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EVALUATION OF THE EFFECT OF ANTIOXIDANT AGENTS OF SHRIMP - SEA JELLYFISH *(Catostylus sp)* bioactive peptide compounds ON MACROSCOPIC CHANGES IN THE LIVER OF WISTAR RATS *(Rattus novergicus)*

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ABSTRACT

Jellyfish contain bioactive compounds that have the potential to act as antioxidants. Bioactive peptides in jellyfish are still rarely studied in research, so exploration regarding the benefits of bioactive compounds in jellyfish needs to be carried out. This research aims to isolate bioactive peptides from Catostylus sp. jellyfish, determine the levels of isolated protein compounds, and evaluate the antioxidant activity of bioactive peptides through in vitro and in vivo tests. The research was carried out using the method of isolating bioactive peptides through ammonium sulfate fractionation and analyzing its activity on rat as test animals. Jellyfish sample preparations were ground and extracted to obtain crude protein, which was then fractionated using ammonium sulfate. The antioxidant activity of bioactive peptides was tested using the DPPH method. Preliminary research findings indicate that bioactive peptides from jellyfish have the potential for significant antioxidant activity, with in vitro tests showing possible free radical inhibiting abilities and in vivo tests showing the potential for increased liver healing response and immune enhancement in rat.

Keywords: Antioxidant, Immunostimulant, In Vivo, In Vitro

Introduction

Indonesia, as a maritime country, has great potential in the utilization of marine resources, one of which is jellyfish. However, the jellyfish processing industry in Indonesia is still lagging behind compared to other countries such as Vietnam which have been more advanced in this field. There are several types of jellyfish that can be consumed, which contain various valuable bioactive compounds such as terpenoids, phenolic derivatives, proteins, and peptides that have various biological benefits (Pangestuti & Arifin, 2018). Bioactive compounds in jellyfish such as proteins, polysaccharides, and peptides can exhibit antioxidant properties (Li et al., 2023) and immunostimulants (Mutalipassi et al., 2021).

Previous research has shown that bioactive peptides extracted from marine animals, including jellyfish, have significant biological activity. These peptides exhibit a wide range of activities such as antioxidant, anti-aging, anti-hypertensive, and immunostimulant. Bioactive peptides from jellyfish have unique characteristics, such as weak antigenicity and good biological compatibility, which make them potential to be developed as functional ingredients in the food and pharmaceutical industries (Kim & Wijesekara, 2010). In addition, this peptide also shows promising anti-cancer and anti-inflammatory activities (Wang et al., 2008).

Peptides in jellyfish are short-chain structures consisting of amino acids that have their main benefits in the health field. Peptides can be used as the basis for the development of promising new drugs, such as the treatment of diabetes and cancer (Luo et al., 2023). Jellyfish peptides have unique characteristics of low antigenicity and good biological compatibility. Peptides with small molecular size tend to have a higher absorption rate through the gastrointestinal tract compared to large-sized peptides resulting from the ability of small peptides to more efficiently pass through the intestinal mucosa (Williams-Noonan et al., 2023). Thus, research on sea jellyfish peptides still needs to be studied to contribute to science and health in the future.

Despite the great potential of jellyfish, more in-depth research on the bioactive peptides of sea jellyfish is still very limited. Therefore, this study aims to explore the potential of jellyfish as a source of bioactive peptides that have important biological activity. The benefit is that it provides new knowledge about the bioactive compounds of jellyfish, which can contribute to the development of new therapies and further understanding of complex biological interactions. The urgency of this research lies in its ability to open up new opportunities in the therapy of diseases related to oxidative stress and the immune system, as well as support the sustainable use of marine resources. It is hoped that the results of this research can make a significant contribution to the field of science and health, as well as support the development of new medicines from marine resources. The expected findings include data on the antioxidant and immunostimulant activity of bioactive peptides from jellyfish, which could enrich our understanding of the therapeutic potential of these bioactive compounds.

