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The Influence of Giving Ether Fractionand Pheophythyn Pigment from Brown Algae (Sargassum polycystumC. Agardh) to Lowering Cholesterol Levels in Vitro

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ABSTRACT: Brown algae are potentially as an antioxidant and anticholesterol. Anticholesterol compounds are compounds which can reduce cholesterol levels in the body. One of the compounds in the brown algae that can serve as anticholesterol is the phaeophytin pigment. Phaeophytin is a pigment result of degradation atoms Mg from chlorophyll which contained in brown algae. It has been reported that a chlorophyll and that derivation has the ability of antioxidants and anticholesterol.The purpose of this research is to know the ability of methanol extracts and isolates phaeophytin pigment of brown algae in lowering cholesterol levels in vitro, knowing the influence of the concentration in lowering cholesterol levels in vitro and knowing the effective concentration that can lowering cholesterol levels in vitro.The extraction methods that used in this research is quick remaserationand methods for the isolation of phaeophytin pigment used chromatography columns. The analysis of the cholesterol concentration is using Liebermann-Burchard methods. Ether fraction and isolates phaeophytin pigment made a series of concentrations 0.1; 0.5; 1; 5; 10; 15 and 20 ppm. Quantitative tests done with a spectrophotometer visible.The research results showed that the ether fractions and phaeophytin pigment from brown algae are able to lowering cholesterol in vitro. Ether fractions and phaeophytin pigment can lowering cholesterol with percent decrease that was almost equal with the difference in concentration. Test of anava shows that there is a meaningful difference between the concentration of the sample. The effective concentration of methanol extract is 15 ppm with percent decrease of cholesterol 40,92% and isolates phaeophytin pigment is 1 ppm with percent decrease of cholesterol 40,35%.

KEYWORDS:brown algae, phaeophytin, cholesterol-lowering, Liebermann-Burchard

I. INTRODUCTION

Algae is one of the marine natural resources that has quite large potential. Brownalgaecontainsalginate, vitamin C, vitamin E (α-tocopherol), minerals, carotenoids, chlorophyll, phlorotannin, sulfatedpolysaccharides, fattyacidsand amino acids[1]alsocontainssecondarymetabolitecompounds , namelyalkaloids, flavonoids, saponinsandsteroids/triterpenoids[2]. The useofbrownalgae in themedicinalsector, algae has thepotential as anantioxidantand anti-cholesterol[3].

Anti-cholesterol compounds are compounds that can break down cholesterol deposits in the body. One ofthecompounds in brownalgaethatcanfunction as an anti-cholesterolisthepigmentpheophytin[1]. Pheophytin is a chlorophyll derivative that is formed when the magnesium metal center in chlorophyll is removed. The effectofheatingandacidsthathave OH-ionswillattract magnesium metal ions in themacrocyclicchlorophyll ring [4]. The structure of pheophytin is shown in figure 1.

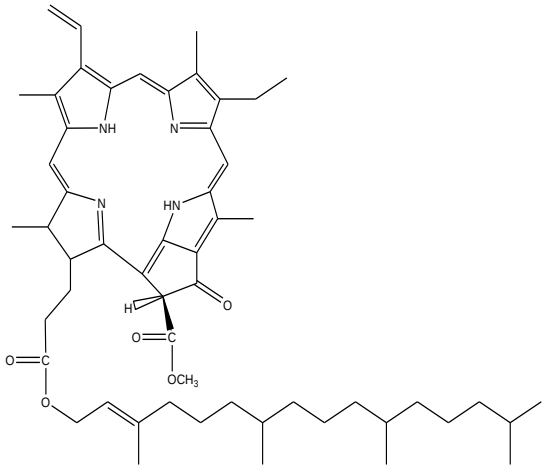

Figure 1. Pheophytin structure

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The use of pheophytin as an anticholesterol was measured in vitro using the Liebermann-Buerchard method. The Liebermann-Burchard reaction is the basis for the photometric determination of cholesterol. A sample of cholesterol dissolved in chloroform is reacted with anhydrous acetic acid and a small amount of concentrated sulfuric acid to produce a characteristic coloration for single sterols [5].The reaction between pheophytin and cholesterol can be seen in Figure 2.

