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The Effect of Different Extraction Methods of Sargassum polycystum Pigments on Antioxidant and Anti-Obesity Activities in Vitro

Novy Dwi Susilowati[1], Lia Kusmita[2], Etty Sulistyowati[3*]
[1] *Magister Farmasi STIFAR Yayasan Pharmasi Semarang,Indonesia*
[2] *S1 Farmasi,STIFAR Yayasan Pharmasi Semarang, Indonesia*
[3] *D3 Analis Farmasi dan Makanan, STIFAR Yayasan Pharmasi Semarang, Indonesia*

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ABSTRACT
The brown algae Sargassum polycystumcontains the pigment fucoxanthin. Fucoxanthinis a bioactive compound useful for antioxidants and anti-obesity. An appropriate extraction method is required to obtain a high content of bioactive compounds with potential as antioxidants and anti-obesity agents.Themethods of maceration and ultrasonic extraction were used to search for bioactive compounds. This study aims to determine the effect of different extraction methods, namely maceration and ultrasonic, of the pigment Sargassum polycystum on antioxidant activity IC50 using the DPPH method and anti-obesity activity with pancreatic lipase enzyme inhibitors in vitro. This research was conducted using fresh Sargassum polycystum samples with two extraction methods, namely rapid maceration and ultrasonic, each for 20 minutes, using 100% acetone as the solvent. A TLC test was conducted to identify the fucoxanthin pigment, obtaining a standard fucoxanthin Rf of 0.69, and a Rf of 0.69 for both the maceration and ultrasonic extracts. The phytochemical screening showed positive results for saponin and steroid compounds. Antioxidant activity test IC50 DPPH method and obesity activity with pancreatic lipase enzyme in vitro, obtained yield percentage results from the rapid maceration method for 20 minutes of 1.20 ± 0.09, with antioxidant activity IC50 111.24 ± 0.17 µg/mL and lipase inhibition percentage 79.23 ± 1.99, yield percentage from the ultrasonic method for 20 minutes of 1.30 ± 0.11 with antioxidant activity IC50 79.65 ± 0.42 µg/mL and lipase inhibition percentage 92.35 ± 4.02. The best results for the effect of yield on antioxidant activity IC50 and lipase inhibition percentage are from the ultrasonic extraction method.

Keywords: anti-obesity, antioxidant, maceration, ultrasonic, Sargassum polycystum

I. INTRODUCTION

Indonesia is an archipelagic country that has abundant biological resources. One of the natural resources found in the sea is brown algae. The brown algae Sargassum polycystum contains the pigment fucoxanthin, which can be an antioxidant and antiobesity agent(Gammone et al., 2015). The dominant pigment from the marine biota'scarotenoid group is fucoxanthin,a xanthophyll group. This pigment is mainly produced by brown seaweed (Phaeophyceae) and is the main factor that determines the brown color of the seaweed (Peng et al., 2011). Fucoxanthin pigment from brown algae can be an antioxidant due to its ability to reduce free radicals(Nursid et al., 2013). In addition, fucoxanthin found in brown algae can act as an anti-obesity agent with a mechanism that inhibits the activity of the pancreatic lipase enzyme(Wan-Loy et al., 2016).

The right extraction method is needed to extract high bioactive compounds that have the potential as antioxidants and anti-obesity. Extraction is the process of separating mixed materials with appropriate solvents. Several extraction methods can be used to extract bioactive compounds in plants, such as maceration extraction, soxhletation, subcritical water extraction, supercritical fluid extraction and extraction with the help of ultrasonic waves (Wiranata et al., 2022). The maceration extraction method is a conventional, generally running rather slowly and producing low yields.Another method that can used is ultrasonic waves, an efficient but simple extraction method.This extraction method uses ultrasonic waves, namely sound waves with frequencies above human hearing (≥ 20kHz)(Kumar et al., 2021).

The selection of extraction methods to extract bioactive compounds in Sargassum polycystum is very important to do so that it can

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produce high-potential bioactive compounds. This study used the rapid maceration methodand ultrasonic for 20 minutes each. The parameters observed in this study wereidentifyingfucoxanthin pigment by thin-layer chromatography, phytochemical screening to determine secondary metabolite compounds, antioxidant activity seen from the IC50 value, and anti-obesity activity by inhibiting pancreatic lipase enzymes.

