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# Protein profiles and DNA isolation of hemolymph gonggong snail (*Strombus* sp.) from Bintan

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**Abstract.** Gonggong snail is one of the famous and highly-priced local seafood in Bintan Island, Riau Islands Province. It is an icon of Tanjung Pinang city. Until now, general research on Bintan gonggong snail is scarce, even if it has the potential to be used for aquaculture, functional food ingredient, and medicine. Research of bioactive peptides are mostly performed using the group of molluscs (gastropods). The research on protein profiles and DNA isolation has never been studied in hemolymph Bintan gonggong snail. This research was aimed to identify protein profiles and DNA isolation in hemolymph Bintan gonggong snail (*Strombus* sp). Protein profiling used SDS-PAGE. DNA isolation used Qiagen DNeasy methods, while PCR used primer histon H2A. Identification on protein profiles displayed that thin-shelled and thick-shelled gonggong snails had a similiarity value of 37 kDa, whereas the other protein profiles were different. The types of protein of Bintan gonggong snails were predicted to be histone proteins. The DNA of hemolymph Bintan gonggong snails had a similiarity value of 75 bp fragment histon. It is predicted as protein histon H2A and will be developed potentially as antimicrobial peptides (AMPs).

**Keyword:** Bintan, DNA, snail

## 1. Introduction

Thousands of bioactive compounds have been identified from marine organisms. This reveals that marine animals are also a source of medicine (pharmaceutical), including antimicrobial peptide bioactive compounds (Antimicrobial Peptides, AMPs) [1]. Antimicrobial Peptides are low molecular weight bioactive peptides in the form of short proteins or peptides produced by cells and tissues in the body as the body's defense system in prokaryotes, plants, animals and humans. AMPs can inhibit or kill microbes [2-3]. Antimicrobial peptides (AMPs) are one of the most important compounds in innate immunity. AMPs are thought to play an important role in invertebrate animals as a major defense against various pathogenic potentials and are naturally used for non-specific body defense systems in order to maintain life in extreme conditions [1-4].

AMPs are found in some marine invertebrates including marine sponges, mollusks and crustaceans [5]. Generally, AMPs in molluscs have histone proteins, which are a form of protein important as antibiotic



peptides [4-6]. Studies on the AMPs from mollusks are focused on solving the problem of conventional antibiotic resistance [7]. AMPs Molluskin found gastropods (H2A histone protein derivatives) such as 25 amino acid residues, with a low molecular weight of 2.84 kDA and  $\alpha$ -helix structures [4].

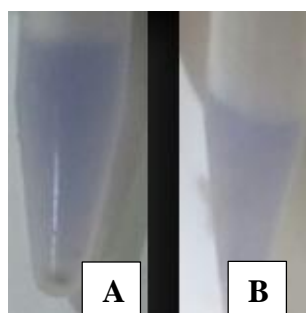
The hemolymph in gastropods is very important in the body's defense system because it contains antimicrobial bioactive peptides [8]. The sea snail gonggong (*Strombus* sp.) is one type of marine biota from the *Prosobrancia* subclass (the same subclass as abalone) that can be found in the Riau Islands Province and that has a high economic value but is not widely known. Gonggong is a one of the marine mollusks from the class of gastropoda (family Strombidae) which lives a lot on the coastal areas of Bintan Island. It is available in the Riau Islands, thus becoming the "icon" of Tanjung Pinang, Riau Islands Province. Generally, in culinary tours at Bintan, gonggong is only processed by boiling with sea water and then eaten with peanut sauce [9].

Studies on gonggong snails are still very limited. The preliminary study of gonggong focused on gonggong meat, which contains amino acids and heavy metals [9-11]. Gonggong meat contains a very high protein level (19.77% w/w), higher than the protein in oysters (9.47%) [9]. Currently the most studied gastropods for antimicrobial bioactive peptides is *Haliotis* sp. (abalone) because it has histone protein in the hemolymph [4, 8, 12]. Generally, studies on histone proteins as antimicrobial bioactive peptides look at mollusks, which contain the amino acids arginine, alanine, serine, glycine, proline, tryptophan, and cysteine [4, 13]. Gonggong contains the amino acids glycine, alanine, arginine, serine leucine and lysine [14]. More than a hundred patents in the pharmaceutical industry are based on sea snails. Based on this information, it is necessary to identify and characterize protein profiles and isolate hemolymph Bintan sea snail gonggong (*Strombus* sp.), which potentially contains histone proteins as natural antibiotics.

