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Bioactivity and Antioxidant Test of Hexadecanoic Acid and β-Sitosterol Isolated from Hydroid *Aglaophenia Cupressina* Lamoureoux

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ABSTRACT

The research on the bioactivity and antioxidant test of hexadecanoic acid and β-sitosterol compunds isolated from hydroid *Aglaophenia cupressina* Lamoureoux had been done. The purpose of research was to determine the bioactivity of hexadecanoic acid and β-sitosterol through toxicity test using *Artemia salina* Leach as antioxidant. The research was conducted at the Laboratory of Environmental and Marine Sciences, Department of Biology and Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Hasanuddin, Makassar. The method used in this study was Brine Shrimp Lethality Test (BSLT) for toxicity and Diphenil picry hidrazil (DPPH) methods test for antioxidant. The results showed that hexadecanoic acid has a high toxicity and β-sitosterol has no toxicity based toxicity tests using *Artemia salina* Leach. The hexadecanoic acid and β-sitosterol did not have antioxidant activity.

Keywords: Bioactivity; *Aglaophenia cupressina* Lamoureoux; hexadecanoic acid; β-sitosterol;
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1. INTRODUCTION

There have been some disease begins by excessive free radical reactions in the body. Free radicals in the body are formed will generate new free radicals through a chain reaction that ultimately numbers continue to grow and invade the body. Due to the effects of free radicals that are not good for health, the body requires a critical component that counteract free radical attack. An important component that is capable of saving the human body's cells from free radical damage is an antioxidant [1].

Lately the use of antioxidant compounds is growing rapidly both for food and medication. The usage as a medicine is growing along with the increasing knowledge of free radical activity against several degenerative diseases such as heart disease and cancer [2].

Indonesia has vast areas of sea more than the land and marine life in the waters of Indonesia is diverse. All of this is a natural source of sea that has not been used mainly for health [3].

Hydroids are marine invertebrates belongs to phylum Coelenterata or Cnidaria which was living or attached to the sponge, rich in chemical compounds that can be used as drugs [4]. According to [5] hydroid Aglaophenia cupressina Lamoureoux contains chemical compounds sesquiterpen, diterpene, alkaloids, prostaglandins, pyridines and tridental A as an antioxidant. Hydroid is marine animal which rich of bioactive compounds, has a self-defense tool that nematocysts, are arranged in capsules, has tentacles and consists of a material that resembles collagen. Poison nematocysts are removed through a long capsule, this is a tool to capture prey and defend themselves [4]. Nematocysts containing some sort of chemical that can cause itching and even skin irritation if touched [6]. According to [7] hydroid has the most promising bioactive compounds to be isolated for the benefit of the treatment of human diseases.

Previous research by [8] showed that hexadecanoic acid compound isolated from hydroid Aglaophenia cupressina Lamoureoux can serve as antimitotic agent. Further study by [9] find that chemical compounds β-sitosterol of Aglaophenia cupressina Lamoureoux has potential as an antimitotic agent. Based on this, the curren study was conducted in order to determine bioactivity of toxicity tests using Artemia salina Leach and activity of antioxidant compounds hexadecanoic acid and β-sitosterol of hydroid Aglaophenia cuppresina Lamoureoux.

2. METHODOLOGY

This experimental research was conducted in the Laboratory of Environment and Marine Study, Department of Biology and Biochemistry laboratory, Chemistry Department, Faculty of Mathematics and Natural sciences, University of Hasanuddin, Makassar.

A. Materials

Materials and equipments used were pipette, test tube, tube rack, balance of digital, rod stirrer, spoon horn, a beaker, a magnifying glass (magnifying glass), a measuring cup, flask, aerator, bottle vial, lamp, micropipette and UV VIS spectrophotometry, hexadecanoic acid and β-sitosterol isolates of *Aglophenia cruppresina* Lamoureoux, dimethyl sulfoxide (DMSO), a solution difenilpikrilhidrazil (DPPH) 0,4 mM, tissue, synthetic sea water, distilled water H_2O, paper labels, methanol, Artemia salina Leach and vitamin C.

