
Antibacterial potential of chitosan extracted from the shells of green mussels (*Perna viridis*; Linnaeus) against *Escherichia coli* and *Staphylococcus aureus*

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Abstract This study focused on the characterization and determination of the antibacterial potential of the chitosan extracted from green mussel shells using microwave irradiation. Results showed that chitosan was successfully extracted from green mussel shells yielding a moisture content of 4% and a percentage yield of 1.92%. The result of FTIR analysis revealed different functional groups of organic compounds such as hydroxyl- (3637.17 cm^{-1} and 3324.31 cm^{-1}), amide- (1652.22 cm^{-1}), alkane/ether- (1026.24 cm^{-1} and 963.82 cm^{-1}), and carbonate-containing compounds (2517.85 cm^{-1} , 1798.47 cm^{-1} , 1405.89 cm^{-1} , 871.51 cm^{-1} and 711.65 cm^{-1}). Analysis from FT-IR spectroscopy revealed the Degree of Deacetylation (DDA) as 64.36%, making it suitable for biomedical applications. This study showed that the chitosan samples extracted from the green mussel shells showed antibacterial potential against *E. coli* and *S. aureus*. While the chitosan treatments were not as potent as the antibiotic Ciprofloxacin and did not differ significantly from each other, they demonstrated greater inhibition compared to the negative control. Therefore, further comprehensive investigation could establish green mussel shell chitosan as a valuable natural source of antibacterial agents.

Keywords: Antibacterial, Chitin, Chitosan, *Perna viridis*

Introduction

Every year, 10 million tons of shell by-products are produced from mollusks and crustaceans (de Alvarenga *et al.*, 2012). In the Philippines, more than 250,000 metric tons of seafood byproducts are produced annually, and within the seafood production sector, 35% to 40% of this waste consists of discarded shells (Gumayan *et al.*, 2023). Unfortunately, improper bio-waste disposal, such as discarded shell waste, leads to detrimental environmental pollution in the coastal regions. Thus, shells represent a major by-product that must not be discarded easily; they should be utilized to their fullest potential as a new raw material.

Perna viridis, or the green mussel, is a bivalve mussel extensively found across the Asia-Pacific region. Referred to locally as “Tahong”, the

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green mussel is one of the economically important bivalves in the Philippines, yielding 25,421 metric tons in 2019 (Philippine Statistics Authority, 2020). The flesh of *P. viridis* serves as a valuable food resource for human consumption. However, the shells of green mussels have not been used optimally and are usually dumped in landfills or incinerated. The shells constitute approximately half of the total mass of green mussels. While some industries use these shells as ornaments, this only represents a small fraction of the overall production of green mussel shells (Musico, 2007). Still, the residues remain a waste in the environment. Finding alternative applications for these bio-waste materials is highly warranted to enhance the sustainability of aquaculture industries by promoting a shift towards a circular economy, wherein waste is transformed into a valuable resource. Consequently, studies have reported that the green mussel shells consist of chitin compounds within the range of 14–35%, which can be derived into chitosan, high-value natural biodegradable and biocompatible polymers (Cadano *et al.*, 2021). Other studies showed that chitin extracts from green mussel shells yielded a weight of 40.8% with a low moisture content (7.38%), which means greater potential chitin and chitosan to be extracted per gram from the collected materials (Safitri and Violando, 2023).

Chitosan is a biopolymer and polysaccharide derived from chitin through deacetylation. Its natural sources come from crustaceans' exoskeletons, fungi's cell walls, some mollusks, and insects. It is a highly favorable biopolymer because of its biocompatibility, biodegradability, and non-toxic nature. It has widespread applications in various industries, including food, cosmetics, pharmaceuticals, and biomedical (Kozma *et al.*, 2022). Studies of the antibacterial properties of chitosan have become more profound over time. Chitosan and its derivatives demonstrate a broad spectrum of activities, effectively eliminating various bacterial strains (Varma and Vasudevan, 2020). However, research on a more efficient extraction process is still ongoing, and mollusks' antibacterial activity still needs improvement. Chitosan from green mussel shells has yet also to be applied optimally. Therefore, the extraction of chitosan from a wide variety of bio-waste, such as green mussel shells, not only encourages alternative sources from industrial production but also presents potential biomedical applications, especially as a basis for assessing the efficacy of new antibacterial agents.

This study focused on the determination and characterization of chitosan extracted from green mussel shells using microwave irradiation as an extraction method. It also aimed to determine the antibacterial potential of the chitosan samples against Gram-positive and Gram-negative bacteria.

Generally, this study aimed to evaluate the antibacterial potential of chitosan extracted green mussel shells (*P. viridis*) against *E. coli* and *S. aureus*. Specifically, it aimed to determine the moisture content and percentage yield of microwave-extracted chitosan and to characterize the

chitosan extracted from green mussel shells using Fourier Transform Infrared (FT-IR) Spectroscopy.

Materials and methods

Time and place of the study

This study was conducted at the Protein Chemistry and Biosensor Laboratory of Isabela State University, Echague, Isabela, Philippines, from April 2024 to June 2024.

Sample collection and preparation

The shells of *P. viridis* (>5.01 cm in length) were collected from a public market in Santiago City, Isabela, Philippines. The mussel samples were taxonomically verified by the Institute of Fisheries as *Perna viridis* only. Two hundred fifty grams (250 g) of shells were collected and stored at 4 °C before processing. The collected samples were rinsed with a mild detergent to remove any barnacles and slime attached to their surfaces. The detergent was fully rinsed to clean the shells and remove impurities. The shells were blot-dried before their initial weight was determined. Then, the shells were oven-dried for 24 hours at 60°C and measured in dry weight. Furthermore, the shells were crushed until smoothed and filtered using a sieve screen with a mesh size of 1 mm.