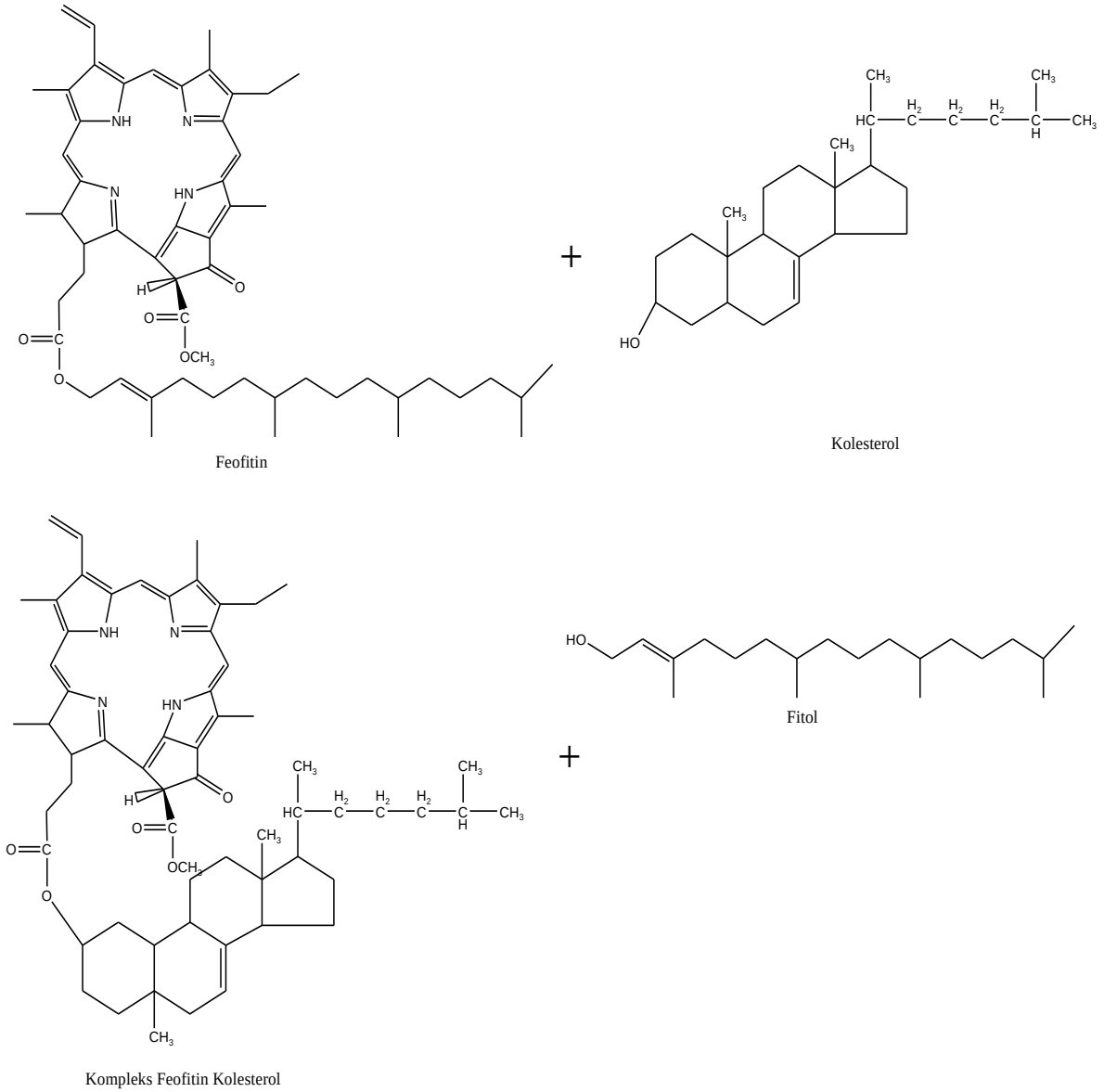

Figure 2. Pheophytin Reaction with Cholesterol

II. MATERIAL AND METHODS

The main material is (Sargassum polycystumC. Agardh.) obtained from Montong Beach, KecamatanUtan, Kabupaten Sumbawa, Sumbawa Besar, Provinsi Nusa Tenggara Barat, Indonesia. The chemicals used were methanol, acetone, diethyl ether, hexane, diethyl ether, cholesterol (sigma), silica GF_{254} and silica gel 60 (Merck).

