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Utilization of Green Mussel Shells Waste of Perna viridis for Bioproduction of Chitin

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ABSTRACT

Chitin is the second most abundant biopolymer in nature after cellulose which is used in the exoskeleton preparation of various arthropods, bivalves, insects, and fungi. The source of chitin as a raw material is very abundant in Indonesia, especially from waste shells which are not commercially useful. The purpose of this study was to perform the chitin extraction procedure from the green shells of Perna viridis and to characterize the chitin content qualitatively. The results showed that the chitin extract had a yield weight of 40.8% with a moisture content of 7.38%. After qualitative screening and characterization of the active components, data were obtained, namely P. viridis chitin extract containing saponins and benzene ringed amino acids (tyrosine, phenylalanine, and tryptophan).

1. Introduction.

Chitin is a polysaccharide found in the exoskeletons of arthropods, bivalves, and insects, as well as certain types of mosses and bacteria. Chitin is also present in fungi's mycelium and spores (Abdulkarim et al., 2013). Chitin, which is made of 10%-30% polysaccharides, acts as a supportive and protecting component, particularly in the hard skin (Nguyen et al., 2020).

Chitin is the second most prevalent biopolymer in nature after cellulose. Chitin's primary structure is a -N-acetyl-D-glucosamine polymer. The units of -N-acetyl-D-glucosamine are in the form of pinarose, with the units of -B pinarose arranged in the cellulose molecule. Chitin is a chemical with the empirical formula (C8H13O5N)n that is only soluble in concentrated mineral acids. Chitin remains linked to other elements, including proteins and minerals (Mirwandhono, Nasution and Yunilas, 2022).

Indonesia has an abundance of raw materials for polysaccharide polymers, particularly from clamshells that become aquaculture trash (Teanchai, Witit-Anun and Chaikhun, 2016). Chitin is synthesized at a rate of up to one billion tons per year on a global scale, although only a small portion is utilized, particularly in various industries (Benhabiles et al., 2012). As a result, a technique for removing clamshells to create chitin is required, as is qualitative characterization of the chitin, which enables the shellfish waste to be used for research and other uses.

2. Materials and Methods.

2.1. Materials.

The primary material in this study, green mussel shell waste of *Perna viridis*, was gathered from the Ujungpangkah Essential Mangrove Ecosystem Area (KEE MUP), Banyuurip Village, Ujungpangkah District, Gresik, Indonesia. Additionally, hydrochloric acid (HCl) (Merck, USA) and sodium hydroxide (NaOH) are used in the extraction operation (ROFA, Idn). A microwave oven (UN55, Memmert, Germany) and a hot plate magnetic stirrer were used (Z168556-1EA, Thermo scientific, USA) to extract the chitin.

2.2. Preparation and Chitin Extraction.

The chitin extraction process was adapted from Abdulkarim et al. (2013). The green shells of Perna viridis were rinsed twice

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with running water and then placed in a 40°C oven for 16 hours. After completely drying the clamshells, they are crushed with a mortar and pestle until totally smooth.

In the demineralization technique, finely powdered shells were macerated in 0.68M HCl solution (1:5; w/v) for 6 hours. Throughout the procedure, the solution was swirled at a constant speed at a temperature of 40°C. After washing with distilled water, the shell powder was dissolved in 0.62M NaOH solution (1:5; w/v) for 16 hours and swirled at a steady speed at 40°C (Deproteination). The chitin was washed three times with distilled water and dried in an oven at 40°C. The yield was determined using the following formula.

$$\text{Yield of chitin } (\%) = \frac{\text{Extract\_weight(gr)} \times 100\%}{\text{Sample\_weight(gr)}}$$

2.3. Moisture Content.

A total of 1 g of the sample was placed in an oven at 105°C for 3 hours before being removed from the oven and cooled in a desiccator for 30 minutes before being weighed.

$$\text{Moisture\_content } (\%) = \frac{[(\text{Mc}+\text{Ms})-\text{Mcs}] \times 100\%}{\text{Ms}}$$

Keterangan:
Mc: The weight of empty dish
Ms: The weight of chitin powder before heated
Mcs: The weight of dish+sample had been heated

2.4. Phytochemical Test.

Phytochemical tests were conducted to determine the bioactive compounds contained in a metabolite. Phytochemical tests include tests for saponins, alkaloids, flavonoids, steroids, triterpenoids, and tannins. An additional test was carried out, namely the xantoprotein test, to determine the presence of protein groups in the extracted chitin. This test was carried out referring to the research of Bruck De Souza et al. (2020).