## 2. Materials and Methods

### 2.1. Sample collection

Samples of Bintan gonggong snails was collected in the coastal areas of Madong Village, Bintan Island, Tanjungpinang, Riau Islands Province (0°59.34 'N, 1°4027.22' E) at four stations using hands (directly or by diving). The samples were brought to the laboratory and acclimatized for 3 days in an aquarium (90 x 40 x 40 cm<sup>3</sup>) before the samples were analyzed. The Bintan gonggong snail used in this study measured 46.53-64.92 mm with outer lip > 2 mm for thick-shelled gonggong, outer lip > 1 mm for mature thin-shelled gonggong [15]. The number of samples used in this study was 200 each. The hemolymph of Bintan gonggong snail was taken without anesthesia using 3 mL sryinge on the leg muscles at horizontal position. The hemolymph was stored without anticoagulant at a temperature of -20°C before it was used for analysis (figure 1).



**Figure 1.** The hemolymph of gonggong snail from Bintan. A= hemolymph of thick-shelled gonggong; B= hemolymph of thin-shelled gonggong.

## 2.2. Protein profiles

The identification of protein profiles in the hemolymph of the gonggong snail used SDS-PAGE [16]. The SDS-PAGE method of protein profile analysis includes: sample preparation, sample added buffer loading (1:1), separation and retaining gel preparation, running conditions (three hours), gel staining (30 men), cleaning color (one hour), and gel photo recording. Preparation of SDS-PAGE used 12.5% separation gel and 3% retaining gel. The volume of sample gonggong snail hemolymph was 3  $\mu\text{L}$ . It was run at 100 V and 13 mA for three hours. Marker used 250 kDa of 3  $\mu\text{L}$ .

## 2.3. Isolation and DNA amplification of gonggong hemolymph

**2.3.1. DNA extraction.** Hemolymph extraction in samples of gonggong snail (thick-shelled gonggong and thin-shelled gonggong) was done randomly on 200 samples. Samples were taken as much as 0.05 mL, then put into an eppendorf tube, then heated to 60°C for 15 minutes, then added with 180  $\mu\text{L}$  ATL buffer and 20  $\mu\text{L}$  proteinase K solution, then homogenized for 3 seconds. The samples were then exported for one second. The samples were again heated to 60°C for 15 minutes, and the addition of 200  $\mu\text{L}$  AL Buffer was then distorted for one second, then heated to 60°C for eight minutes, then added with 96% ethanol as much as 200  $\mu\text{L}$ , then stored in a freezer for 30 minutes at -20°C.

The samples were pipetted to spin 2 mL column, then centrifuge 8,000 rpm for one minute (remove the lower liquid), then add AW 1 buffer as much as 500  $\mu\text{L}$ . The samples were centrifuged at 8000 rpm for 1 minute (dispose of the lower liquid), then added 500  $\mu\text{L}$  of AW 2 buffer then centrifuged 8000 rpm for one minute and continued 14,000 rpm for three minutes (removing the lower liquid). Finally, a sample of 100  $\mu\text{L}$  of AE buffer was added and incubated for teen minutes at room temperature and then centrifuged 8000 rpm for one minute so that total DNA (Qiagen D-Neasy Method).

**2.3.2. DNA amplification of gonggong hemolymph.** This study used 2  $\mu\text{L}$  DNA from each sample extraction. The intensification of sample was carried out using Polymerase Chain Reaction (PCR). All reactions were performed at a volume of 25  $\mu\text{L}$ , consisting of 10  $\mu\text{L}$  mixture (Mytaq, dNTP, DNA polymerase and buffer), Histone H2 Primer (F) and Histone H2 Primer 2  $\mu\text{L}$ , NFW (ddH<sub>2</sub>O), respectively with a volume of 9  $\mu\text{L}$ .

