ANTIOXIDANT ACTIVITY POTENCY OF CHITOSAN FROM HARUAN

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Introduction

Periodontal disease is an inflammation of the supporting tissues of the teeth consisting of gingiva, cementum, periodontal ligament and alveolar bone. Periodontal disease has a high prevalence in all age groups in Indonesia, which is 96.58%. The main factors causing periodontal disease are a number of pathogenic microorganisms, including gram-negative bacteria Porphyromonas gingivalis, Treponema denticola, Agregatibacter actinomycetemcomitans (Aa), Fusobacterium nucleatum, Prevotella intermedia. Periodontitis occurs due to interactions between periodontal tissue, plaque and saliva. The interaction between these factors creates various immunological processes both protective and tissue damage. Periodontal tissue damage can be caused by direct damage due to toxins produced by bacteria in plaque or by the local inflammatory response and the activity of inflammatory mediators. [2,3,4]

Kim (2010) in his research said organisms exposed to bacterial attacks will trigger an immune response between bacterial pathogens and hosts. These bacteria will cause the release of cytokines such as interleukin-1 alpha (IL-1α) and β, interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor-alpha (TNF-α) thereby increasing the amount of polymorphonuclear production (PMN). The produced PMN has a protective role against

periodontal tissue. PMN that is functionally activated will show an increase in free radicals in the form of Reactive Oxygen Species (ROS) in the process of phagocytosis against bacterial infections. Excessive ROS will cause destruction of gingival tissue, periodontal ligaments and alveolar bone through various means including damaging DNA and stimulating the formation of pro-inflammatory cytokines.[5]

Haruan fish (Channa striata) is one of the freshwater fish species of South Kalimantan which is known to have properties in helping accelerate wound healing.[6] Recently, new methods in the utilization of fish scales waste have increasingly been developed, including in the field of biomedicine in the pharmaceutical, food and medical, namely chitin contained in fish scales.

Chitin is found in cells below the dermal layer of fish scales.[7] Chitin which undergo chemical and enzymatic deacetylation will produce chitosan. Chitosan is a non-toxic biodegradable polymer and has a high molecular weight. Damayanti (2016) also added that chitosan has special properties in terms of biocompatibility, biodegradation, biological activities such as antibacterial, does not cause allergies, and its ability to form fibers and films.[8] This is in line with research by Widyaningrum (2019) and Dania (2020), which proves that chitosan from fish scales have potential as an antibacterial agent because they contain aminopolysaccharide groups that can inhibit the growth of gram-positive and gram-negative bacteria.[9,10]

Research relating to the potential of chitosan from fish scales today shows positive results. Sumanthi et al, in their research carried out extraction and characterized the results that chitosan obtained from fish scales in addition to being antimicrobial turned out to also be antioxidants. Tanvir Muslim et al. also conducted research by extracting and then characterizing chitin from Labeo rohita fish scales and obtained chitosan from chitin on fish scales have some potential in the medical field both as antibacterial and tissue engineering in bone repair.[11,12]

Based on the description above, it can be seen that fish scales contain chitin which can be synthesized into useful chitosan in the biomedical field. There has been no research on the synthesis and antioxidant potential of chitosan derived from freshwater fish scales, especially haruan fish (Channa striata). This research is needed to determine the antioxidant potential of chitosan from Haruan fish scales.

Material and Method

The research method to be conducted is true experimental design with a post-test only with control group design that involves qualitative and quantitative testing. The study was conducted with quantitative tests to calculate the amount of antioxidant activity of chitosan in fish scales using DPPH radical reduction method using a UV-Vis spectrophotometer.

The sampling technique to be investigated using simple random sampling in 2 groups with a total of 8 samples, namely 4 positive control samples of DPPH solution reacted with ascorbic acid with a standard concentration of 4,6,8, and 10 ppm and 4 samples of the treatment group solution DPPH which reacted with chitosan from Haruan fish scales concentration of 200,250,300 and 350 ppm. Each group was tested using 3 repetitions (triplo) to get the average value in each plot so that the IC50 results from DPPH radical reduction using linear regression calculations.