2.4.1. Saponins.

Boil the sample that has been dissolved in 20 mL of distilled water and filter it through filter paper. Take the filtrate and shake the solution until a stable foam is formed. Add 5 mL of 0.5 mol/L KOH alcohol and shake vigorously. Wait up to 10 minutes. Next, one drop of 2N HCl was added. If a stable emulsion is formed, then there is fat in the substance in the form of saponins.

2.4.2. Alkaloids.

Add 1 mL of chloroform and 1 mL of Ammonia into the test extract and homogenize. Filter with filter paper. Take the filtrate and drop it with 3-4 concentrated H2SO4, then shake until two layers are formed. The top layer was transferred in three test tubes having a uniform volume. Each solution was then dripped with 3-4 drops of Mayer, Dragendorff, and Wagner reagents. The formation of a precipitate indicates that the sample contains alkaloids. Mayer's reagent showed a white precipitate, Dragendorff's reagent showed an orange-red precipitate, and Wagner's reagent showed a brown precipitate.

2.4.3. Flavonoids.

2 mL of extract was added with 5 mL of distilled water, boiled for 5 minutes, and filtered. Next, 1 mL of filtrate was taken, and 0.01 gram of Mg powder and 0.2 mL of concentrated HCl were added. Shake vigorously. A positive test is indicated by the formation of a red, yellow, or orange color.

2.4.4. Steroid and Triterpenoid.

Add 10 drops of glacial CH3COOH and 2 drops of concentrated H2SO4 into the solution. Next, the solution was shaken gently and left for 8 minutes. Positive solutions containing steroids are indicated by blue/green color, and red/purple colors indicate positive solutions containing triterpenoids.

2.4.5. Tannin.

1 mL of extract was added with 10 drops of 10% FeCl3 and waited for 3 minutes. The extract is known to be positive for tannin if it produces a blackish-green or blue-black color.

2.4.6. Xantoprotein.

Enter the test solution that has been mixed with 2.5 mL of concentrated nitric acid and observe the white precipitate formed. Then, heat the solution carefully until the color of the solution changes to yellow. Let stand a few moments until the solution is completely cool and add a few drops of NaOH. A substance is known to be positive when the solution changes color to orange.

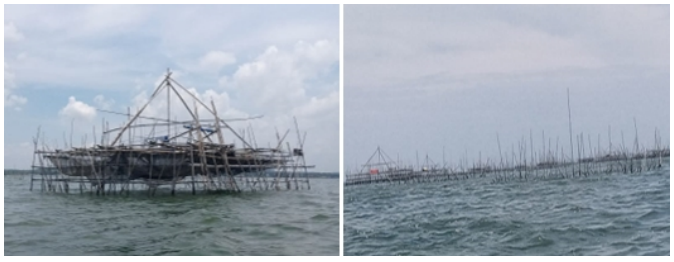
3. Results and Discussion.

3.1. Green Mussel (Perna viridis) Shells Waste.

Perna viridis, sometimes known as the green mussel, is a form of shellfish whose habitat is still heavily controlled by the maritime environment, especially water limiting constraints. Green mussel shells waste was taken from the cultivation in the Mangroves area of Ujungpangkah, Gresik Regency, Indonesia. It is often done in shallow marine waters in the mangrove habitat, with bamboo erected in the sea, seeds, and naturally accessible feed in the waters (Figure 1). Harvesting is usually done after 6-7 months of growing.

P. viridis is widely dispersed in the Indo-Pacific waters of tropical countries such as Indonesia (Tan and Ransangan, 2017). This clam has a female shape that is longer yet slim, and males that are smaller and tend to widen dependent on the distance between the ligament and the umbo (Gayle et al., 2016). Male species have white to cream internal morphology, while female species have yellow to orange internal morphology (Al-Barwani et al., 2013).

Figure 1: Green mussels farming at Ujungpangkah Mangroves Gresik, Indonesia.



Source: Authors.