Two hundred grams of sample were grounded and extracted by using a mixture solution of acetone: methanol (7 : 3, v/v). During the extraction, calcium carbonate was added as a neutralizing agent and sodium ascorbate as an

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antioxidant to prevent further oxidations. The extraction was done as quick as possible to avoid further oxidations or enzymatic degradations. Then, the extract was filtered and the residue was re-extracted until the color of residue became pale as an indicator of complete pigment extraction. The resulting extract was partitioned with diethyl ether. The diethyl ether layer was dried with nitrogen gas [6,7].

Isolation was carried out using the dry ether fraction dissolved in n-hexane: acetone (7:3), then put into column chromatography with a stationary phase of silica gel-60 of 30 g and a mobile phase of n-hexane: acetone (7: 3) 250 mL. The blackish gray isolate was collected in a vial until it was no longer grey, the isolate was then dried with N2 gas.

Measurements were carried out using a visible spectrophotometer at a wavelength of 350-700 nm. Pipetted 5.0 mL of each concentration of cholesterol standard solution (160, 200, 240, 280 and 320 ppm) and sample solution (0.1; 0.5; 1; 5; 10; 15 and 20 ppm), added 5 .0 mL chloroform p.a. Pipet 5.0 mL of the mixture then react with 2.0 mL anhydrous acetic acid and 0.1 m concentrated H2SO4. Leave for 13 minutes in a dark place. Cholesterol absorbance was measured using a visible spectrophotometer at the maximum wavelength (667.0 nm).

III. RESULT AND DISCUSSION

This study aims to determine the effect of administering methanol extract and pheophytin pigment isolate from brown algae (Sargassum polycystum C. Agardh.) on reducing cholesterol in vitro. The sample used in this study was fresh brown algae. The fast remaceration method was chosen because pheophytin is not resistant to the effects of light and oxygen. The solvent used is methanol, because this solvent is able to attract more compounds in brown algae and is more optimal than acetone and ethanol [8]. During the extraction process, CaCO3 is added as a neutralizing agent and sodium L. ascorbate as an antioxidant.

The results of the dry methanol extract were identified and qualitative phytochemical tests were carried out. This qualitative phytochemical testing was carried out with the aim of determining the secondary metabolite compounds contained in the ether fraction. The results of the identification carried out showed that the ether fraction of brown algae was positive for containing steroids. Pheophytin is a degradation agent of chlorophyll which is a type of steroid, therefore a confirmation test was carried out to find out whether the extract contained pheophytin.

The confirmation test for pheophytin pigment content was carried out using thin layer chromatography (TLC). The mobile phase used is n-hexane: acetone (7:3), where acetone is semipolar and n-hexane is non-polar. Based on figure 3, there are 5 spot on the TLC plate with Rf 5=0.98 (yellow) α-carotene pigment, Rf 4= 0.78 (blackish gray) pheophytin pigment, Rf 3= 0.48 (light green) pigment chlorophyll a, Rf 2= 0.41 (dark green) chlorophyll c pigment, Rf 1= 0.31 (yellow orange) fucoxanthin pigment. Spot 4 with Rf = 0.78 was identified as containing pheophytin because it was close to the Rf value of pheophytin in the literature [9,10], namely Rf = 0.77 (Figure 3.).

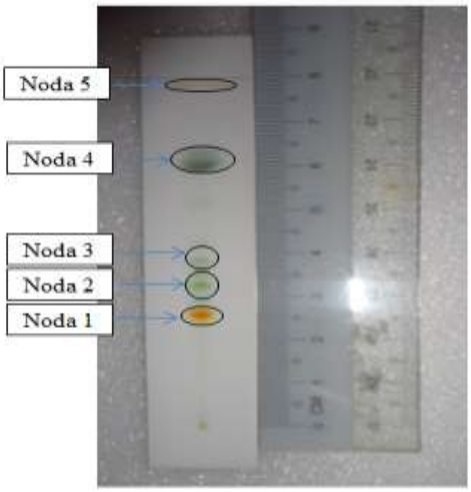

Figure 2. TLC chromatogram of pigment ether fractions from Sargassum polycystum C. Agardh.