Extraction of chitin and chitosan using microwave Irradiation

The extraction of chitin and chitosan from green mussel shells was performed using microwave technology by El Knidiri *et al.* (2016) with slight modifications. The microwave-assisted process was carried out using a domestic oven and the contact time was fixed at 8 minutes. After the samples were filtered in a sieve screen, samples were demineralized using a 3M hydrochloric acid (HCl) solution with a ratio of 1:10. Solution was heated in a domestic microwave oven for 8 minutes at a power of 500 W. After heating, samples were filtered using a vacuum distillation pump and washed with distilled water until relatively neutral. The demineralized samples were oven-dried overnight at 80°C. The demineralized samples were then treated with alkaline treatments using 10% sodium hydroxide (NaOH) solution in 1:10 ratio. It was then heated for 8 minutes in different power ranging from 160-350 W separately. The samples were first heated for 5 minutes at 160 W and followed by 350 w for 3 minutes. After heating, samples were filtered and washed thoroughly with distilled water until excess NaOH was removed. The samples were oven-dried overnight at 80°C.

The resulting product is known as chitin from the green mussel shells sample.

To obtain chitosan from the chitin samples, deacetylation was carried out using 50% sodium hydroxide (NaOH) in a 1:20 ratio. The sample was heated for 8 minutes at 350 W. After the process, samples were filtered and rinsed with distilled water and oven-dried at 80°C overnight.

The extracted chitosan was purified using the precipitation method. Samples were dissolved in 2% acetic acid in a 1:100 ratio (w/v) with constant stirring for 4 hours. The obtained solution was placed in a separate flask to remove insoluble particles. Then, 0.5 mol/dm³ NaOH was added. The solution was kept for at least 10 minutes until the chitosan precipitated. The precipitates were then filtered, washed, and oven-dried at 110°C for 1 hour (Ali *et al.*, 2019).

Fourier –Transform Infrared (FT IR) spectroscopy analysis

The obtained chitosan samples were analyzed using Fourier-Transform Infrared (FT-IR) Spectroscopy. At least 0.5g of the chitosan sample was sent to Advance Device and Material Testing Laboratory (ADMATEL) in Bicutan, Taguig City, Philippines, to characterize and determine the functional groups present and the degree of deacetylation of the chitosan samples.

Preparation of treatment concentration

The obtained chitosan samples were dissolved in 2 & % acetic acid to form a stock solution of 1mg/mL. From there, different concentrations of chitosan samples were prepared in 100 µg/mL, 200 µg/mL, 300 µg/mL, and 400 µg/mL, respectively.

Procurement of bacterial cultures

Escherichia coli (ATCC 8739) and *Staphylococcus aureus* (ATCC 6535) strains were obtained in the Protein Chemistry and Biosensor Laboratory of Isabela State University, Echague, Isabela. The glycerol bacterial strain stocks were revived in nutrient broth under aerobic conditions at 37°C for 24 hours, followed by incubation in a shaker for another 24 hours. Afterward, selective and differential media confirmed the bacteria, whereas Eosin-Methylene Blue Agar (EMBA) was used to culture *E. coli*. In contrast, Mannitol Salt Agar (MSA) was used for *S. aureus*. The density of the bacterial suspensions of *S. aureus* and *E. coli* against various chitosan treatments was adjusted using the 0.5 McFarland standard (equivalent to 1.5 x 10⁸ CFU/mL) to ensure consistency during susceptibility testing using a UV-Vis Spectrophotometer. Subsequently, the testing of the bacteria against various chitosan treatments on Petri dishes using Mueller

Hinton Agar (MHA), as per protocols outlined by previous studies by Hilles *et al.* (2019), Wei *et al.* (2010), and Vennila *et al.* (2011).

Antibacterial assay of the chitosan extract

The antibacterial activity of extracted chitosan on the Gram-positive and Gram-negative bacteria was tested using Agar Well Diffusion on Mueller-Hinton agar. In preparation, the MHA plates were swabbed uniformly with *E. coli* and *S. aureus* strains. Then, bores were made from the plates using a sterile borer with a 6 mm diameter, creating six equitized wells. Fifty (50) μL of different treatment concentrations of chitosan (100 $\mu\text{g}/\text{mL}$, 200 $\mu\text{g}/\text{mL}$, 300 $\mu\text{g}/\text{mL}$, and 400 $\mu\text{g}/\text{mL}$) were aspirated into the respective wells, along with Ciprofloxacin as the positive control and distilled water as the negative control. Treated plates were incubated overnight at 37°C. The diameters of the zones of inhibition were measured and recorded in millimeters (mm) using a vernier caliper to examine their antibacterial potential.

Experimental design

Using a Complete Randomized Design (CRD), the different treatments were prepared in triplicate to confirm the validity of the results. The treatments included a negative control (T0), positive control (Ciprofloxacin), and varying concentrations of chitosan solution: T2 with 100 $\mu\text{g}/\text{mL}$, T3 with 200 $\mu\text{g}/\text{mL}$, T4 with 300 $\mu\text{g}/\text{mL}$, and T5 with 400 $\mu\text{g}/\text{mL}$, respectively.