P. viridis develops gonads at the age of three months of cultivation, which is defined by hard and thickened gonads, until the fourth to sixth month, which is marked by greater gonad sizes with widening cell walls. The gonads of *P. viridis* range in color from dark orange to deep red (Noor et al., 2019).

The meat of green mussels is normally consumed, but the shells of green mussels are discarded. The green mussel shell is distinctive for its brownish-green color and elongated anteriorly and rounded posteriorly (Sze and Lee, 2000). (Figure 2).

Figure 2: Unused green mussel shells waste.

Source: Authors.

Green mussels are present in the majority of MUP's coastal areas, particularly in the water area surrounding Banyuurip Village, which provides an ideal environment for P. viridis green mussels. The area's characteristics are still influenced by tidal currents with muddy substrates (Macintosh, Ashton and Havanon, 2002).

3.2. Chitin Yield.

The shells that have been recovered are next cleaned in running water under preparation for the chitin extraction method. Its goal is to eliminate particles from the shell. After drying, the shells were mashed and the chitin isolation procedure was resumed. The goal of administering HCl is to soften the shells and dissolve the minerals stored within them. The shell begins to lose color throughout this phase. Furthermore, the use of NaOH is intended to lower the protein content of the shell. As a result of this process, the color of the shell extract changes to grayish-white.

Following the chitin extraction technique, 50 grams of chitin extract were extracted from the total weight of the 122.5-gram green mussel shell sample. The obtained yield value is 40.8 percent. The chitin extract was a grayish-white powder (Figure 3). The moisture content test revealed a value of 7.38%.

Figure 3: Chitin *P. viridis* powder.

Source: Authors.

3.3. Active Components of crude extract of P. viridis chitin.

The components of the active compounds contained in the crude extract of green mussel chitin *P. viridis* were observed by phytochemical testing with the addition of a qualitative xantoprotein test. Phytochemical tests carried out included saponins, alkaloids, flavonoids, steroids, triterpenoids, and tannins. The test results can be seen in Table 1.

Table 1 shows that the crude extract of chitin was detected to contain compounds from the saponin group. Saponins are a compound containing fat that is easily detected from their ability to form foam because they are a group of glycosides bound to the C3 position and two sugar chains attached to the C3 and C17 positions (Vincken et al., 2007). These compounds are generally used as drugs and natural surfactants. Some of the biological characteristics of saponins include hemolytic ability, cytotoxic activity, antioxidant, hyperglycemia to antibacterial (Grienke, Silke and Tasdemir, 2014; Moses, Papadopoulou and Osbourn, 2014).

Table 1: The active component of chitin from green mussel shells *P. viridis*.

The Active Component	*Result*	*Color Indicator*
Saponins	+	White emulsion foam
Alkaloids		
- *Wagner*	-	Chocolate precipitate
- *Meyer*	-	White precipitate
- *Dragendorff*	-	Orange-red precipitate
Flavonoids	-	Red/Yellow/Orange
Steroids	-	Blue/Green
Triterpenoids	-	Red/Purple
AA Benzene Ring	+	Yellow to Orange

Source: Authors.

Xantoprotein testing aims to determine the presence of amino acids (AA) containing a benzene ring (tyrosine, tryptophan, and phenylalanine), where these three amino acids have high antioxidant activity (Safitri, Herawati and Hsu, 2017). The test results show that if the solution contains this benzene ringed amino acid, a white precipitate will form, turning yellow when heated (Abu Bakar Putri, 2016).

Conclusions.

As a conclusion, the crude chitin extract from P. viridis green mussel shell waste yielded 40.8 percent by weight with a water content of 7.38 percent. The qualitative screening of active components revealed that chitin extract contains saponins and benzene-ringed amino acids (tyrosine, phenylalanine, and tryptophan), all of which possess bioactive properties and so have the potential for use as emulsifiers and functional foods.

References.

Abdulkarim, A. et al. (2013) 'Extraction and Characterisation of Chitin and Chitosan from', Civil and Environmental Research, 3(2), pp. 108–115.