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The results of this TLC test were then used as the basis for using a suitable eluent in column chromatography to isolate pheophytin pigment. The mobile phase used is the same, namely n-hexane: acetone (7: 3) and the stationary phase is silica gel 60 GF254.

The isolate resulting from column chromatography was then subjected to a TLC test using the mobile phase n-hexane: acetone (7:3) to prove that the isolate was indeed a pheophytin pigment. The results of isolate identification using TLC showed blackish grayspots with Rf = 0.76. Based on the results, the Rf isolate showed the same results as the literature [9,10], namely 0.77. The TLC results are shown in Figure 4.

Figure 4. Thin Layer Chromatography of Pheophytin Isolate

The pheophytin isolate test was also carried out by measuring the spectral pattern using a visible spectrophotometer with a range of 350-700 nm. The results obtained from measuring the isolate pattern were almost the same as those in the literature. Based on [11] pheophytin has several wavelengths, namely at 410, 505, 535, 606, and 665 nm in acetone. The results of measuring the spectral pattern of the isolate in acetone solvent also show several wavelengths, namely at 414, 505, 538, 611, and 668.5 nm which can be seen in Figure 5. Based on the results of this spectral pattern, it can be said that the isolate contains the pigment pheophytin.

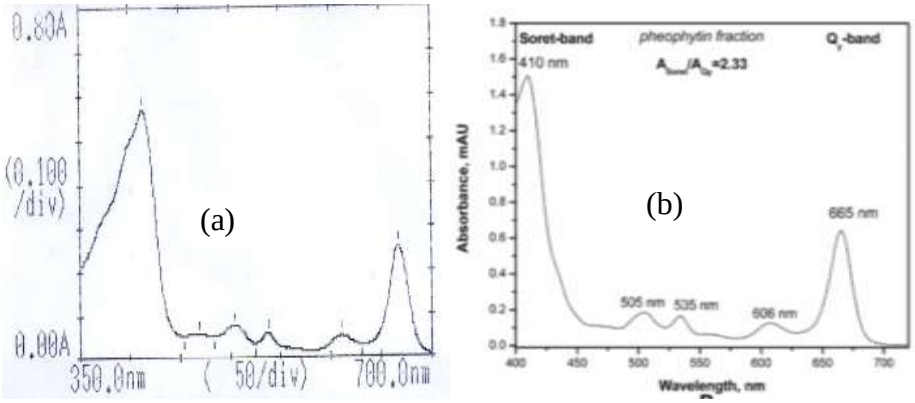

Figure 5. Pheophytin isolate spectrum (a) and pheophytin from literature (Sanja dkk, 2012) (b).

After obtaining the ether fraction and pheophytin isolate from brown algae, an anti-cholesterol test was carried out using the Liebermann-Burchard method. The Liebermann-Burchard method was chosen because this method is the basis for photometric determination of cholesterol, the process is simpler and this method is specifically used to measure steroid compounds, one of which is cholesterol. By adding anhydrous acetic acid and concentrated sulfuric acid, a green compound is formed which is then measured using a visible spectrophotometer. The cholesterol concentration used in this study was 320 ppm. The concentration of 320 ppm was chosen because it was assumed to be the hypercholesterolemia concentration which was adjusted to the results of standard cholesterol orientation which could be measured in vitro using a visible spectrophotometer.

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The reaction between cholesterol with anhydrous acetic acid and concentrated sulfuric acid will form a blue complex to produce cholestadine which then becomes green to produce ergocalciferol. The reaction between pheophytin pigment and cholesterol uses the principle of the transesterification reaction, where if an ester is reacted with alcohol it will form a new ester and alcohol which is a complex of pheophytin cholesterol and phytol. A graph of the average percent reduction in cholesterol by methanol extract samples and pheophytin pigment isolates can be seen. seen in figure 6.