Statistical analysis

To determine and analyze the significant differences in the inhibition zones of the different treatments of chitosan samples against *E. coli* and *S. aureus*, the one-way analysis of variance (ANOVA) in Complete Randomized Design (CRD) was used. Furthermore, the level of significance was determined at $p < 0.05$. Tukey's Honest Significant Difference (HSD) post-hoc test was also used to analyze which among the mean values have exhibited statistically significant differences when compared to the various treatments.

Results

Moisture content and percentage yield of chitosan extracted from mreen mussel

The moisture content was determined from the initial wet weight and dry weight of the green mussel shells. The percentage yield of the chitosan

was also determined using the initial weight of the green mussel shells to the chitosan obtained after the extraction. The results of the moisture content of the shells and the percentage yield of chitosan are summarized in Table 1.

Table 1. Moisture content and percentage yield of chitosan from green mussels

Sample	Total weight (g)	Moisture content (%)	Percentage yield (%)
Green mussel shells	250g	4%	1.92%

The result showed that the green mussel shells have a moisture content of 4%. It also shows the percentage yield of chitosan, wherein 1.92% of chitosan was extracted successfully from 250 grams of green mussel shells (Table 1).

Characteristics of extracted chitosan using Fourier-Transform Infrared Spectroscopy

Analysis from the FT-IR spectroscopy showed the functional groups present in the extracted chitosan samples. The presence of certain functional groups confirmed that the sample obtained was chitosan. Depending on the infrared absorption frequency, the specific molecular group prevailing in the sample was determined through the spectrum data in the automated software of spectroscopy. The FT-IR spectrum is shown in Figure 1, which indicates characteristic bands formed in the frequency range between 4000 and 600 cm^{-1} . The peak assignments obtained from the FT-IR results are summarized in Table 2, showing the frequencies, structures, and types of bonds.

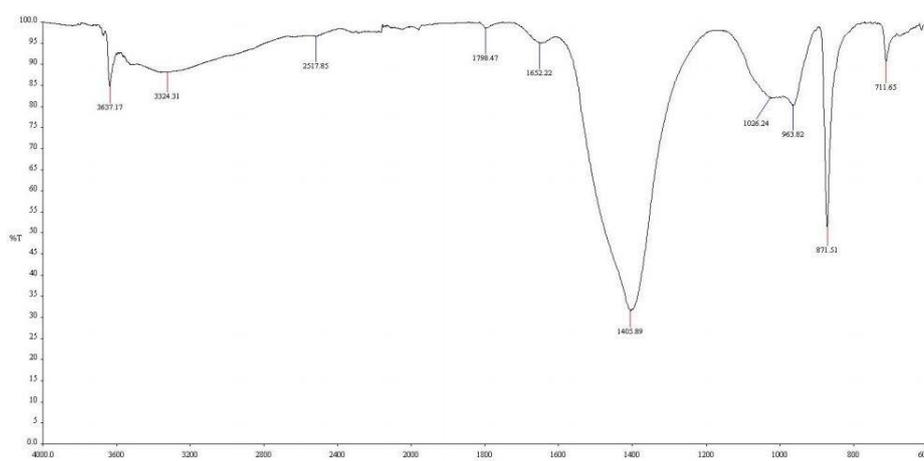


Figure 1. FT-IR spectrum of extracted chitosan from green mussels (*Perna viridis*)

Table 2. Peak Assignments in the infrared spectrum of the chitosan sample

Frequencies (cm ⁻¹)		Structure/ Compound Type*	Bonds*
Carbonate (Standard)*	Standard Group Frequencies*		
	3650-3200	3637.17 3324.31	Hydroxyl O–H Stretch
2513		2517.85	Carbonate O–C=O Stretch
1796		1798.47	
	1650-1600	1652.22	Amide I (C=O) stretch in the NHCOCH ₃ group
1411		1405.89	Carbonate Symmetric CO ₃ ²⁻ Stretch
	1310-950	1026.24 963.82	Alkane/Ether C–H Bend / C–O Stretch
875		871.51	Carbonate C–O Bend
710		711.65	

The FT-IR spectrum of extracted chitosan showed a variety of absorption bands, indicating the presence of different functional groups in the sample. Distinct peaks include a sharp peak at 3637.17 cm⁻¹ and a broad peak at 3324.31 cm⁻¹, characteristic of O–H stretching, typical of hydroxyl groups, matching the standard group frequency range of 3650-3200 cm⁻¹. The absorption band at 1652.22 cm⁻¹ is attributed to C=O stretching, indicative of the amide group. The peaks within the 1026.24 cm⁻¹ and 963.82 cm⁻¹ range are assigned to C–H bending or C–O stretching, suggesting the presence of alkane/ether groups.

The spectrum also recorded absorption at 2517.85 cm⁻¹ and 1798.47 cm⁻¹, corresponding to carbonate groups. This signified the presence of O–C=O stretching, which is typical for carbonates. The strong band at 1405.89 cm⁻¹ indicated symmetric CO₃²⁻ stretching. Finally, the 871.51 cm⁻¹ and 711.65 cm⁻¹ peaks corresponded to C–O bending in carbonates.

Antibacterial potential of chitosan extracted from green mussels

The antibacterial potential of chitosan from green mussel shells was tested against *Escherichia coli* and *Staphylococcus aureus* using the Agar Well Diffusion Method. It is expressed as the diameter in millimeters (mm) formed around the treatments. The zones against the bacteria are visually shown in Figure 2 and Figure 3.