B. Stock Solution

Stock Solutions Test made by dissolving 0.05 mg of the test compound into a 10 mL solvent (5 mL DMSO + 5 mL of distilled water) thus obtained 5000 ppm stock. This solution is used to test the toxicity and antioxidants.

C. Toxicity Test - Brine Shrimp Lethality Test (BSLT) Using Artemia Salina Leach.

Preparation of larvae *Artemia salina* Leach performed by incubating 50-100 mg

the eggs into 1000 mL of synthetic sea water, equipped with aeration and light. The eggs hatch after about 24 hours and 24 hours old larvae were used to test the toxicity of the compound [10].

Solution Test were made with the concentration of 1000, 100, and 10 ppm into a test tube. Furthermore, each filled with 1 ml of synthetic sea water. Each tube contained 10 *Artemia salina* Leach, then added with sea water made to 5 mL. Each treatment was done in 3 replicates. DMSO was used as the control solution with addition of distilled water. Test tube was placed under lighting for 24 hours and the number of dead larvae of *Artemia salina* Leach was counted [11].

Toxicity effects were analysed using the following formula:

$$\text{Mortality} = \frac{\text{the number of dead larvae}}{\text{the total number of larvae}} \times 100\ \%$$

If the existing control larvae were dead, then the percentage of mortality was calculated by the formula Abbot [12]:

$$\%\ \text{mortality} = \frac{T-K}{\text{The number of test larvae}} \times 100\%$$

Description:

T= Number of dead treated larvae

K= number of dead controllarvae

Results of percent mortality then used to find the value of probit in the probit table. Furthermore, the results of the probit analysis of probit associated with log concentration to obtain the equations that will be used in the search for value of LC_{50}. The value of LC_{50} will show toxicity to the test compound.

D. Antioxidant Activity Test with DPPH

The DPPH stock solution made by dissolving 0.008 gram of DPPH crystals in 67.2 mL of methanol to obtain 0.4 mMa concentration. The making process of DPPH solution carried on under conditions of low temperature and shielded from sunlight.

The 5000 ppm stock test solution which had previously been made pipette as much as 0.4 mL then added with 1.6 mL of methanol to obtain a 1000 ppm concentration.

1000 ppm test solution was made at a 20, 30 and 40 ppm concentration. Vitamin C as a positive control was made at a 2, 3, 3 and 4 ppm concentration. Each concentration is added 1 mL of 0.4 mM DPPH. Then methanol was added to make volume to 5 mL. Furthermore, the reference solution is prepared by reacting 4 mL of methanol with 1 mL of 0.4 mM DPPH solution in a test tube. Each solution was homogenized using a vortex and incubated at 37oC for 30 minutes. Determination of the wave length (λ) of maximum absorption of DPPH solution is performed as follows: l, 0 mL of 0.15 mM DPPH plus 1.0 mL of absolute methanol, shaken homogeneous, measured absorbance in the wave length range of 400-600 nm [13]. Then measuring the absorbance of the reference solution is measured to perform the

calculation of percent inhibition. Furthermore, measuring the absorbance of the test solution and the reference solution. The antioxidant activity expressed as a percent inhibition, which was calculated by using formula:

$$\% \text{ inhibition} = \frac{(\text{blank} - \text{sample}) \text{ absorbance}}{\text{blank absorbance}} \text{ x } 100\ \%$$

Concentration of sample and the percent of inhibition respectively plotted on the x and y axis and obtained a linear regression equation. This equation is used to asses the value of LC_{50}.

3. Result And Discussion

A. Toxicity test with method of Brine Shrimps Lethality Test (BSLT)

The toxicity test of hexadecanoic acid compounds is shown in Figure 1

Based on Figure 1, the percentage of larvae that died at 10 ppm concentration is lower than 1000 ppm which has a higher number of larvae. According to [14] the mortality rate of dead shrimp larvae would correspond to a predetermined level of concentration. The higher the concentration specified then the death of shrimp larvae will be greater. Probit analysis results for the concentration of 10 ppm was 4.36, 100 ppm and 1000 ppm were 6.08 and 4.56.