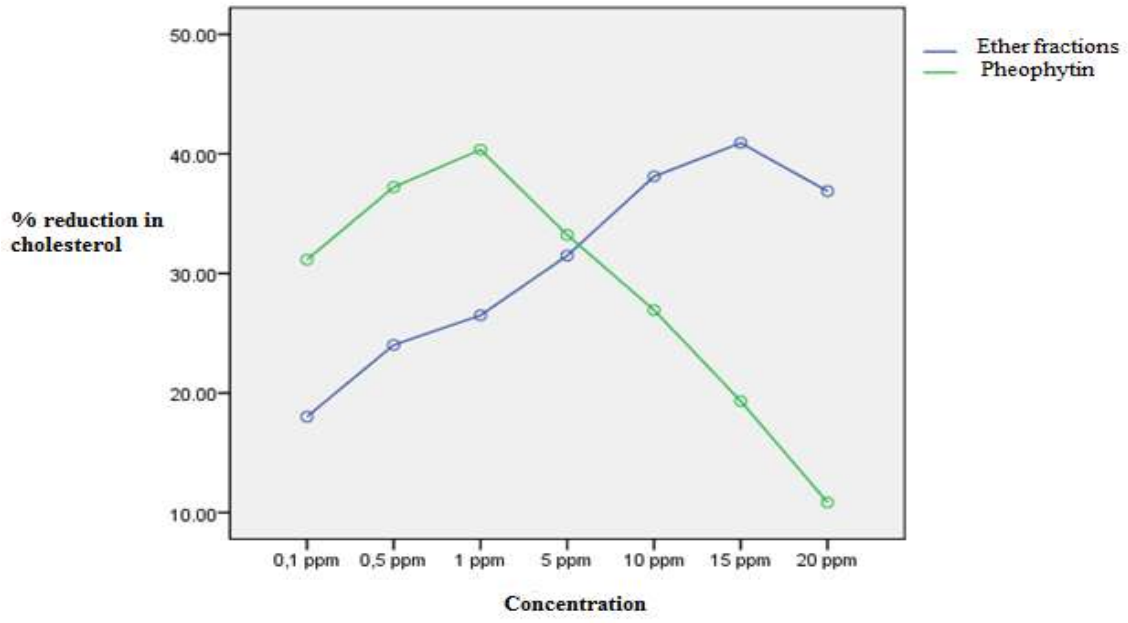

Figure 6. Graph of the average percentage reduction in cholesterol

Statistical data analysis was carried out using SPSS version 16. The results of the ANOVA test showed a significant difference between concentrations of 0.1; 0.5; 1; 5; 10; 15 and 20 ppm from the ether fraction and pheophytin pigment isolate by looking at the significance value of 0.000 < 0.05. The effective concentration that can reduce cholesterol in the ether fraction is 15 ppm, which is 40.92%, while in the pheophytin pigment isolate it is 1 ppm, which is 40. 35%. In this study, the use of pheophytin pigment isolate was more effective than ether fraction because with a smaller concentration it could reduce cholesterol by almost the same percentage. However, if we look at the percentage reduction produced, the use of methanol extract becomes more effective because even with a large concentration, ether fraction is able to reduce cholesterol by a fairly large percentage too.

This can be caused because the fraction contains other compounds which also have the potential to act as anti-cholesterol agents such as carotenoids, chlorophyll and their derivatives where the resulting effect is antagonistic to pheophytin when it reacts with cholesterol, causing a decrease in activity. requires greater concentration to produce results. effective. In contrast to the pheophytin pigment isolate which is a single compound that reacts perfectly with cholesterol so that its activity is maximum, where a small concentration can provide effective results.

IV. CONCLUSION

The results of this research, it was concluded that the ether fraction and pheophytin isolate from brown algae (Sargassum polycystumC. Agardh.) had an effect on reducing cholesterol levels in vitro, the concentration of the ether fraction and pheophytin pigment isolate from brown algae had an effect on reducing cholesterol levels in vitro, the effective concentration that can reduce cholesterol levels in vitro in the ether fraction is 15 ppm, which is 40.92%, while in the pheophytin isolate it is 1 ppm, which is 40.35%.

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