Antioxidant Activity of Astaxanthin Flour Extract of Mud Crab (*Scylla Serrata*) with Different Acetone Concentrations

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Abstract. Mud crabs one of Indonesia's economically valuable fishery commodities, the high demand for crab from world and domestic trade will have an impact on the resulting waste in the form of shell waste. Crab shells contain bioactive compounds that have the potential to be antioxidant astaxanthin. This study consisted of four stages, namely (1) astaxanthin extraction (2) yield (3) terpenoid test (4) antioxidant test (DPPH). The objectives of this study were (1) to determine the amount of astaxanthin yield, (2) to determine the terpenoid test, (3) to determine the potential for antioxidant activity in mangrove crabs. The data obtained were tabulated in the form of tables and graphs, which were analyzed descriptively. The results showed that the yield with different acetone concentrations produced the highest yield of A 65%, namely 4.46 g, A 50%, namely 3.36 g and A 80% in the amount of 1.71 g. The antioxidant activity showed that treatment with the concentration of acetone extraction was able to produce the IC value of 50 lowest with acetone concentration of 65% compared to the extraction concentration of 50%. The IC value of 50 of 805.837 ug / mL is still classified as having weak or inactive antioxidant activity.

1. Introduction

Crustacea groups such as shrimp, lobster and crab contain active compounds in the form of carotenoids pigments that have potential as antioxidants, especially in the form of red pigments, namely astaxanthin. Astaxanthin is a type of carotenoid that is found in salmon and crustaceans by giving species a characteristic pink color [1].

According to [2] astaxanthin is a strong and safe type of carotenoid without pro-oxidants such as β-carotene, lycopene, zeaxanthin, and lutein. Astaxanthin Has 550 times as strong as vitamin E and 40 times β-carotene as a singlet oxygen extinguisher, and 1000 times stronger than vitamin E against lipid peroxidation. This compound has a superior position in the cell membrane and exhibits 3 important effects, namely: antioxidant, anti-inflammatory, and immune properties.

Astaxanthin is able to neutralize free radicals and oxygen properly by accepting or donating electrons without becoming a pro-oxidant [3]. Astaxanthin also contributes to the world of cosmetics, namely improving the structure of collagen tissue. On the skin, free radicals cause lines and wrinkles by destroying the collagen that gives skin youth and elasticity. When antioxidants neutralize free radicals, they protect against damage and can also help repair collagen tissue [4].

The objectives of this study were (1) determine the amount of astaxanthin yield, (2) to determine the terpenoid test, (3) determine the potential for antioxidant activity in mangrove crabs. The data obtained were tabulated in the form of tables and graphs, which were analyzed descriptively.

2. Research Method

This research was conducted from February to July 2020. The research was conducted at the Chemical Laboratory of Fisheries and Marine Products, Riau University and at the College of Pharmaceutical Sciences Riau. The main ingredient in this research is mud crab shells (*Scylla serrata*). The tools used...
are erlenmeyer, blender, maceration bottle, vacuum rotary evaporator, analytical balance, Whatman paper, saucer, dropper, measuring cup, UV-Vis spectrophotometer. This research was conducted in four experimental stages, namely: (1) extraction of akstaxanthin, (2) yield (3) terpenoid test, (4) analysis of 2,2- difenil-1-pikrilhidrazil (DPPH) for antioxidant activity.

**Astaxanthin extraction**

![Flowchart of astaxanthin extraction](image)

**Figure 1.** Flowchart of astaxanthin extraction

### 2.1 Calculation of the yield

The yield can be determined by comparing the percentage of the final weight with the initial weight of the material. The yield is analyzed by following formula [5]:

\[
\text{Yield} = \frac{\text{final product weight (g)}}{\text{initial raw materials weight}} \times 100\%
\]
2.2 **Terpenoid test (astaxanthin)**

The extract sample is placed into a vapor plate then placed in a fume hood, dropped Lieberman Burchard solvent, and a positive test result is obtained when the color of the extract changes to red, green, and violet after reacting with Lieberman Burchard's solvent.

2.3 **Antioxidant test** [7]

Antioxidant is tested using the DPPH (1,1-Diphenyl-2-picryl hidrazyl) method at a wavelength of 517 nm [6].

- A sample of 2 mg was dissolved in 2 mL of methanol so that the sample concentration was 1000 µg/mL.
- In line A, 100 µL of samples were inserted (the plate consisted of 12 AH rows each). A total of 50 µL of methanol was added to each well in line.
- Line A pipette as much as 50 µL put into line B, line B pipette 50 µL put into line C and carried out until line F, line F pipette 50 µL then discarded, so that we get concentrations of 1000, 500, 250, 125, 62.5 and 31.25 mg/mL. Whereas the GH line is filled with 50 µL of methanol, specifically in the H line only wells 1-6 are filled.
- The AG line was added with DPPH of 80 µL with a concentration of 40 µg/mL, then incubated for 30 minutes. Free radical scavenging activity was measured as a decrease in DPPH absorbance with a microplate reader and data processing. The positive control used as a comparison was ascorbic acid with a concentration of 50 µg/mL.