The inclusion criteria for including subjects in the study sample were haruan fish scales obtained directly from Kampung Kuin Cerucuk Banjarmasin, South Kalimantan, which had not been decomposed by special markings / colors covered by clear mucus and not much dirt attached.

The data obtained were further analyzed using the Shapiro-Wilk normality test, the Levene's Test homogeneity test, and the Independent T-Test.

Sample Collection

The collection of Haruan fish scale was done directly from Kampung Kuin Cerucuk Banjarmasin. Collected fish scales have had a gross weight of 3 kg. Fresh Haruan fish scales were put in plastic and stored in the ice box to the next stage. The next stage was separating the Haruan fish scales with other impurities which are then washed using soap.

Sample Preparation

At this stage, the fish scales were dried until the fish scales become brittle to facilitate the process of isolating chitin. Drying was done by roasting and using an oven for 24 hours at a temperature of 50°C. The scales were then crushed by blending until they become powder and stored in an airtight container.[13,14]

Chitin Isolation

Isolation of chitin from Haruan fish scales powder was consisted of two stages, namely deproteinization using 4% NaOH (1: 5 m / v) which boiled for 1 hour, then washed with distilled water until the pH was neutral and dried at 50°C for 24 hours. The next step was demineralization by mixing and stirring in 1 M HCl solution (1: 5 m / v) for 24

hours at 30°C, then washed with distilled water until the pH was neutral and dried at 50°C for 24 hours.14,15

Chitin Test

The Van Wesslink color reaction is useful for detecting the presence of chitin in the sample. The positive presence of chitin when reacted with a 1% solution of potassium-iodide (I2-KI) will give a yellowish-brown color, then add 1 M sulfuric acid (H^2SO^4) turns into a purplish red or red violet color.16

Chitosan preparation

Chitosan preparation was done by breaking the acetyl group contained in chitin extract from Haruan fish scales. The deacetylation process was carried out by dissolving chitin in 50% NaOH (1: 4 m / v) and heated at 80°C for 120 minutes on a hotplate. The results obtained were filtered and washed with distilled water until the pH was neutral then dried at 50°C for 24 hours using an oven.14,17

Quantitative Antioxidant Activity Testing on chitosan from fish scales using DPPH Method

Antioxidant activity test on ascorbic acid was carried out by making several concentrations of 4,6,8 and 10 ppm. Each concentration was mixed with 1 ml of DPPH methanol solution with an incubation time of 30 minutes then the absorbance was read at a wavelength of 517 nm. Each chitosan sample was taken at 0.1-1 mg and then dissolved in 0.2% acetic acid. Then the solution was mixed into 1 ml of DPPH methanol solution to obtain a final solution of 10 mM DPPH. The solution was then stirred and allowed to stand in a dark place for 20 minutes. The solution was read absorbance at a maximum wavelength of 517 nm. Ascorbic acid was used as a standard with the same concentration. Scavenging ability from chitosan was measured using the following formula:

$$\%\text{inhibisi} = \frac{\text{Absorbansi kontrol} - \text{absorbansi sampel}}{\text{absorbansi kontrol}} X\ 100\%$$

IC50 value is a concentration where the sample can inhibit free radicals by 50% obtained by using the linear regression equation y = a + bx.

Table 1. Antioxidant Activity Based on IC50 Value

Aktivitas	Nilai IC_{50}
Sangat Aktif	<50 ppm
Aktif	50-100 ppm
Sedang	101-250 ppm
Lemah	250-500 ppm
Tidah Aktif	>500 ppm

Result

After the data was collected, then the data was entered into a table and statistical calculations are performed with SPSS 22 for windows.

The results of the inhibition percentage normality test of chitosan from Haruan fish scales and ascorbic acid using Shapiro-Wilk showed all treatment groups had p values > 0.05, which means the data were normally distributed. The results of the Levene's Test homogeneity test showed a value of p > 0.05 which means that the data variance was homogeneous. The data obtained were normally distributed and homogeneous followed by the Independent T-Test parametric analysis with a confidence level of 95%. The results of the Independent T-Test parametric analysis test showed a value of p = 0,000 (p <0.05) which showed a significant difference in average between each treatment group.

