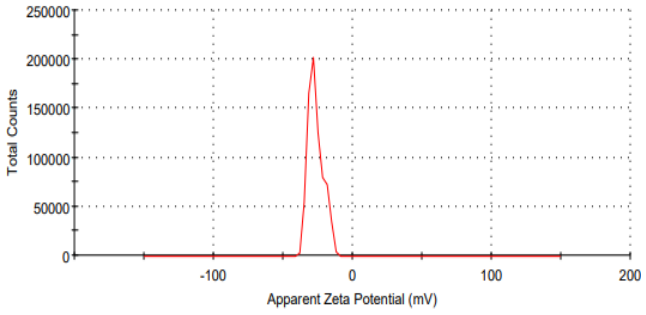

Figure 3. Zeta potential of nanochitosan

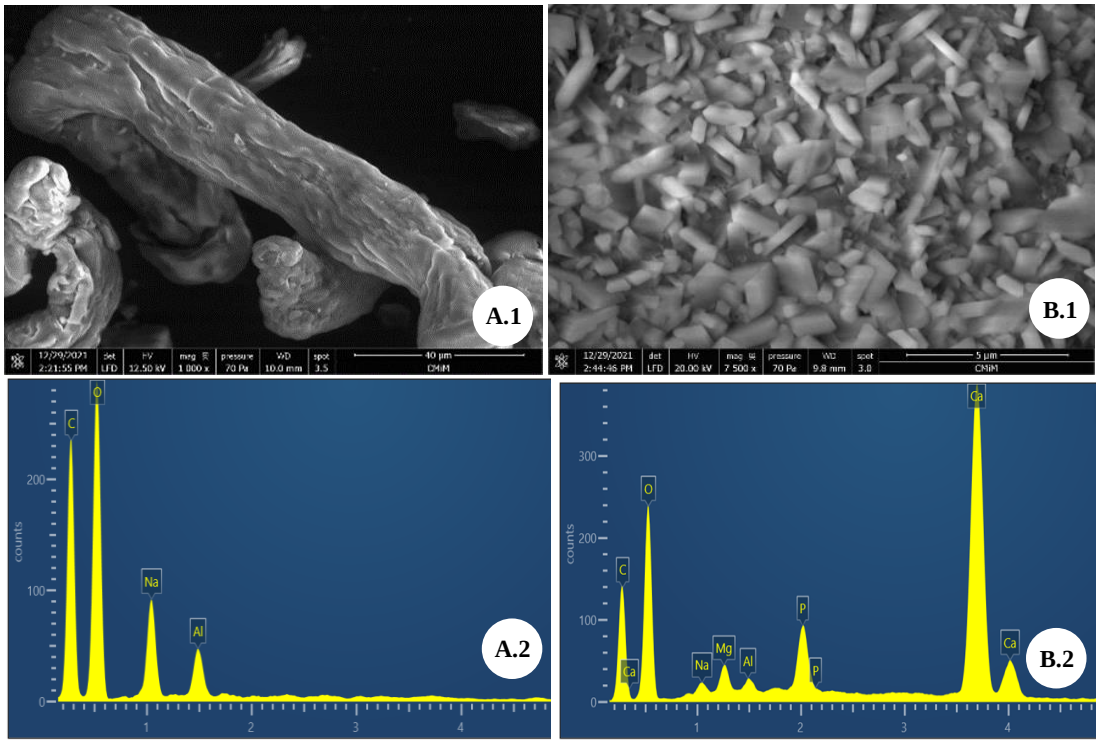

Figure 4. Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX) (A1, A2) chitosan, (B1, B2) nanochitosan