II. RESEARCH METHODS

Place and time of research

This research was conducted at the STIFAR Laboratory of the Semarang Pharmacy Foundation, and it was implemented from May toAugust 2024.

Materials and tools

The main material in this study was fresh Sargassum polycystum from Montong village, Sumbawa, West Nusa Tenggara. The chemicals used were 100% acetone, n-hexane, ether, 1,1-diphenyl -2 picrylhydrazyl (DPPH), methanol, 1% DMSO, Porcine pancreatic Lipase (PPL), pNPB (Paranitrophenyl Butyrate), phosphate buffer pH 7.2, distilled water, silica gel GF 254. The tools used were chemical glassware, analytical scales, ultrasonic bath (Branson 1800), rotary vacuum evaporator, UV-Vis spectrophotometry (Shimadzu 1780),multimode reader (Synergy HTX).

Sampling

Fresh Sargassum polycystum sample, taken from Montong village, Sumbawa, West Nusa Tenggara, Indonesia.

Extract preparation

The fresh Sargassum polycystumwaswet sorted, then the extraction was carried out using the rapid maceration method and ultrasonic for 20 minutes each. Extraction of the rapid maceration method for 20 minutes, weighing 25.0 grams of sample, cut into small pieces and pounded in a mortar stamper, then put into an Erlenmeyer flask, then added a little CaCO3 and 100 mL of 100% acetone, shaken in a dark room protected from light, then filtered, rapid maceration was carried out again by adding 50 mL of solvent until the solvent liquid was colorless. The macerate was collected and concentrated with a rotary evaporator at a temperature of 30 ° C, then evaporated with nitrogen gas(Maulina et al., 2018).

Ultrasonic extraction method weighed as much as 25.0 grams of sample, cut into small pieces and pounded in a mortar stamper, then put into a beaker glass, then added a little CaCO3 and 100 mL of 100% acetone, ultrasonic for 20 minutes, in a dark room protected from light, then filtered, ultrasonic again with the addition of 50 mL of solvent until the solvent liquid is colorless. collected and concentrated with a rotary evaporator at 30 ° C, then evaporated with nitrogen gas(Oliyaei et al., 2021).

Pigment identification by Thin Layer Chromatography

The extracts from rapid and ultrasonic maceration for 20 minutes each were subjected to pigment identification compared with the fucoxanthin standard using a mixture of hexane:ether:acetone (6:3:2) mobile phase and silica gel GF 254 stationary phase.(Kusmita et al., 2023).

Phytochemical Screening

Phytochemical screening was conducted to determine the group of secondary metabolite compounds found in Sargassum polycystum extract. Phytochemical tests were conducted using the method from(JB Harborn, 1987), including:

1.) Flavonoid Test

Sargassum polycystum extract of 0.05 mg was put into a test tube then dissolved in 1-2 mL of methanol, the sample was added with 0.1 mg magnesium powder and 3 drops of concentrated HCl and 1-2 mL of amyl alcohol, shaken vigorously. Positive results are indicated by the red or orange amyl alcohol layer.

2.) Alkaloid Test

Sargassum polycystum extract of 0.05 mg was dissolved in 5 drops of HCl. This test was conducted using three reagents, namely Dragendroff, Meyer, and Bauchardat. The test result was declared positive if Dragendroff formed an orange precipitate, Meyer formed a white precipitate and Bauchardat formed a brown precipitate.

3.) Saponin Test

Sargassum polycystum extract of 0.05 mg was put into a test tube containing 5 mL of distilled water, boiled for 5 minutes. Shaking was carried out for 10 seconds and then left for 10 minutes, 1 drop of HCl was added, the presence of saponin was indicated by the presence of stable foam.

4.) Tannin Test

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Sargassum polycystum extract of 0.05 mg was put into a test tube, 1% gelatin and 10% NaCl were added, the presence of tannin was indicated by the presence of white precipitate.