Research Methods

Bioactive Peptide Isolation

The jellyfish sample was cleaned with seawater and 100 mL was added *Buffer* A. Then use *Blender* to blend and sonicate for 30 minutes. After that, the sample was centrifuged at a speed of 500 rpm for 30 minutes at a temperature of 4oC to produce a crude extract of protein that would be analyzed using the Lowry method modified from the research of Ahmad et al. (2014). Then hydrolysis is carried out by diluting the highest protein extract into a 3% solution. Hydrolysis was carried out by a ratio of enzyme and substrate 3:100 at 37oC with pH 2. The solution is incubated in a test tube for half an hour with variations in hydrolysis time (0; 0.5; 1; 2; 3; 4; 5; and 6 hours) to obtain optimal conditions. After hydrolysis, the enzyme is inactivated by boiling the solution in water for 10 minutes and centrifugating at a speed of 10,000 rpm at a temperature of 4oC for 20 minutes modified from (Teng et al., 2023). After that, protein levels were measured in crude extracts and dialysis results were carried out using the Lowry method of dimodulation from (Suhendi et al., 2023). Then, the standard series is made from a standard solution of BSA (*Bovine Serum Albumin*) 0.2 % with concentrations (0.04; 0.05; 0.06; 0.07; and 0.08 mg/ml). A total of 5 mL of the standard solution and sambel were taken, then as much as 750 µL of each solution was added 1 mL of Lowry A reagent and left in the room for 10 minutes. Next, 1 mL of Lowry B reagent is added and left at room temperature for 65 minutes. Finally, the absorbance of the solution is measured at wavelength with a UV-Vis Spectrophotometer.

In Vitro *Antioxidant Activity Test*

The in vitro *antioxidant activity test* was carried out by the DPPH method by making a standard series of ascorbic acid made by pipetting a 5 ppm concentration of ascorbic acid solution of 0.25 mL, 1 mL, 2 mL, and 4 mL in different test tubes to obtain standard series of 0.25 ppm, 0.5 ppm, 1 ppm, 2 ppm, and 4 ppm. Askorban acid solution is added 1 mL of DPPH 0.4 mM solution. Then methanol p.a was 3.75 mL, 3.5 mL, 2 mL, and 0 mL so that the total volume of each solution was 5 mL. Incubation in a dark room was carried out at room temperature of 30 minutes, as tight as a standard series measured at a maximum length of 515 nm modified from (Sutriningsih, 2018). The determination of the antioxidant activity of protein fractions is carried out in a similar way. Protein fractionation with a concentration of 500 ppm was pipetted at 0.1 mL, 0.2 mL, 0.4 mL, 0.8 mL, and 1.6 mL, respectively, to create a measuring series of 10 ppm, 20 ppm, 40 ppm, 80 ppm, and 160 ppm. Each measuring series solution is added 1 mL of 0.4 mM DPPH solution and p.a methanol until the total volume reaches 5 mL. The control solution was made by pipetting 1 mL of DPPH 04 mM solution into a test tube and adding p.a methanol to a total volume of 5 mL. The measuring series of each fraction and control solution were incubated at room temperature for 30 minutes in a dark room. Absorption was measured at a maximum wavelength of 515 nm modified from (Sutriningsih, 2018).

In Vivo *Antioxidant Activity Test*

The in vivo *antioxidant activity test* was carried out by grouping 8 rats with a body weight of 180 grams with an age of 2-3 months into 4 groups, namely normal control without treatment and negative control given a toxic dose of paracetamol 500 mg/kgBB, treatment group 1 was given paracetamol as much as 500 mg/kgBB and Jellyfish Bioactive Peptide Extract as much as 250 mg/kgBB, and treatment group 2 was given paracetamol as much as 500 mg/kgBB and Jellyfish Bioactive Peptide Extract as much as 500 mg/kgBB. Test animals will be given treatment for 14 days in the form of induction of paracetamol at a toxic dose of 500 mg/lgBB and on days 15 to 21 they will be given bioactive peptide extracts. After 24 hours on day 21, blood will be drawn from the rats in the group and checked for SGPT and SGOT levels of serum rats. Serum in mice was taken from the blood vessels in the eyes of mice that were accommodated with Eppendorf tubes and centrifuged at a speed of 3000 rpm for 15 minutes (Putri, W. C. P., Yuliawati., and Rahman, 2021).