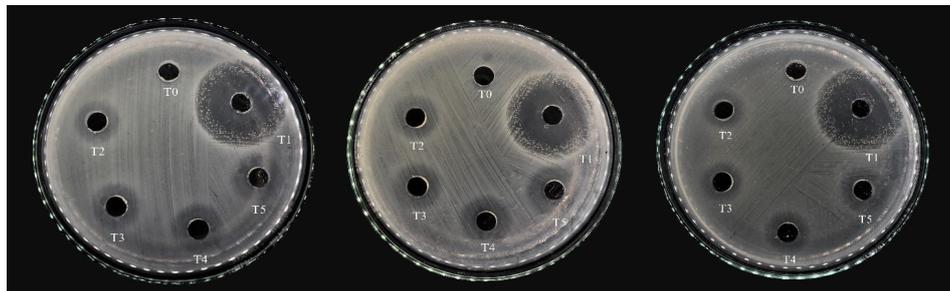


Figure 2. Zone of Inhibition of Chitosan Treatments against *E. coli* in 3 replicates

Note: T0= distilled water (negative control); T1= Ciprofloxacin (positive control); T2= 100 µg/mL treatment; T3= 200 µg/mL treatment; T4= 300 µg/mL treatment; T5= 400 µg/mL treatment.

Zone of Inhibition of six treatments against *E. coli* is shown in Figure 2. The results showed that among the six treatments, Ciprofloxacin (positive control) and all the chitosan solution treatments (100,200,300 and 400 µg/mL) showed inhibition zones; while the distilled water (negative control) was showed no zone of inhibition. Moreover, statistical analysis through one-way analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) post-hoc test revealed the significant differences among the treatments as shown in Table 3.

Table 3. Statistical Analysis Result: The mean inhibition zones of chitosan treatments against *E. coli*

Treatments	Means
T ₀ - Distilled water (negative control)	0.71 _c
T ₁ - Ciprofloxacin (positive control)	25.11 _a
T ₂ - 100 µg/mL of chitosan solution	8.30 _b
T ₃ - 200 µg/mL of chitosan solution	8.36 _b
T ₄ - 300 µg/mL of chitosan solution	9.56 _b
T ₅ - 400 µg/mL of chitosan solution	9.94 _b

Note: Means with the same letter are not significantly different

The mean inhibition zones for the six different treatments against *E. coli* is shown in Table 3. The results showed that Ciprofloxacin (25.11_a) has the highest mean inhibition among other treatments, followed respectively by the 400 µg/mL of chitosan solution (9.94_b), 300 µg/mL of chitosan solution (9.56_b), 200 µg/mL of chitosan solution (8.36_b), and 100 µg/mL of chitosan solution (8.30_b). The distilled water (negative control) has the least mean value of 0.71 mm.

Based on the results shown, statistics revealed that T₁ (Ciprofloxacin) was significantly different from all other treatments, exhibiting the highest mean inhibition. This suggested that Ciprofloxacin had significantly affected as compared to the other treatments, indicating its effectiveness as an

antibiotic control. Meanwhile, T₀ (distilled water) showed no significant effect.

The study also showed that the different chitosan treatment concentrations (T₂, T₃, T₄, and T₅) were not significantly different but different from both T₁ and T₀. This indicated that the chitosan treatments had similar effects on the measured outcome, forming a statistically homogenous group despite numerical differences in their means. Hence, these findings suggested the antibacterial potential of chitosan. Although the chitosan treatments at different concentrations (100 µg/mL to 400 µg/mL) was not showed significant differences among each other, they demonstrated a significant antibacterial effect compared to the negative control. This suggested that even at the lowest concentration tested, chitosan can inhibit bacterial growth.

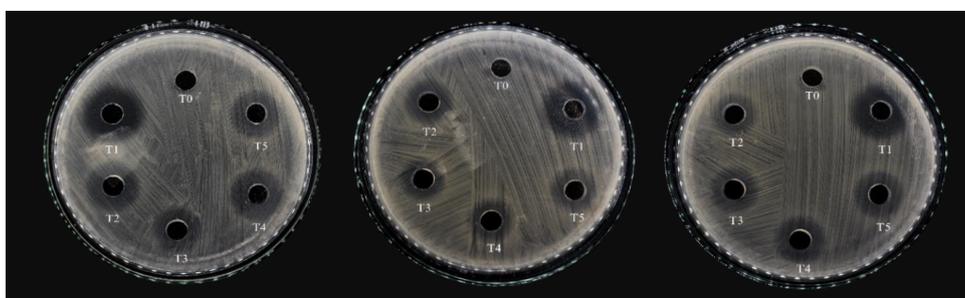


Figure 3. Zone of Inhibition of Chitosan T treatments against *S. aureus* in 3 replicates

Note: T₀= distilled water (negative control); T₁= Ciprofloxacin (positive control); T₂= 100 µg/mL treatment; T₃= 200 µg/mL treatment; T₄= 300 µg/mL treatment; T₅= 400 µg/mL treatment.

Zone of inhibition of six treatments against *S. aureus* is shown in Figure 3. The results showed that among the six treatments, Ciprofloxacin (positive control) and all the chitosan solution treatments (100,200,300 and 400 µg/mL) showed inhibition zones; while the distilled water (negative control) showed no zone of inhibition. Moreover, statistical analysis through one-way analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) post-hoc test revealed the significant differences among the treatments as shown in Table 4.

The mean inhibition zones for the six different treatments against *S. aureus* is shown in Table 4. The results showed that Ciprofloxacin (12.48_a) had the highest mean inhibition among other treatments, followed respectively by the 400 µg/mL of chitosan solution (9.23_b), 300 µg/mL of chitosan solution (9.04_b), 100 µg/mL of chitosan solution (8.39_b), and 200 µg/mL of chitosan solution (8.35_b). The distilled water treatment (negative control) had the least mean value of 0.71 mm.