5.) Steroid and terpenoid test

Sargassum polycystum extract of 0.05 mg was dissolved in 10 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid. Positive results for steroids are indicated by green color, positive terpenoids are indicated by red color.

Antioxidant activity test using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method

Antioxidant activity testing using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The test solution was made at concentrations of 50, 100, 150, 200, and 250 µg/mL dissolved in methanol. Determination of antioxidant activity was carried out by putting 0.2 mL of sample solution into a test tube, adding 4.0 mL of 0.7 mM DPPH for each concentration. Furthermore, the mixture was homogenized by vortexing for 1 minute and incubated for 30 minutes. The absorbance of the solution was read with a UV-Vis spectrophotometer at a wavelength of 515. The calculation of the percentage of antioxidant activity can use the formula equation 1(Marinova, 2011).

$$\% \text{ antioxidant activity} = \frac{\text{Abs .kontrol} - \text{Abs .sampel}}{\text{Abs .kontrol}} \text{ x } 100\ \% \quad (1)$$

DataSample concentration with % antioxidant activity is made in a linear regression equation to determine the sample concentration that can reduce free radicals by 50% (IC50). Vitamin C is used as a comparison or positive control

Antiobesity activity test by inhibition of pancreatic lipase enzyme

A new 96-well micro plate was prepared and mapping was carried out on each well to facilitate the activity test. The extract sample, positive control and negative control were each pipetted as much as 50 µL then added 50 µL of PPL enzyme and incubated at 37°C for 10 minutes. The mixture was then added with 50 µL of pNPB substrate and incubated again at 37°C for 10 minutes. The absorbance was measured on a multimode reader at the maximum wavelength. Each sample in 1% DMSO was also measured for its absorbance to be included in the calculation without the addition of PPL enzyme and pNPB substrate (blank). Inhibition of lipase activity was expressed as the percentage decrease in activity when PPL was incubated with the test compound. Lipase inhibition or lipase inhibition (%) was calculated based on the equation formula 2(Alias, 2017)

$$\% \text{ Lipase Inhibitory Activity} = 100 - \left[\frac{(B - b)}{(A - a)} \text{x } 100\right] \quad (2)$$

Information :

A = negative control absorbance with the addition of enzyme and substrate

a = absorbance of negative control without addition of enzyme and substrate

B = absorbance of inhibitor with addition of enzyme and substrate

b = absorbance of inhibitor without addition of enzyme and substrate

Inhibitor = extract sample and positive control

III. RESULTS AND DISCUSSION

Extract Yield

The results of this study indicate that the treatment of different extraction methods of Sargassum polycystum extract carried out by rapid maceration and ultrasonic extraction methods for 20 minutes each with 100% acetone solvent. The rapid maceration extraction method for 20 minutes obtained a yield of 1.20 ± 0.09% and ultrasonic extraction obtained a yield of 1.30 ± 0.11%. The yield of the ultrasonic method is greater than the maceration method. The ultrasonic method increases mass transfer from the liquid-liquid extraction process by producing cavitation, in the material, several bubbles appear during sonication and appear so much that they eventually collapse. The increase in cavitation bubbles and their collapse will damage the material cells and increase the release of solutes(Oliyaei et al., 2021). The yield results of the two methods of rapid and ultrasonic maceration for 20 minutes each showed an insignificant effect (P>0.05) as seen in Figure 1.

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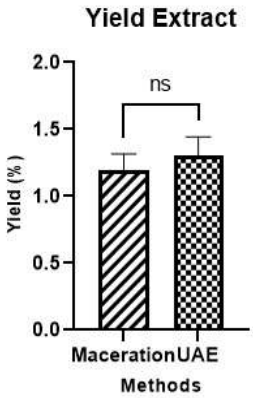

Figure 1. Yield of Sargassum polycystum Extract

Figure 2. TLC results of fucoxanthin standards and Sargassum polycystumextract

Identification of pigments by Thin Layer Chromatography (TLC)

The extract obtained from the two extraction methods was subjected to pigment identification using the TLC method. The pigment from the Sargassum polycystum extract to be identified was the fucoxanthin pigment used as a standard fucoxanthin comparison. Based on the TLC results, the standard fucoxanthin stain was shown to be orange and the maceration extract sample stain and ultrasonic extract stain were orange. Both extraction methods can attract fucoxanthin compounds with an Rf of 0.69 which is parallel to the fucoxanthin standard, the results of the TLC analysis can be shown in Figure 2.