Results and Discussion

Results of Protein Crude Extract Content Measurement Data

The results of the extraction of jellyfish were obtained from crude protein extracts with protein levels of *Catostylus sp.* by 2.85% (Figure 1) with the regression equation y = 3.7665x + 0.1659 with the value of the determination coefficient (R2) = 0.9941. The R2 value on the measurement graph using a spectrophotometer indicates how well the data produced in the spectrophotometric analysis (Smith et al., 2023). Jellyfish have low calories, contain 95% water, and 4% - 5% protein, especially collagen (Hsiesh et al., 2001), but the protein content of jellyfish depends on the type of jellyfish.

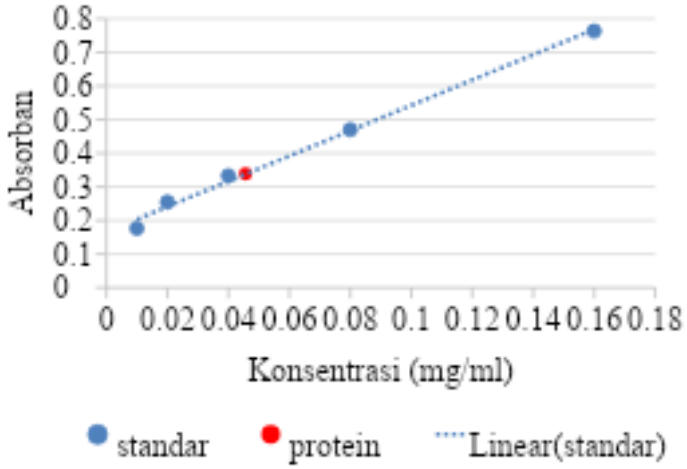

Figure 1. Protein Crude Extract Content Measurement

Results of Protein Content Measurement Data After Hydrolysis

Hydrolysis testing of protein levels with different time variations of (0, 30, 90 and 120) minutes produced the optimum value for hydrolysis for 90 minutes. The optimal time of 90 minutes for hydrolysis is because the peptide concentration obtained is larger, which is 2.23%, compared to the time (0.60 and 120) minutes (Figure 2). The concentration decreased after hydrolysis for 120 minutes because hydrolysis using the pepsin enzyme had passed the optimal time to hydrolyze proteins into peptides. Studies have shown that pepsin activity increases as the incubation time increases until it reaches its peak at a certain time, which typically ranges from 60 to 120 minutes depending on experimental conditions (Munaeni et al., 2023).