The equation for hexadecanoic acid is y = 0.1x + 4.8. LC_{50} Value of hexadecanoic acid is 100 mg/mL which indicates that these compounds have very strong toxicity, which means that the compound has a very high bioactivity. Examples of bioactivity in hexadecanoic acid compound is to have antimicrobial properties that can inhibit the growth of bacteria and some fungi [15]. According to Rusdy [8] hexadecanoic acid can serve as an anticancer by inhibiting the rate of cell division and growth (mitotic effect).

LC_{50} value is a value that indicates the magnitude of the concentration of a test substance that can cause 50% of deaths the number of test animals after 24 hours of treatment. A compound is said to be very strong if the value LC_{50} less than 250 mg/mL, strong if the value LC_{50} between 250-500 mg/mL, moderate if the value LC_{50} ranged between 500 to 750 mg/mL, and weak if the value LC_{50} ranges between750 to 1000 mg/mL [16].

Results in Figure 1 shows that the percentage of dead larvae at a concentration of 10 ppm lower than 1000 ppm which has a higher number of larvae. Results of probit analysis for β-sitosterol at a concentration of 10 ppm was 4.26, 5.71 at 100 ppm, and 1000 ppm was 4.56. Linear regression equation for β-sitosterol is y = 0.15x + 4.543. LC_{50} value for β-sitosterol is 1096.47 ug / mL. This shows the β-sitosterol has no significant toxicity bioactivity of compounds β-sitosterol low. The example of β-sitosterol compounds bioactivity in previous studies can be used as antimitotic and antifungal agents.

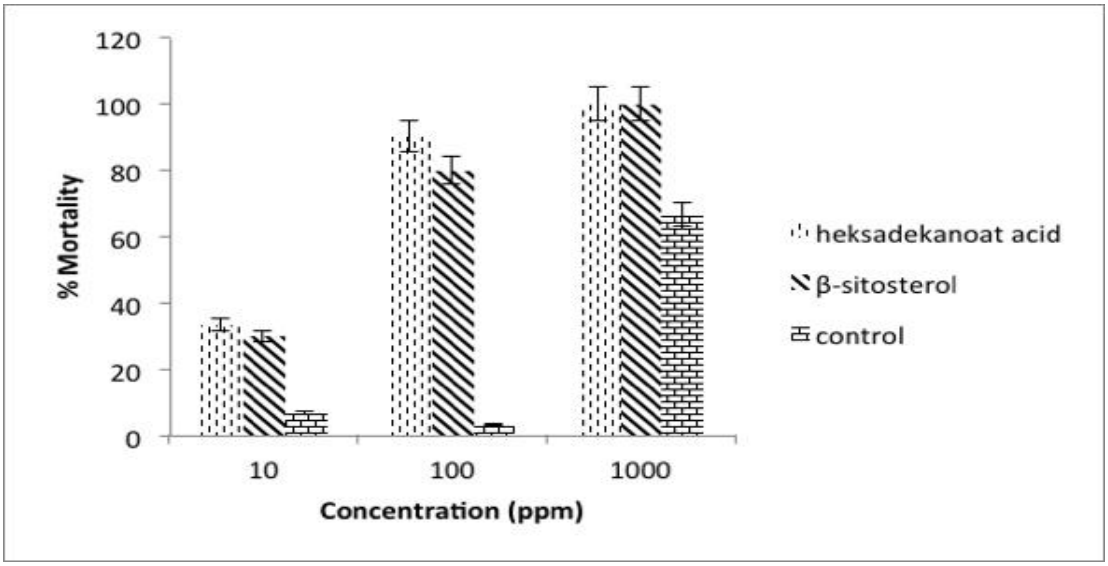

Figure 1. The mortality of larvae at different concentration

A. Test antioxidants with DPPH method

Test results hexadecanoic acid, β-sitosterol and vitamin C as antioxidant compound is described in Table 1.