Table 4. Statistical analysis result: The mean inhibition zones of chitosan treatments against *S. aureus*

Treatments	Means
T ₀ - Distilled water (negative control)	0.71 _c
T ₁ - Ciprofloxacin (positive control)	12.48 _a
T ₂ - 100 µg/mL of chitosan solution	8.39 _b
T ₃ - 200 µg/mL of chitosan solution	8.35 _b
T ₄ - 300 µg/mL of chitosan solution	9.04 _b
T ₅ - 400 µg/mL of chitosan solution	9.23 _b

Note: Means with the same letter are not significantly different

On the other hand, treatments T₂ (100 µg/mL chitosan solution), T₃ (200 µg/mL chitosan solution), T₄ (300 µg/mL chitosan solution), and T₅ (400 µg/mL chitosan solution) were not significantly differed from each other. These results suggested that the chitosan solutions, regardless of their concentrations, produced similar effects. Nonetheless, these findings suggested the antibacterial potential of chitosan against *S. aureus*. Although the chitosan treatments at different concentrations (100 µg/mL to 400 µg/mL) was not significantly differed among each other, they all showed a significant antibacterial effect compared to the negative control. This indicated that chitosan inhibited bacterial growth even at the lowest concentration tested.

Discussion

In this study, 250 grams of green mussel shells were used to extract chitosan using microwave-assisted technology. The obtained chitosan was 1.92% which is comparable to the 0.079% yield achieved from the green mussel shells by Kaewprachu and Jaisan (2023). However, the yield is lower compared to the yield of 11.60 % achieved by the study of Pratama *et al.* (2023)—similarly, the study of Varma and Vasudevan (2020) showed a higher yield of 10.21%, also extracted from green mussel shells.

The low yield of chitosan observed in this study can be attributed to several factors related to the extraction process. Key parameters such as the concentration of acids and bases, temperature, and extraction duration play a crucial role in determining the chitosan yield (Hu *et al.*, 2020). In this study, the fixed time of 8 minutes with 50% NaOH might have led to an incomplete removal of acetyl groups, contributing to the low yield. This duration may not have been sufficient for a complete overall conversion to chitosan.

Precipitation, filtration and washing may also lead to the potential loss of material during the extraction and purification process. These stages are critical for obtaining high-quality chitosan, but they can also lead to incomplete recovery of the product. The washing of distilled water until relatively neutral pH, for example, could have led to the loss of chitosan particles, further reducing the yield. The delicate balance between

deacetylation and particle stability during washing is important, as extended contact time between chitin and NaOH typically increases the conversion of acetyl groups to sodium acetate, potentially enhancing the yield (Djaeni, 2003).

Based on the results, the characterization of chitosan with FT-IR showed similar results to those obtained from other studies. In the study of George *et al.* (2011), they have identified a broad absorption band in the 3000-3500 cm^{-1} range, indicating O–H stretching frequencies. Specifically, in this study, the O–H stretching frequencies were observed at 3637.17 cm^{-1} and 3324.31 cm^{-1} , confirming the presence of chitosan in the sample (Gumayan *et al.*, 2023). The C=O stretch vibration around 1652.22 cm^{-1} corresponds to the presence of the amide I band, which corresponds to the residual acetyl groups in chitosan. This indicates that the sample is partially deacetylated chitosan. This finding aligns with Moosa *et al.* (2016), who observed similar C=O stretch at 1662.64 cm^{-1} for the standard chitosan and 1654.92 cm^{-1} for the shrimp wastes. Moreover, the C–O stretch observed at peaks 1026.24 cm^{-1} indicates the skeletal vibrations present in the backbone of chitosan (Dziedzic and Kertmen, 2023). The peaks at 963.82 cm^{-1} also conform to the C–O skeletal in the chitosan backbone in the study of Moosa *et al.* (2016).

Despite slight variations in the exact peak locations compared to other studies, these peaks correspond to the same functional groups identified in this study. This consistency across different studies reinforces the characterization of the sample as chitosan, affirming the presence of key functional groups such as O–H, C=O, and C–O stretching and bending vibrations. The presence of these groups indicates that the chitosan sample retained its essential chemical features even after the extraction process.

On the other hand, a few peaks in the spectrum revealed the presence of carbonate compounds in the chitosan sample. This indicates the presence of impurities such as calcium carbonate (CaCO_3) in the green mussels during the extraction process. The study of Kaewprachu and Jaisan (2023) suggests that the amount of CaCO_3 present in green mussels ranges within 78%, which could affect chitosan's extraction process and physicochemical properties. This is supported by Alishahi *et al.* (2011), who stated that the mineral content in the starting materials significantly influences the duration of the demineralization process; higher mineral content necessitates a more extended demineralization period.

The degree of deacetylation (DDA) is an essential parameter for determining the quality of chitosan. The higher the purity of the chitosan, the higher the DDA. The DDA of the chitosan extracted from green mussels was based on the FT-IR spectrum, studied, and calculated to be 64.36%. In the study of Kaewprachu and Jaisan (2023), the green mussel shells exhibited a DDA of 52.56%, which is less than the results of this study. A much lower DDA value (48.68%) was also obtained in the green mussel shells from the study of Pratama *et al.* (2023). The higher DDA value of

chitosan extracted in this study might primarily be attributed to the microwave extraction methods, which were not used in the studies of Pratama *et al.* (2023) and Ray (2011). The microwave-assisted extraction can significantly impact the purity of chitosan. Microwave heating has been shown to accelerate reaction rates significantly compared to conventional heating methods, leading to higher degrees of deacetylation in a shorter timeframe (El Knidri *et al.*, 2016). The rapid heating rate associated with microwave extraction can enhance the contact between chitin and the deacetylating agent, resulting in a higher DDA of the extracted chitosan. Nonetheless, the value of DDA for chitosan that can be applied for use ranges from 40-98% (Ray, 2011). This finding indicates that the chitosan obtained in this study can be used for different applications because it has a more than 40% deacetylation degree.