The value of % inhibition is calculated by the following formula:

\[
\% \text{ inhibition} = \frac{A\text{Control} - A\text{Sample}}{A\text{Control}} \times 100\%
\]

Description:

- A control = DPPH absorbance
- A sample = Absorbance - DPPH

The sample concentration and percent inhibition values are plotted on the x and y axes respectively in the linear regression equation. The linear regression equation obtained in the form of the equation is \( y = a + bx \) is used to find the IC50 (50% inhibitor concentration) value from each sample by determining the y value of 50 and the x value to be obtained as IC50. The IC50 value states the amount of concentration of the sample solution needed to reduce DPPH free radicals by 50%.

3. **Result And Discussion**

3.1 **Astaxanthin Yield**

The results of the amount of crude shell flour extract can be seen in Figure 2.

![Figure 2. Astaxanthin extraction](image)

Based on the Figure 2, the color produced by each treatment is different, where the higher the concentration used produces the darker the color of the extract is, and the higher the concentration used resulting in a low yield of each extract. This is influenced by the acetone content in the solvent,
the greater the acetone fraction, the more it evaporates when it is evaporated during the rotary evaporator process causing the extract to be low. The basic principle of extraction is like dissolves like where the strength of a compound in the solvent is based on the similarity of polarity between the solvent and the extract compound so that the bioactive compound is easier to get out of plant cells [8]. The yield of astaxanthin can be presented in Table 1

Table 1. Astaxanthin yield of mangrove crab shells

| Treatment | Total extract | Yield | Average of yield (g) |
|-----------|---------------|-------|----------------------|
| A50%      | 1,9172        | 1,7381| 3,82                 |
|           | 2,2899        | 2,1443| 4,58                 |
|           | 0,8704        | 0,8582| 1,74                 |
| Rata-rata |               |       | 3,29                 |

Based on Table 1, the yield of mangrove crab shell extracts extracted with different acetone concentrations shows that a concentration of 65% produces the highest yield of 4.46, while a concentration of 80% produces the lowest yield, namely 1.71 g. This is influenced by the presence of heat coming out of the acetone solvent during the soaking process of the crab shells, so that the carotenoid pigments are degraded. [8] stated that the difference in the total carotenoids from each sample was due to the carotenoids having conjugated double bonds which made them very sensitive to oxidative degradation when exposed to air and heat.

3.2 Terpenoid Test

Astaxanthin is an active compound that causes antioxidant activity in the sample. Its presence can be detected by conducting a terpenoid test. Astaxanthin is a terpenoid compound that is built up by eight isoprene and consists of 40 carbons [9]. Astaxanthin is a lipid-soluble pigment. The results of the astaxanthin test are presented in Figure 3.

Based on the Figure 3, that the A50% extract has a color change from orange yellow to a slightly greenish color after being reacted with Lieberman Burchard solvent in Figure 3, as well as the A65% and A80% extracts. A slight color change can be said that the shell extract contains astaxanthin compounds, but with a slight green color change it can be said that the extract contains very little astaxanthin.

3.3. Antioxidant activity

The results of the mangrove crab shell extract were carried out by means of the maceration method which is expected to increase the antioxidant activity. To determine the presence of antioxidant activity in a material, an antioxidant test can be performed the DPPH method clearly.
Figure 4. Graphic IC$_{50}$ inhibition value at concentration of A 50%, B 65%, C 80%

Figure 4 shows the linear equation $y = ax + b$, where $y = 28.158x - 138.43$ $R^2 = 0.9998$ so that the IC$_{50}$ value is 805.837. The equation shows that treatment with the concentration of acetone extraction is able to produce the lowest IC$_{50}$ value with acetone concentration of 65% compared to the extraction concentration of 50%. The IC$_{50}$ value of 805.837 ug / mL is still classified as having weak or inactive antioxidant activity.

[10] states that a compound is said to be active as an antioxidant very strong if the IC$_{50}$ value is <50 ug / mL, the strength for IC$_{50}$ is 50-100 ug / mL, whereas if the IC$_{50}$ is 100-150 ug / mL, it is weak if the IC$_{50}$ is 150-200 ug / mL and very weak if the IC$_{50}$ is >200 ug / mL. The DPPH test results show that the IC$_{50}$ value is quite large, which is not entirely correct if it is stated to have weak or inactive antioxidant activity. [11], this can happen if the solvent used to dissolve the extract has polarity properties that are different from the extract. The solvent used to dissolve DPPH in this test is methanol which has polar properties, so it can be assumed that the non-polar bioactive components in this extract do not dissolve completely in this solvent. The amount of bioactive components dissolved in the type of solvent will be different and will affect the resulting IC50 value.

4. Conclusion and Suggestion
4.1. Conclusion
1. The yield of mangrove crab shell flour extract with different acetone concentrations resulted in different yields of A50%, namely 3.69 g, A65% namely 4.46 g and A 80% namely 1.71 g.
2. The average yield of astaxanthin produced with different acetone concentrations in mangrove crab shell flour was 3.29 g.
3. The best acetone concentration, namely 65%, resulted in antioxidant activity with the smallest IC value of 805.837 ug / mL indicating that the antioxidant activity was still relatively weak.

4.2. Suggestion
Carotenoids extraction process would be doing with several other methods, such as the pressurized liquid extraction (PLE) method, and the supercritical fluid extraction method to the ratio of the number of carotenoids produced, then for the antioxidant activity of the IC50 value can be tested by the Cupric Ion Reducing Antioxidant Capacity Method (CUPRAC) or Ferric Reducing Antioxidant Power (FRAP).

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