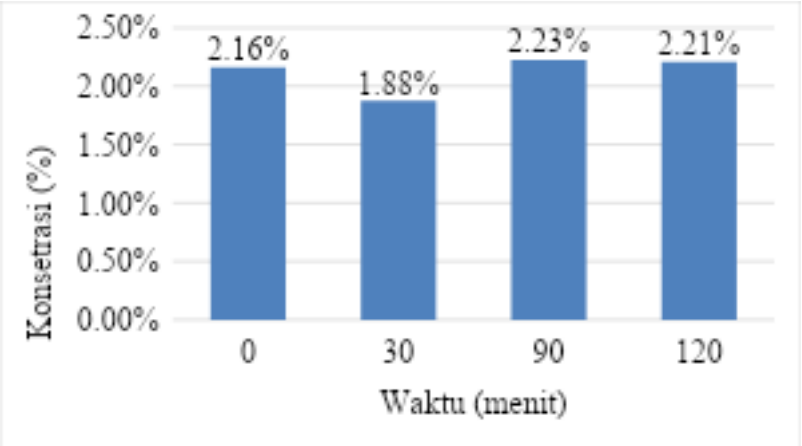

Figure 2. Protein Content Measurement After Hydrolysis

Peptide Level Measurement Data Results After *Cut Off*

The results of protein content measurement in the sample cut-off membrane test will be divided into 4, namely >10 KDa, 5-10 KDa, 3-5 KDa and <3 KDa (Figure 3). Peptides measured after *Cut Off* by the Lowry method using Uv-Vis spectrophotometry obtained the largest peptide concentration in a sample with a molecular weight of >10 of 2.55%, compared to a sample with a molecular weight of < 10 kDa (Figure 3). Peptides with a molecular weight of >10 kDa are among the large peptides, although peptides are generally known as short chains of amino acids with low molecular weight, there are some cases where larger molecules are classified as peptides (Cottrell, 2011). Peptides with a molecular weight of >10 kDa tend to have a more

complex structure and function in a variety of biological processes, including as hormones and growth factors (Chait & Kent, 1992). This peptide with a large molecular size is a neurotoxic peptide found in marine animals, including the species of jellyfish (Cottrell, 2011). After obtaining concentration data in each sample, the 4 samples were tested for antioxidants using the DPPH method.

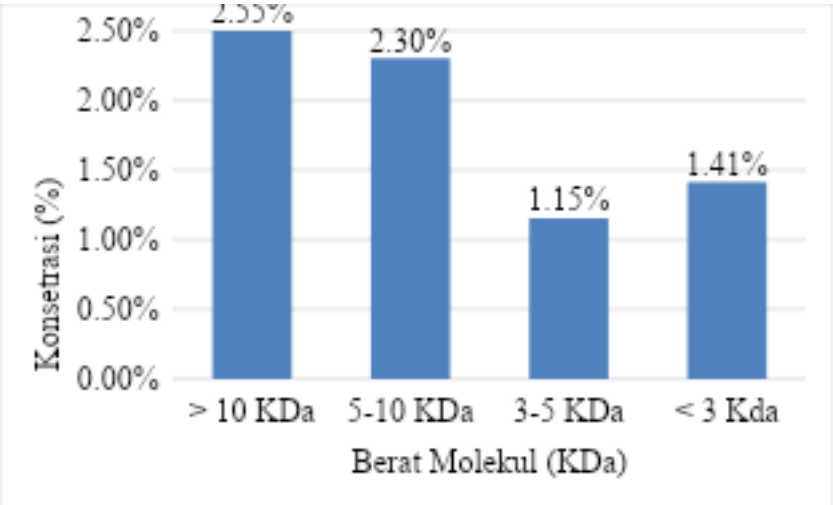

Figure 3. Measurement of Peptide Levels After *Cut Off*

In Vitro *Antioxidant Test Data Results*

Measurement of antioxidant activity was carried out by measuring the activity of a standard solution of ascorbic acid. The main function of ascorbic acid in this case is as a positive control that allows the determination of the antioxidant ability of other compounds or ingredients relative to known standards (Padayatty & Levine, 2016). The IC50 value is obtained from the linear regression curve equation (Figure 4) where the value of the equation y = 22.469x + 44.337 is then calculated by entering the value of 50 as the value of y. The lower the IC50 value, the more effective the compound is in fighting oxidative stress (Cadenas & Packer, 2002). The IC50 that is considered good for ascorbic acid is generally in the low concentration range, indicating its ability to effectively inhibit oxidative stress. This information is important in evaluating the antioxidant potential of a new compound or formulation. IC50 for ascorbic acid in some studies has been recorded at around 10-50 ppm (Misra & Fridovich, 1972). The percentage value of inhibition is calculated by the formula:

$$\%\ Inhibisi = \frac{Absorban\ Kontrol - Absorban\ Sampel}{Absorban\ Kontrol}$$

Table 1. Results Measurement of absorbance value and % inhibition value of Ascorbic Acid solution with DPPH