Table 2. Percentage of Ascorbic Acid Inhibition

Larutan Baku (ppm)	%inhibisi	Absorbansi	IC50 (ppm)
4	42,68	0,5027	5,776
6	51,46	0,4257	
8	59,60	0,3543	
10	63,63	0,3190	

It can be seen from table 2. above that the higher the concentration of ascorbic acid, the higher the percent inhibition of ascorbic acid.

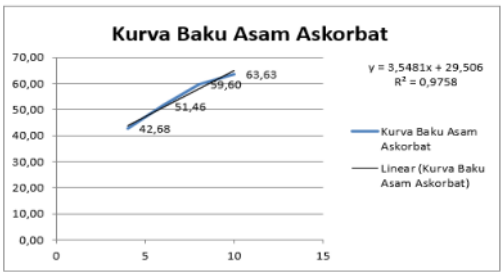


Figure 1. Standard Curve Relationship between Ascorbic Acid Concentration and Percentage of Inhibition.

Viewed from Figure 1, the IC50 value of ascorbic acid has an average IC50 value of 5.776 ppm. In Figure 1, ascorbic acid has a linear equation y = 3.548x + 29.50 and linearity r = 0.975.

Table 3. Inhibition Percentage of Chitosan from Haruan (Channa striata) Fish Scales

Larutan Baku (ppm)	%inhibisi	Absorbansi	IC50 (ppm)
200	27,024	0,6400	356,958
250	34,512	0,5743	
300	38,883	0,5360	
350	50,513	0,4340	

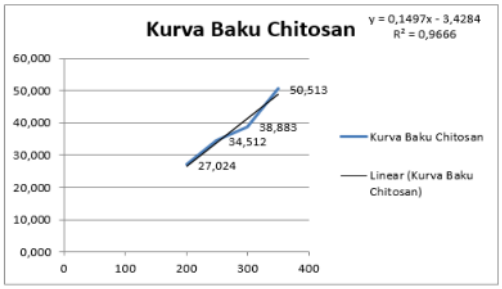


Figure 2. Standard Curve Relationship between Concentration of Chitosan from Haruan (Channa striata) Fish Scales with Percentage of Inhibition

It can be seen from Figure 2 that antioxidant activity of chitosan from Haruan fish scales had an average IC50 value of 356.98 ppm with a linear equation seen from Figure 5.2, which is y = 0.149x-3.428 and linearity r = 0.966.

After calculating the average between chitosan from Haruan fish scales and ascorbic acid, it is obtained the comparison value of antioxidant activity based on IC50 parameters as shown in the following figure:

Figure 3. Comparative Values of Antioxidant Activity in Ascorbic Acid and Chitosan from Haruan Fish Scales Based on IC50 Parameters

Figure 3 above states that the IC50 value of chitosan from Haruan fish scales was higher than the IC50 value of ascorbic acid. This showed that the antioxidant properties of ascorbic acid were still superior to chitosan from fish scales.

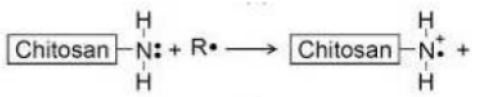
Discussion

Based on the results of the study, it can be seen that the greater the concentration of the chitosan test solution, the percentage of DPPH free radical inhibition by the test solution was increased. The regression equation obtained from the graph of the relationship between chitosan concentration of Haruan fish scales and inhibition percentage of DPPH was used to find the IC50 value. The amount of antioxidant activity was indicated by the IC50 value, which is the concentration of the test solution needed to inhibit 50% of DPPH free radicals. The smaller the IC50 value means the more active or higher antioxidant activity of a sample that is tested to be an antioxidant compound. [18] Specifically, a compound can be said to be a very strong antioxidant if the IC50 value is less than 50 ppm, strong for IC50 is 50-100 ppm, moderate if it is 100-150 ppm, and weak if IC50 value is 151-200 ppm.[19]