Based on the results, statistical analysis indicates that T₁ (Ciprofloxacin) is significantly different from all other treatments, demonstrating the highest mean inhibition. This suggests that Ciprofloxacin had a markedly greater impact than the other treatments, indicating its effectiveness as an antibiotic control. Conversely, T₀ (distilled water) is also significantly different from the other treatments, indicating that the negative control had no effect compared to the active treatments, as expected.

On the other hand, treatments T₂ (100 µg/mL chitosan solution), T₃ (200 µg/mL chitosan solution), T₄ (300 µg/mL chitosan solution), and T₅ (400 µg/mL chitosan solution) did not differ significantly from each other. These results suggest that the chitosan solutions, regardless of their concentrations, produced similar effects. Nonetheless, these findings suggest the antibacterial potential of chitosan against *S. aureus*. Although the chitosan treatments at different concentrations (100 µg/mL to 400 µg/mL) did not show significant differences among each other, they all showed a significant antibacterial effect compared to the negative control. This indicates that chitosan inhibits bacterial growth even at the lowest concentration tested.

These findings conform to other studies wherein extracted chitosan from shrimp shells exhibited an antibacterial effect against *S. aureus*, a Gram-positive bacterium (Gerasimenko *et al.*, 2004; Resmi *et al.*, 2021). It is also supported by the study of Zaghoul and Ibrahim (2019), which found out that chitosan showed a bacteriostatic effect on *S. aureus*. The results showed a higher inhibition zone than the study by Varma and Vasudevan (2020), which exhibited 6 mm inhibition at 200 µg/mL chitosan treatment. Conversely, Paul *et al.* (2014) showed 12 mm inhibition at 120 µg/mL of chitosan from Indian prawns, which is higher than all the chitosan treatments in this study.

These findings aligned with other studies wherein chitosan extracted from peregrine shrimp and caridean shrimp shells exhibited antibacterial activity against *E. coli*, a gram-negative bacteria (Küçükgülmez *et al.*, 2012; Benhabiles *et al.*, 2012; Zaghoul and Ibrahim, 2019). Other studies also tested the antibacterial activity of chitosan against *E. coli* and found a

diameter of 10mm (Al-Nabulsi *et al.*, 2020; Alfaif *et al.*, 2020), which, in this study, only exhibited a range between 8.30- 9.94 mm.

The antibacterial activity of extracted chitosan from crab shells against *E. coli* was recorded at 100µg/ml (6 mm) (Duraishamy *et al.*, 2022); another study from crab-shell chitosan in the same bacteria showed inhibition at 400 µg/mL (8mm), which are less than the results of the study. In the study of Varma and Vasudevan (2020), the antimicrobial activity of chitosan from horse mussel shells was tested against *E. coli*. It showed greater inhibition (9 mm) at a concentration of 200 µg/ mL, which is comparable to this study, with at least 9 mm in 300-400 µg/ mL chitosan treatment.

The study revealed the antibacterial potential of chitosan in *E. coli* and *S. aureus*, which was observed through their mean inhibition zones. Although the chitosan treatments in both bacteria were not as potent as the Ciprofloxacin (positive control), they were significantly higher than their respective negative controls, confirming the antibacterial effect of chitosan.

Chitosan is a natural polymer with a broad antibacterial activity, including Gram-positive and Gram-negative bacterial strains (Zou *et al.*, 2016). It has demonstrated activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Lactobacillus brevis*, and *Escherichia coli* (Ngo *et al.*, 2015). The antibacterial activity of chitosan and its derivatives might be attributed to the electrostatic interactions between the polycationic structure of chitosan and the anionic groups found on the bacterial cell surface, leading to the alteration of the cell wall of the Gram-positive or outer membrane of Gram-negative bacteria (Confederat *et al.*, 2021). Specifically, the electrostatic interactions between the NH_3^+ sites on chitosan, which are positively charged, and the negatively charged microbial cell membranes. This interaction disrupts the cell membrane's permeability, leading to the leakage of intracellular contents.

In its polycationic form, chitosan exhibits antimicrobial properties against Gram-positive and Gram-negative bacteria, with its mechanism of action varying according to the distinct cell surface structures of each bacterial type (Guarnieri *et al.*, 2022). In Gram-negative bacteria, chitosan interacts with anionic structures on their surface, such as lipopolysaccharides. When the environmental pH is below 6.5, the increased negative charge on the cell surface facilitates the binding of cationic chitosan to phospholipids (Cheung *et al.*, 2015). Meanwhile, chitosan interacts directly with the cell layer of Gram-positive bacteria, consisting of a thick peptidoglycan layer and teichoic acid (Hosseinnejad and Jafari, 2016). Other mechanisms of chitosan's antibacterial effect, as described by Guarnieri *et al.* (2022), include altering cell wall calcium levels, destabilizing peptidoglycan, disrupting osmotic balance and energy stability, chelating metal ions, and inhibiting RNA and protein synthesis. These suggest that chitosan may have more complex intracellular and extracellular effects against bacteria that must be further studied.