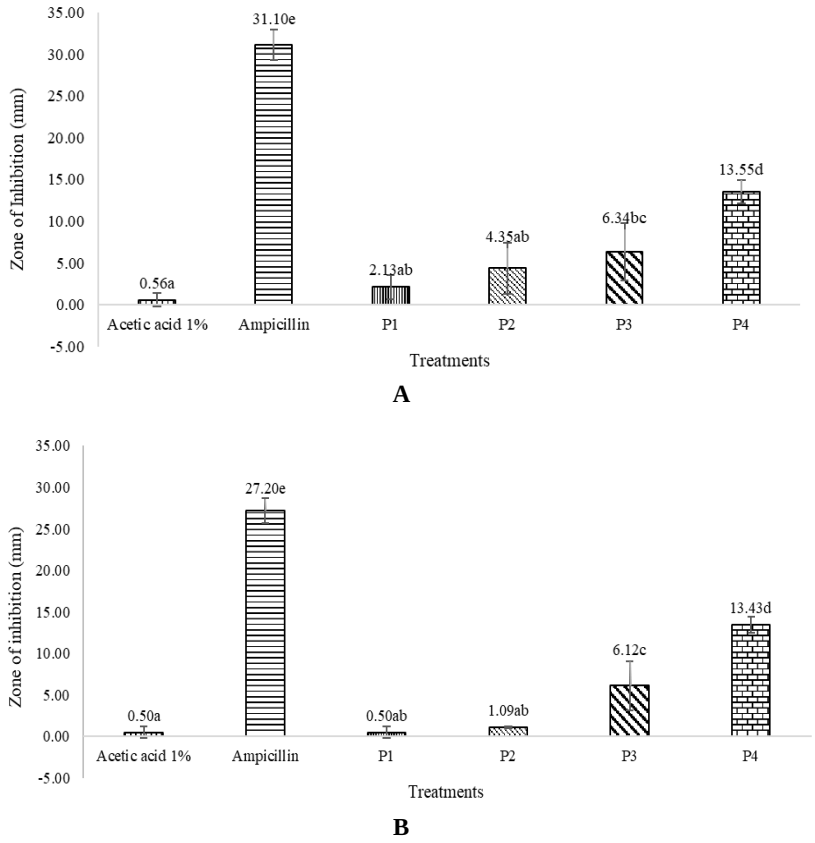

Figure 5. Inhibition zone of mud crab shell chitosan against bacteria: A. *Staphylococcus aureus*; B. *Staphylococcus epidermidis* treated with ampicillin (positive control), 1% acetic acid (negative control), P1 (0.25%), P2 (0.50%), P3 (0.75%), P4 (1%). Note: letters from the same alphabet indicate non-significant at $p < 0.05$. The data were expressed as mean ± standard deviation of three replications

The antibacterial activity of nanochitosan against *S. epidermis* is shown in Figure 5b. The results showed that the concentration of nanochitosan significantly affected the zone of inhibition. The higher the given concentration of nanochitosan, the higher the zone of inhibition formed. The results of further LSD test analysis showed that 0.25% (P1) and 0.50% (P2) treatments were not significantly different, but both P1 and P2 were significantly different with 0.75% (P3) and 1% (P4). Treatment with 0.75% (P3) was also significantly different from 1% (P4). The above results show that the best antibacterial activity of nanochitosan against *S. epidermis* was achieved at a concentration of 1%.

The antibacterial activity of nanochitosan against *S. aureus* has also been scientifically reported by Ngan et al. (2014), who achieved a minimum inhibitory concentration (MIC) of 0.002% or 20 ppm. Abdeltwab et al. (2019) found that nanochitosan also exhibits better antibacterial and anti-fungal activity compared to chitosan. In this study, the highest zone of inhibition against *S. aureus* was 30 mm at 23 ppm concentration. Alqahtani et al. (2019) reported that nanochitosan mixed with diclofenac sodium showed significant antibacterial activity against *S. aureus* with MICs of 35 ppm and 18 ppm.

Qi et al. (2004) showed that the antibacterial activity of nanochitosan using the tripolyphosphate ion gelation method (TPP) was superior to that of chitosan solution or doxycycline antibiotics. Chandrasekaran et al. (2020) found that nanochitosan showed polycations with a higher surface charge density than chitosan when fighting bacteria. This causes the bacterial cell walls and membranes to be damaged and then leakage of intracellular molecules occurs, resulting in the death of the bacterial cells. The size of nanochitosan also affects the response of antibacterial activity.

Atomic force microscopy (AFM) results showed that nanochitosan disrupts cell membranes and causes cytoplasmic leaks in microbial cells (Abdeltwab et al. 2019). According to Chao et al. (2019), mechanism of chitosan in inhibiting gram-positive bacteria, such as *S. aureus* and *S. epidermis*, is by binding to the plasma membrane, enzymes and proteins. According to Alqahtani et al. (2019), the exact mechanism of chitosan's antimicrobial activity is still unknown, but the widely believed theory is the electrostatic theory. Chandrasekaran et al. (2020) observed that several factors can affect the antimicrobial activity of nanochitosan, such as bacterial species, growth curve, pH, concentration, zeta potential, molecular weight, and degree of acetylation.

The use of nanochitosan to inhibit bacterial activity is widespread, including as a food coating to extend shelf life (Javaherzadeh et al. 2020; Chao et al. 2019; Ramezani et al. 2015). Nanochitosan is also used to treat wounds on the skin (Zmejkoski et al. 2021). Also, nanochitosan is used in synergy with *Zataria multiflora* boiss essential oil to maintain the shelf life of chicken breast meat during frozen storage (Hematizad et al. 2021). It is concluded from the present study that nanochitosan exhibits the potential as a promising antibacterial agent.

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