Abu Bakar Putri, A. (2016) 'ANALISIS KADAR ALBUMIN IKAN SIDAT (Anguilla marmorata dan Anguilla bicolor) DAN UJI AKTIVITAS PENYEMBUHAN LUKA TERBUKA PADA KELINCI (Oryctolagus cuniculus) ANALYSIS OF ALBUMIN LEVELS OF THE EELS (Anguilla marmorata and Anguilla bicolor) AND EXAMINATION OF', Galenika Journal of Pharmacy Journal of Pharmacy, 2(2), pp. 90–95.

Al-Barwani, S. M. et al. (2013) 'Incidence of Hermaphrodite in Green Mussel Perna viridis Along the West Coast of Peninsular Malaysia', Asian Journal of Animal and Veterinary Advances, 8(2), pp. 376–382.

Benhabiles, M. S. et al. (2012) 'Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste', Food Hydrocolloids, 29(1), pp. 48–56. doi: 10.1016/j.foodhyd.2012.02.013.

Bruck De Souza, L. et al. (2020) 'Phytochemical Analysis, Antioxidant Activity, Antimicrobial Activity, and Cytotoxicity of Chaptalia nutans Leaves', Advances in Pharmacological and Pharmaceutical Sciences, 2020. doi: 10.1155/2020/3260745.

Gayle, C. et al. (2016) 'Morphological variations of green mussel (Perna viridis) in Bula, General Santos city using geometric morphometric analysis', Journal of Biodiversity and Environmental Sciences J. Bio. Env. Sci, 8(2), pp. 2220–6663. Available at: http://www.innspub.net.

Grienke, U., Silke, J. and Tasdemir, D. (2014) 'Bioactive compounds from marine mussels and their effects on human health', Food Chemistry, 142, pp. 48–60. doi: 10.1016/j.foodchem.2013.07.027.

Macintosh, D. J., Ashton, E. C. and Havanon, S. (2002) 'Mangrove rehabilitation and intertidal biodiversity: A study in the Ranong mangrove ecosystem, Thailand', Estuarine, Coastal and Shelf Science, 55(3), pp. 331–345. doi: 10.1006/ecss.2001.0896.

Mirwandhono, E., Nasution, M. I. A. and Yunilas (2022) 'Extraction of chitin and chitosan black soldier fly (Hermetia illucens) prepupa phase on characterization and yield', IOP Conference Series: Earth and Environmental Science, 1114(1). doi: 10.1088/1755-1315/1114/1/012019.

Moses, T., Papadopoulou, K. K. and Osbourn, A. (2014) 'Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives', Critical Reviews in Biochemistry and Molecular Biology, 49(6), pp. 439–462. doi: 10.3109/10409238.2014.953628.

Nguyen, V. B. et al. (2020) 'Utilization of Crab Waste for Cost-Effective Bioproduction of Prodigiosin', Marine Drugs, 18(11). doi: 10.3390/MD18110523.

Noor, N. M. et al. (2019) 'Biological aspects of green mussels Perna viridis cultivated on raft culture in Pasaran coastal waters, Indonesia', AACL Bioflux, 12(2), pp. 448–456.

Safitri, N. M., Herawati, E. Y. and Hsu, J. L. (2017) 'Antioxidant Activity of Purified Active Peptide Derived from Spirulina platensis Enzymatic Hydrolysates', Research Journal of Life Science, 4(2), pp. 119–128. doi: 10.21776/ub.rjls.2017.004.02.5.

Sze, P. W. C. and Lee, S. Y. (2000) 'Effects of chronic copper exposure on the green mussel Perna viridis', Marine Biology, 137(3), pp. 379–392. doi: 10.1007/s002270000350.

Tan, K. S. and Ransangan, J. (2017) 'Feeding behaviour of green mussels, Perna viridis farmed in Marudu Bay, Malaysia', Aquaculture Research, 48(3), pp. 1216–1231. doi: 10.1111/are.12963.

Teanchai, K., Witit-Anun, N. and Chaikhun, S. (2016) 'Characterization and analyzation of chitosan from Anadara granosa Shell', Key Engineering Materials, 675–676, pp. 463–466. doi: 10.4028/www.scientific.net/KEM.675-676.463.

Vincken, J. P. et al. (2007) 'Saponins, classification and occurrence in the plant kingdom', Phytochemistry, 68(3), pp. 275–297. doi: 10.1016/j.phytochem.2006.10.008.