The IC50 value for the chitosan test sample from Haruan fish scales in this study was 356,958 ppm. Therefore, it can be concluded that the chitosan from Haruan fish scales is an antioxidant which has weak activity and has not exceeded the antioxidant activity of ascorbic acid. However, chitosan from Haruan fish scales have a higher IC50 value when compared to other types of chitosan such as chitosan from squid's endoskeleton, which is 484.05 ppm, in Nursyamsiah research (2017).[20] In addition, at a maximum concentration of chitosan from Haruan fish scales, 350 ppm, it had a 50.513% inhibition percentage. Whereas in Sumathi research (2017), at a maximum concentration of 1000 ppm fish scales in general in India only had 49.05% inhibition

percentage.[21] This showed that chitosan from Haruan fish scales have better inhibition percentage than chitosan from fish scales in general.

According to Kurniasih et al 2018, the amine group, NH2, in chitosan was responsible for the capture of free radicals. This is supported by the research of Putri (2020), which showed chitosan from Haruan (Channa striata) fish scales which through the deacetylation process with 50% sodium hydroxide at 80°C has 85.25% degree of deacetylation higher than SNI (≥75%). The higher the degree of deacetylation value, the greater the number of positively charged amine groups formed, so that the ability to capture free radicals is also greater.[22] The amine group (NH2) also acts as an antibacterial property of the chitosan from Haruan fish scales. The reaction from the capture of free radicals by chitosan is[23]:



Chitosan form fish scales solution contains antioxidants as evidenced by the ability of the solution to reduce the activity of free radicals such as hydrogen peroxide, superoxide anions and Cu2+ ions by binding to these free radical ions. The mechanism of action of antioxidants in inhibiting the oxidation process or stopping the chain reaction of free radicals can be caused by two kinds of mechanisms. The first mechanism is the release of electrons from antioxidants by relying on the nature of free radicals that is a species that is relatively unstable. It has unpaired electrons in its outer orbitals so is reactive in finding electron pairs. They will be able to interact directly with the NH3+ chitosan group that can donate electrons to oxidant ions (free radicals), so that free radicals turn into a stable molecule. The second mechanism is the release of hydrogen ions from antioxidants by binding to free radicals by chitosan. Radical groups such as OH- from DPPH solution can react with hydrogen ions from ammonium groups (NH3+) in chitosan in an acidic atmosphere, so it produces a more stable molecule and antioxidant compounds.[23, 24]

Chitosan has two hydroxyl groups, while ascorbic acid has four hydroxyl groups, so the antioxidant activity of ascorbic acid is stronger than the antioxidant activity of chitosan from Haruan fish scales. The mechanism of antioxidant activity of ascorbic acid, that is ascorbic acid can directly react with hydroxyl anions by donating one electron to form a non-reactive semihydroascorbate compound and subsequently undergo a disproportionation reaction to form an unstable dehydroascorbate. Dehydroascorbate will be degraded to form oxalic acid and treonic acid.[25]

The human body naturally has an antioxidant system to counteract free radicals in a sustainable manner, but if the number of free radicals in the body is excessive, additional antioxidants are needed from food intake.[26]

From the results obtained, chitosan from Haruan fish scales can be measured for antioxidant activity using the DPPH method by Brand-William (1985) but must receive some special treatment such as the use of weak acid solvents. This is caused by the nature of chitosan that is not found in other materials such as soluble in organic weak acids at a certain pH, or in mineral acids with certain concentrations. Its solubility is also influenced by the molecular weight and the degree of deacetylation. Chitosan molecular weight and degree of deacetylation can affect the solubility of chitosan in an acidic atmosphere.[27] If the solvent or treatment used is not suitable then chitosan may not act as an antioxidant and therefore another method that is more appropriate to the nature of chitosan is needed. Chitosan Haruan (*Channa striata*) fish scales has been shown to have antioxidant activity with a maximum concentration of 350 ppm and have a 50.513% inhibition percentage.

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