Concentration (PPM)	Ln Concentration	Absorbance		% Inhibition
		Control	Standard	
4	1,386294361	0,784	0,146	81,38
2	0,693147181	0,784	0,37	52,81
1	0	0,784	0,459	41,45
0,5	-0,693147181	0,784	0,531	32,27
0,25	-1,386294361	0,784	0,676	13,78

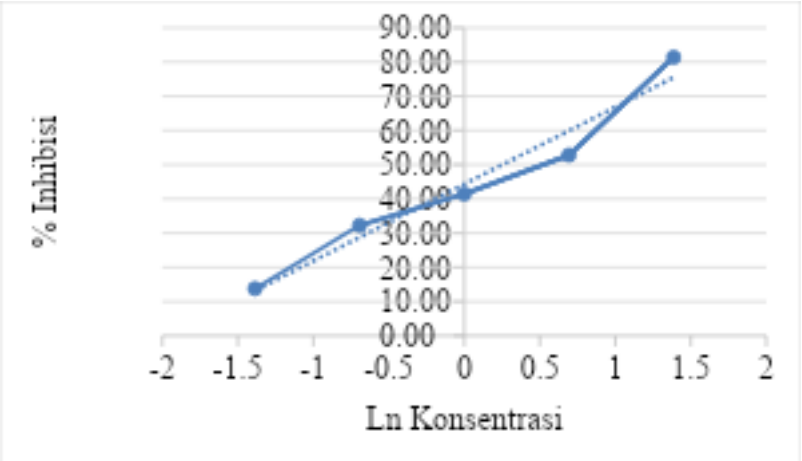

Figure 4. IC50 Measurement of Ascorbic Acid Standard Solution

The results of peptide absorbance measurements were used to measure the percentage of inhibition where the largest percentage of inhibition was peptides with a molecular weight of >10 kDa (Table 2). The results of measuring the concentration of >10 kDa samples had the best IC50 value of 7.79 ppm compared to other samples that had a larger IC50 value as seen in Table 2.

Table 2. Results Measurement of absorbance value and % value of Inhibition of Peptide solution with DPPH

Sample	Concentration (PPM)	Absorbance		% Inhibition	IC50
		Control	Sample		
10 kDa	160	0,784	0,248	68,37	7,97
	80	0,784	0,261	66,71	
	40	0,784	0,316	59,69	
	20	0,784	0,352	55,1	
	10	0,784	0,377	51,91	
5-10 kDa	160	0,784	0,448	42,86	117,85
	80	0,784	0,256	67,35	
	40	0,784	0,592	24,49	
	20	0,784	0,774	1,28	
	10	0,784	0,712	9,18	
3-5 kDa	160	0,784	0,548	30,1	2413,87
	80	0,784	0,703	10,33	
	40	0,784	0,753	3,95	
	20	0,784	0,777	0,89	
	10	0,784	0,782	0,26	
<3 kDa	160	0,784	0,364	53,57	281,7
	80	0,784	0,593	24,36	
	40	0,784	0,719	8,29	
	20	0,784	0,729	7,02	
	10	0,784	0,704	10,2	

A sample of >10 kDa is considered very active and has the potential to be an antioxidant, because based on the standard IC50 value, a sample that has very strong antioxidant activity if the IC50 value is less than 50 ppm, strong if the value is 50-100 ppm, medium if the value is 101-150 ppm, and weak if the value is between 151-200 ppm (Zuhra et al., 2008). However, there is another theory that says that a sample has antioxidant properties if the IC50 value is less than 200 ppm and if the IC50 value obtained ranges from 200-1000 ppm, then the substance is less active but still has the potential to be an antioxidant substance (Pasaribu et al., 2021), so that bioactive peptides weighing <10 kDa are less active but have the potential to be antioxidants.