The PCR was carried out under the following conditions: initial temperature of 94°C for five minutes, pre-denaturation at 94°C for five minutes, DNA denaturation at 94°C for 25 seconds, annealing at 57°C for 25 seconds, and extension at 72°C for 25 seconds, the final elongation temperature was 72°C for five minutes, and the total reaction was 40 cycles. The target amplification is at 75 bp from the H2 histone protein. The sequencing using histone primer H2A is 5' ATGTCTGGACGAGGAAAGGGAGGA -3', while the sequence of the H2B protein is 5' TACTTGGCAGGTTTCTGGGTCT -3' [4]. The PCR reaction was visualized using 2% agarose gel and electrophoresis lasting for 30 minutes at 200 V. This reaction produces a single band of 75 bp.

## 3. Results and Discussion

Live Bintan gonggong snails (thick-shelled gonggong and thin-shelled gonggong) have been stable in the acclimatization process supported by the environment. The water quality variables were measured in acclimatization process (table 1).

The quality of water determined the physiology of Bintan gonggong snails. If the condition is unstable, they will experience stress [11]. The aquatic conditions of Bintan gonggong snails includes: salinity 26-32‰, pH 7.1-8.0, dissolved oxygen 4.5-6.5 ppt, and temperature 26-30°C [10]. Acclimatization of samples were be uniform because they were taken from 4 different stations in Madong Village, Bintan Island, Tanjung Pinang, Riau Islands Province. Hemolymph was taken from Bintan gonggong snails every day in acclimatization process (table 2).

**Table 1.** Water quality in the acclimatization process.

Observation	Aquarium of thin shelled gonggong			Aquarium of thick shelled gonggong		
	Days-1	Days-2	Days-3	Days-1	Days-2	Days-3
pH	7.67	7.51	7.56	7.73	7.58	7.78
Temperature (°C)	27.8	27.8	28.8	26.7	26.6	26.4
DO (ppt)	6.9	6.1	6.3	6.7	6.4	6.4
Salinitas (‰)	27	29	29	27	27	28

**Table 2.** Hemolymph volume of Bintan gonggong snails in acclimatization process (mL).

Sample	Day 1	Day 1-Day 2	Days 3
Thin shelled-gonggong	0.6	0.6	0.3
Thick shelled gonggong	0.4	0.3	0.25

Table 2 showed that the hemolymph volume in the thin-shelled gonggong is more than the thick shelled gonggong. However, the hemolymph volumes of the thin-shelled gonggong decreased on the third day of acclimatization process, whereas that of the thick shelled gonggong was stable. It was suspected that hemolymph volumes of gonggong were influenced by the amount of nutrients available in the environment. The acclimatization conditions in the aquarium had less nutrients (muddy substrate and *Enhallus* sp. seagrass were the least) that they affected the amount of blood (hemolymph). Hemolymph volume in mollusks is influenced by the environment [8, 15]. Hemolymph in this study was collected on August 11, 2017. Hemolymph concentration increased in gastropods in July-August [7], thus the quality of hemolymph in the gonggong snails are good quality.

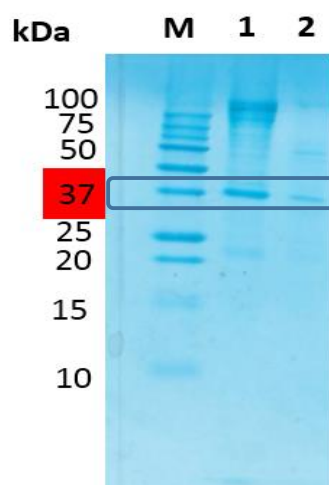
### 3.1. Protein profiles characterization in gonggong snails hemolymph

Hemolymph is a blood cell in gastropods (2000 cells/mL) that contains proteins. It carries oxygen throughout the body of the mollusks and is innately immune [17]. Protein profiles in the gonggong hemolymph can be seen in figure 2.