On the other hand, the differences in the results in this study from previous studies could be related to various intrinsic factors, including the source of chitosan, its molecular weight, degree of deacetylation, viscosity, and the solvent used. On the other hand, extrinsic factors include pH, temperature, organic matter, and the type of bacterial cultures (Küçükgülmez *et al.*, 2012). The degree of deacetylation is particularly crucial among these factors. Chitosan samples with a higher DDA are more effective in inhibiting bacterial growth. This phenomenon is likely due to the higher proportion of positively charged protonated free amine groups, considered the main reactive site in chitosan (Liu and De Yao, 2002; Zaghoul and Ibrahim, 2019). Furthermore, the antibacterial activity of chitosan is effective only in an acidic environment, typically attributed to its limited solubility at pH levels above 6.5. In acidic conditions, chitosan is more positively charged, enhancing its affinity for bacterial cells and thus its antibacterial efficacy (Qin *et al.*, 2006).

In conclusion, the study successfully extracted chitosan from green mussel shells with a low moisture content of 4% and a yield of 1.92%. The DDA (64.36%) of the extracted chitosan is comparably higher than previous studies that also used green mussel shells as a source of chitosan. The study also revealed that the extracted chitosan has an antibacterial potential against *E. coli* and *S. aureus*, wherein all chitosan treatments produced inhibition zones. Although they are less potent than the antibiotic (Ciprofloxacin) and are not significantly different from each other, they exhibited more significant inhibition than the negative control. Thus, the chitosan from green mussel shells could be a natural source of antibacterial agents if thoroughly investigated.

Based on the results obtained from the study, it is recommended to optimize the extraction process from mussel shells using microwave irradiation for greater quantity and quality of chitosan; further characterize the chitosan using other methods (such as X-ray Diffraction (XRD) analysis, Elemental analysis, and UV-VIS spectroscopy); and to explore the antibacterial potential of chitosan from mussel shells on other bacteria.

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References

- Alfaifi, M. Y., Alkabli, J. and Elshaarawy, R. F. (2020). Suppressing of milk-borne pathogenic using new water-soluble chitosan-azidopropanoic acid conjugate: Targeting milkpreservation quality improvement. *International Journal of Biological Macromolecules*, 164:1519-1526.

- Ali, M., Shakeel, M. and Mehmood, K. (2019). Extraction and characterization of high purity chitosan by rapid and simple techniques from mud crabs taken from Abbottabad. *Pakistan Journal of Pharmaceutical Sciences*, 32:171-175.
- Alishahi, A., Mirvaghefi, A., Tehrani, M. R., Farahmand, H., Shojaosadati, S. A., Dorkoosh, F. A. and Elsabee, M. Z. (2011). Enhancement and characterization of chitosan extraction from the wastes of shrimp packaging plants. *Journal of Polymers and the Environment*, 19:776-783.
- Al-Nabulsi, A., Osaili, T., Sawalha, A., Olaimat, A. N., Albiss, B. A., Mehyar, G. and Holley, R. (2020). Antimicrobial activity of chitosan coating containing ZnO nanoparticles against *E. coli* O157: H7 on the surface of white brined cheese. *International Journal of Food Microbiology*, 334:108838.
- Benhabiles, M. S., Salah, R., Lounici, H., Drouiche, N., Goosen, M. F. A. and Mameri, N. (2012). Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. *Food hydrocolloids*, 29:48-56.
- Cadano, J. R., Jose, M., Lubi, A. G., Maling, J. N., Moraga, J. S., Shi, Q. Y. and VinceCruzAbeledo, C. C. (2021). A comparative study on the raw chitin and chitosan yields of common bio-waste from Philippine seafood. *Environmental Science and Pollution Research*, 28:11954-11961.
- Cheung, R. C. F., Ng, T. B., Wong, J. H. and Chan, W. Y. (2015). Chitosan: an update on potential biomedical and pharmaceutical applications. *Marine drugs*, 13:5156- 5186.
- Confederat, L. G., Tuchilus, C. G., Dragan, M., Sha'at, M. and Dragostin, O. M. (2021). Preparation and antimicrobial activity of chitosan and its derivatives: A concise review. *Molecules*, 26:3694.
- de Alvarenga, R. A. F., Galindro, B. M., de Fátima Helpa, C. and Soares, S. R. (2012). The recycling of oyster shells: An environmental analysis using Life Cycle Assessment. *Journal of environmental management*, 106:102-109.
- Djaeni, M. (2003). Optimization of chitosan preparation from crab shell waste. *Reaktor*, 7:37-40.
- Duraisamy, P., Vinod, A. R. and Keerthika, S. (2022). Extraction, characterization, antimicrobial activity of chitosan extracted from crab shell and preparation of chitosanbased bioplastic film for food packaging. *Journal of Advanced Scientific Research*, 13:263-268.
- Dziedzic, I. and Kertmen, A. (2023). Methods of chitosan identification: history and trends. *Letters in Applied NanoBioScience*, 12:94.
- El Knidri, H., El Khalfaouy, R., Laajeb, A., Addaou, A. and Lahsini, A. (2016). Eco-friendly extraction and characterization of chitin and chitosan from the shrimp shell waste via microwave irradiation. *Process Safety and Environmental Protection*, 104:395-405.
- George, T. S., Guru, K. S., Vasanthi, N. S. and Kannan, K. P. (2011). Extraction, purification and characterization of chitosan from endophytic fungi isolated from medicinal plants. *World Journal of Science and Technology*, 1:43-48.
- Gerasimenko, D. V., Avdienko, I. D., Bannikova, G. E., Zueva, O. Y. and Varlamov, V. P. (2004). Antibacterial effects of water-soluble low-molecular-weight chitosans on different microorganisms. *Applied Biochemistry and microbiology*, 40:253-257.
- Gumayan, E. G., Dimzon, I. K. D. and Guerrero, R. A. (2023). Chitosan from crab shell waste for soft lithography of bioplastic diffraction gratings. *Applied Optics*, 62:2487-2492.
- Zaghloul, E. and Ibrahim, H. A. (2019). Comparative study on antimicrobial activity of commercial and extracted chitin and chitosan from *Marsupenaeus japonicus* shells. *Egyptian Journal of Aquatic Biology and Fisheries*, 23:291-302.
- Hilles, A. R., Mahmood, S. and Hashim, R. (2019). Evaluation of the antibacterial activities of skin mucus from Asian swamp eel (*Monopterus albus albus*). *Indian Journal of Geo Marine Sciences*, 48:1855-1859.
- Hosseinnejad, M. and Jafari, S. M. (2016). Evaluation of different factors affecting antimicrobial properties of chitosan. *International journal of biological macromolecules*, 85:467-475.
- Hu, X., Tian, Z., Li, X., Wang, S., Pei, H., Sun, H. and Zhang, Z. (2020). Green, simple, and effective process for the comprehensive utilization of shrimp shell waste. *ACS omega*, 5:19227-19235.
- Kaewprachu, P. and Jaisan, C. (2023). Physicochemical Properties of Chitosan from Green Mussel Shells (*Perna viridis*): A Comparative Study. *Polymers*, 15:2816.