In Vivo *Antioxidant Test Data Results*

In vivo antioxidant tests were carried out by measuring SGPT/SGOT on the blood serum of mice that had been obtained. Each rat from each group was given blood collection treatment through *retro orbital* and will be tested for damage to the liver organ by the SGPT/SGOT method. SGPT enzyme (*Serum Glutamic Pyruvic Transaminase*) is an enzyme formed in hepatocytes (liver cells), while SGOT enzyme (*Serum Glutamic Oxaloacetic Transaminase*) is an enzyme found in the liver, skeletal muscle, heart muscle, kidneys, brain pancreas, white blood cells and red blood cells (Andini & Rahman, 2022). SGPT levels in normal rats ranged from 17.5 to 30.2 U/L (Saputri et al., 2017). The results of the SGPT/SGOT test can be seen in Table 3.

Table 3. Results of Measurement of SGPT and SGOT levels

Group	Average SGPT Levels	Average SGOT Levels
Usual	112.5	231
Negative	114	179
Treatment 1	113.5	182.5
Treatment 2	87	128

SGPT measured by the normal group as a comparison had a higher than normal SGPT level. However, in the test results, it was found that the average SGPT level from treatment group 1, namely jellyfish bioactive peptide extract with a dose of 250 mg/kgBB, was closer to the normal limit than the dose of 500 mg/kgBB. This indicates that a dose of 250 mg/kgBB is more effective at triggering antioxidant effects in white rats.

The SGOT Enzyme test can provide information about liver damage but is not as specific as the SGPT test. According to (Abdulkadir & Tungadi, 2017), the concentration of SGOT will increase when severe necrosis occurs in the liver. The normal concentration of SGOT in normal white rats ranged from 45.7-80.8 U/L (Krysanti & Widjanarko, 2014). Based on the results, the effect of the administration of toxic doses of paracetamol made the levels of SGOT in rats far from the normal limit. Then, the effect of giving jellyfish bioactive peptide extracts at doses of 250 mg/kgBB and 500 mg/kgBB was close to normal levels of SGOT. The dose of 250 mg/kgBB is an effective dose in healing the liver due to damage by paracetamol.

Furthermore, tests were carried out by making morphological observations of the liver of white rats after being given a treatment that can be seen in figure 5.

(A)	(B)	(C)

(D)

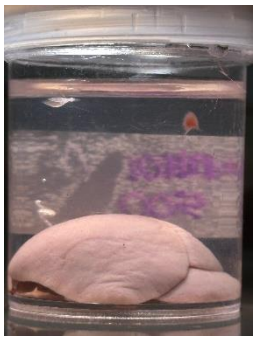

Figure 5. Macroscopic structure of the liver after treatment in four groups: A (normal control); B (negative control); C (Dose treatment of jellyfish bioactive peptide extract 250 mg/kgBB); D (Dose treatment of jellyfish bioactive peptide extract 500 mg/kgBB)

Based on macroscopic observations to see the morphology of the liver of each treatment rat, it was found that normal control showed a healthy liver without damage, while negative control showed poor liver quality due to damage by toxic doses of paracetamol. Treatment 1, namely jellyfish bioactive peptide extract at a dose of 250 mg/kgBB in mice, provided a brighter morphological appearance of the liver compared to the dose of 500 mg/kgBB and negative control. Treatment group 1 (dose 250 mg/kgBB) was more effective than treatment group 2 (dose 500 mg/kgBB). The liver can be damaged because of its function as an organ that neutralizes toxic substances (Bire et al., 2018). A normal liver will be brownish-red caused by blood flow into (Fortes, 2017).

Conclusion

Based on the results of the study, bioactive peptide extracts from sea jellyfish isolated from catostylus sp. showed the presence of antioxidant and immunostimulant activities that have been tested in in vitro antioxidant tests by obtaining results that >10 kDA has the potential to be active as an antioxidant. Then, based on in vivo antioxidant tests and in vivo immunostimulant tests, a dose of 250 mg/kgBB was obtained as the best dose as an antioxidant in mice

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