Based on Table 1, hexadecanoic acid and β-sitosterol have lowest inhibitory activity (% inhibition) in 20 ppm concentration and highest concentration in 40 ppm concentration. This is in according to study conducted by [17], in which percentage inhibitory activity (% inhibition) against free radical increased with increasing concentration. Percent inhibition is the ability of a material to inhibit the activity of free radicals, which are associated with the concentration of a test substance. Percent inhibition is obtained from the difference between the absorbance of DPPH absorbance with the absorbance of samples as measured by UV-VIS spectrophotometer [18].

The equation was used to calculate the value of IC_{50} from hexadecanoic acid compound is y = 0.124x - 1.758 and r = 0.980. The value of IC_{50} for hexadecanoic acid compound was 412.53 ppm.

The equation was used to calculate the value IC_{50} of the compound β-sitosterol is y = 0.153x - 0.328 and r = 0.950. The value IC_{50} for β-sitosterol compound was 385.451 ppm. R value on hexadecanoic acid and β-sitosterol is positive value with approaching +1 value illustrates that with increasing concentration of the sample, the greater the inhibitory activity of free radicals.

Inhibition Concentration (IC_{50}) is the concentration of antioxidants that can cause a 50% DPPH loss radical character or the concentration of antioxidants that give 50% percent inhibition [19].

Sample	Concentration (ppm)	Absorbance (λ)	Activity of inhibition (% inhibition)	Value of LC_{50}
Hexadecanoic acid	20	0.638	0.62	412.53 ppm
	30	0.628	2.18	
	40	0.622	3.12	
β-sitosterol	20	0.365	2.54	385.451 ppm
	30	0.357	4.68	
	40	0.353	5.61	
Vitamin C	2	0.7	42.62	2.24 ppm
	3	0.636	47.87	
	4	0.550	54.92	

Table 1. The result of antioxidant test

The value IC_{50} of compounds hexadecanoic acid and β-sitosterol is included in the compounds which have no antioxidant activity. According to [19] a compound to be a very powerful antioxidant if the value IC_{50} is less than 50 ppm, strong if the value IC_{50} between 50-100 ppm, moderate if the value IC_{50} ranged between 100 to 150 ppm, and weak if the value IC_{50} ranged between 150 to 200 ppm.

According to [20] hexadecanoic acid or palmitic acid are included in the group of carboxylic acid which having low antioxidant power and according to [21] β-sitosterol is a steroid compound which has a hydroxyl group (OH) can provide antioxidant activity. However, in this research, hexadecanoic acid and β-sitosterol did not show any antioxidant activity. This may due to fact these compound has been kept in for long period in the cooler. According to [22], Low temperature can inhibit enzyme activity and metabolism reaction but causes a decrease in the activity of antioxidant compounds. Therefore it is required a study of the antioxidant activity in the shelf life of a compound.

Testing the antioxidant activity with using Vitamin C as a positive control. Vitamin C is used as a positive control because the compounds contained in vitamin C has the ability to reduce well or counteract free radicals and have a high antioxidant activity because it contains hydroxyl groups.

Based on data from Table 1, the inhibitory activity of free radicals of vitamin C in 2 ppm shows low inhibitory activity and the concentration of 4 ppm shows the highest inhibitory activity. Table 1 also indicates that the antioxidant activity of vitamin C is higher than hexadecanoic acidand β-sitosterol, although the concentration of vitamin C used is lower than the concentration used for the two other compounds.

The equation that used to calculate the value IC_{50} of vitamin C is $y = 6.15x + 36.12$ and the value of $r = 0.992$. The value of IC_{50} of vitamin C is 2.24 ppm. This value indicates that vitamin C has a strong antioxidant activity, in contrast to compounds

hexadecanoic acid and β-sitosterol which has a value IC_{50} is greater so showing they have no antioxidant activity.

4. CONCLUSION

Hexadecanoic acid compounds have a high toxicity and β-sitosterol has no toxicity based toxicity tests using *Artemia salina* Leach.

Hexadecanoic acid compound and β-Sitosterol did not have antioxidant activity.

5. RECOMENDATION

There are required further researchs about the antioxidant testing for the shelflife of a compound.

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