- Kozma, M., Acharya, B. and Bissessur, R. (2022). Chitin, chitosan, and nanochitin: extraction, synthesis, and applications. *Polymers*, 14:3989.
- Küçükgülmez, A., Gülnaz, O., Celik, M., Yanar, Y., Eslem Kadak, A. and Gerçek, G. (2012). Antimicrobial activity of the chitosan extracted from *Metapenaeus stebbingi* shell wastes. *Journal of Polymers and the Environment*, 20:431-437.
- Liu, W. G. and De Yao, K. (2002). Chitosan and its derivatives—a promising non-viral vector for gene transfection. *Journal of controlled release*, 83:1-11.
- Moosa, A. A., Ridha, A. M. and Kadhim, N. A. (2016). Use of biocomposite adsorbents for the removal of methylene blue dye from aqueous solution. *American Journal of Materials Science*, 6:135-146.
- Musico, Y. L. F. (2007). The Potential of Calcium Carbonate from Philippine Green Mussel Shells as Extender in the Manufacture of Flat Latex Paints. *TIP Research Journal Quezon City*, 4:1-1.
- Ngo, D. H., Vo, T. S., Ngo, D. N., Kang, K. H., Je, J. Y., Pham, H. N. D. and Kim, S. K. (2015). Biological effects of chitosan and its derivatives. *Food hydrocolloids*, 51:200216.
- Paul, N., Jayan, A., Sasikumar, C. S. and Cherian, S. M. (2014). Extraction and purification of chitosan from chitin isolated from sea prawn *Fenneropenaeus indicus*. *Asian Journal of Pharmaceutical and Clinical Research*, 7:201-204.
- Philippine Statistics Authority (2020). Fisheries Statistics of the Philippines 2017– 2019. Philippine Statistics Authority: Diliman Quezon, Philippines, 28.
- Pratama, G., Munandar, A., Surilayani, D., Rizky, J. A., Hasanah, A. N., Haryati, S. and Aditia, R. P. (2023). Characteristics of crab shells and green mussel shells as potential chitosan material from Karangantu, Banten, Indonesia. In *IOP Conference Series: Earth and Environmental Science*, 1137:012033.
- Qin, C., Li, H., Xiao, Q., Liu, Y., Zhu, J. and Du, Y. (2006). Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate polymers*, 63:367-374.
- Ray, S. D. (2011). Potential aspects of chitosan as pharmaceutical excipient. *Acta Poloniae Pharmaceutica*, 68:619-622.
- Resmi, R., Yoonus, J. and Beena, B. (2021). Anticancer and antibacterial activity of chitosan extracted from shrimp shell waste. *Materials Today: Proceedings*, 41:570-576.
- Safitri, N. M. and Violando, W. A. (2023). Utilization of Green Mussel Shells Waste of *Perna viridis* for Bioproduction of Chitin. *Journal of Maritime Research*, 20:106-109.
- Varma, R. and Vasudevan, S. (2020). Extraction, characterization, and antimicrobial activity of chitosan from horse mussel *modiolus modiolus*. *ACS omega*, 5:20224-20230.
- Vennila, R., Kumar, K., Kanchana, S., Arumugan, M., Vijayalakshmi, S. and Balasubramaniam, T. (2011). Preliminary investigation on antimicrobial and proteolytic property of the epidermal mucus secretion of marine stingrays. *Asian Pacific Journal of Tropical Biomedicine*, 1:239-243.
- Wei, O., Xavier, R. and Marimuthu, K. (2010). Screening of antibacterial activity of mucus extract of Snakehead fish, *Channa striatus* (Bloch). *European Review for Medical and Pharmacological Sciences*, 14:675-681.
- Zou, P., Yang, X., Wang, J., Li, Y., Yu, H., Zhang, Y. and Liu, G. (2016). Advances in characterisation and biological activities of chitosan and chitosan oligosaccharides. *Food chemistry*, 190:1174-1181.

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