Figure 2 showed that the molecular weight of the protein in the hemolymph of the gonggong snails have a similarity of 37 kDa, whereas the other proteins profile were different. The protein profile in Bintan gonggong snails were predicted to be histone protein. Histone protein has molecular weight 11-37 kDa, such as 333 amino acids [18]. Histone proteins (H2A) in gastropods are antimicrobial bioactive peptides [4, 19].

### 3.2. Amplification of gonggong snails hemolymph

Proteins in the hemolymph of the gonggong snails (thick-shelled and thin-shelled) are predicted as histone proteins, so isolation is needed to prove histone proteins. The results of DNA amplification of gonggong snails hemolymph is illustrated in figure 3.



**Figure 2.** Protein profiles in the hemolymph of gonggong snails. Line 1 = hemolymph thick-shelled gonggong ; Line 2 = hemolymph thin-shelled gonggong; M = marker.



**Figure 3.** DNA amplification of histone fragments in gonggong snails hemolymph. M = Marker; 1 = hemolymph thick-shelled gonggong; 2 = hemolymph thin-shelled gonggong.

Figure 3 showed that the DNA of Bintan gonggong snails hemolymph is a histone fragment target of 75 bp. In mollusks there are H2A histone proteins (75 bp), which include 25 amino acids,  $\alpha$ -helix structures, and positively charged amino acids (arginine and lysine). They are as antimicrobial peptides. They are as candidate for natural antibiotics [4, 12].

Until now, research on antimicrobial bioactive peptides gastropods (AMPs) is the most common, such as research on AMPs in sea snails (*Biomphalaria glabrata*) is taken naturally in hemocytes (hemolymph) [1, 20]. In 2006 AMPs were conducted on *Littorina littorea* through hemolymph [17]. Research on these AMPs has also been done on other species of abalone (*Haliotis discus hannai*) from *Haliotidae* family, and hemolymph was obtained by AMPs named *Defensin* (hd-def), which could kill Gram-positive and Gram-negative bacteria. *Defensin* molecular weight (hd-def) is 4,323 kDa and is rich in cysteine amino acids [21]. Research in 2009 was done on an abalone species (*Haliotis discus-discus*), and it named *Abhisin* (Histon H2A protein). *Abhisin* AMPs come from hemolymph on abalone, which contains the amino acids arginine and lysine, hydrophobic amino acid of +13 cationic charges. *Abhisin* has a low molecular weight of 4.32 kDa (40 amino acids) [12]. In 2009, AMPs *Defensin* from the hemolymph of an abalone species (*Haliotis Haliotidae*) was also investigated. It was found to have a lower molecular weight of 4 kDa [22]. Furthermore, in 2010 AMPs *Defensin* was also found in the hemolymph of abalone gastropods (*Haliotis discus-discus*) with a molecular weight of 4.9 kDa (49 amino acids), which has hydrophobic amino acid of +5 cationic charges., and a  $\alpha$ -helix structure with 3 disulfide bridges and has 198 base pairs [19]. The latest AMPs from gastropods were derived from the

hemolymph of the *Cenchritis muricatus* species (*Littorinidae* family). The AMPs were called AMPs peptide Cm-p5, with an  $\alpha$  helix structure that could kill fungi [23].

Thus, in the gastropod class there are 2 sub-classes with the ability of AMPs. This includes *Prosobrancia* and *Planorbidae*. Bintan Gonggong snails are included in the *Strombidae* family from the *Prosobrancia* sub-class, so it can be assumed that gonggong has the potential to be an antimicrobial peptide (AMPs) because they have histone protein (H2A). They can be used as functional food in Bintan Island, which means that they will need purification and screening as antimicrobial peptides.

#### 4. Conclusion

Protein in the hemolymph of gonggong snails from Bintan was predicted to be histone. DNA isolation of gonggong snails hemolymph resulted in fraction H2A histone protein because they have band of 75 bp, which means that they will be needed purification and screening as antimicrobial peptides. They can be used as functional food in Bintan Island, Riau Islands Province.

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