Information :
SF : Standard Fucoxanthin
ME : Maceration extract
UE : Ultrasonic extract.
Based on the results obtained, both extraction methods can extract the target compound, namely fucoxanthin.

Phytochemical Screening

A phytochemical screening test of Sargassum polycystum extract using fast maceration and ultrasonic methods for 20 minutes each was conducted to determine the content of secondary metabolites contained in the extract. Sargassum polycystum extract showed positive results containing secondary metabolite compounds saponin and steroids. The results of the phytochemical screening test of secondary metabolites in the acetone extract of Sargassum polycystum are shown in Table 1.

Table 1. Results of the Phytochemical Screening From Sargassum polycystum Extract

Secondary metabolites	Maceration extract	Ultrasonic extraction
Flavonoid	-	-
Alkaloid	-	-
Saponins	+	+
Tannin	-	-
Steroid	+	+
Terpenoid	-	-

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Results positive saponin in Sargassum polycystum extract from two extraction methods showed the formation of stable foam for 10 minutes. For steroid testing, it showed positive results with the formation of green color, steroid compounds will experience dehydration with the addition of concentrated strong acid (H2SO4), which forms a salt that gives a color reaction(Minarti et al., 2019)

Antioxidant Activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method

Antioxidants are substances that can prevent the occurrence of free radical autooxidation reactions in lipid oxidation.(Septiana et al., 2013). Based on the DPPH method, the IC50 value of Sargassum polycystum extract with the maceration extraction method was 111.24 ± 0.17 and ultrasonic 79.65 ± 0.42, shown in Figure 3.

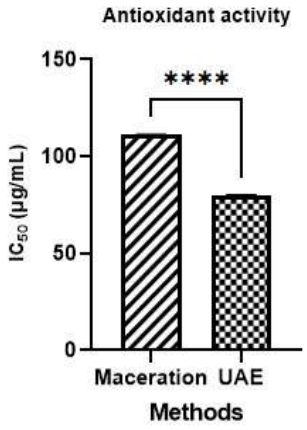

Figure 3. Antioxidant activity

The IC50 results showed a significant difference (P<0.0001). The standard used in antioxidant activity was ascorbic acid, with an IC50 value of 9.40 ± 0.61. The IC50 value of the Sargassum polycystum extract using the maceration method, when compared to ascorbic acid, was categorized as a weak antioxidant.In contrast, the ultrasonic method was categorized as a moderate antioxidant. According toDiah et al., (2014)states that the IC50 value of less than 50 µg/mL has strong antioxidant activity, 50-100 µg/mL is moderate, 150-200 µg/mL is weak and more than 200 µg/mL is very weak. A low IC50 value indicates the strong ability of the extract to act as a hydrogen atom donor(Diachanty et al., 2017).

Antiobesity activity by inhibition of pancreatic lipase enzyme

The pancreatic lipase enzyme inhibition activity test was carried out in vitro using a p-NPB substrate. The principle of this method is that the lipase enzyme will hydrolyze the p-NPB substrate into p-nitrophenol and butyric acid. The resulting p-nitrophenol acid will be yellow, and its absorbance can be measured at a wavelength of 405 nm. The higher the measured absorbance, the higher the measured absorbance, the higher the pancreatic lipase activity contained in the sample. The test was carried out on Sargassum polycystum extract with two extraction methods, maceration and ultrasonic, each for 20 minutes. The results of the pancreatic lipase inhibition percentage test using the maceration method were 79.23 ± 1.99 and for the ultrasonic method 92.35 ± 4.02 can be seen in Figure 4. The results of the pancreatic lipase inhibition percentage activity showed a significant difference (P <0.05). The standard used was orlistat.

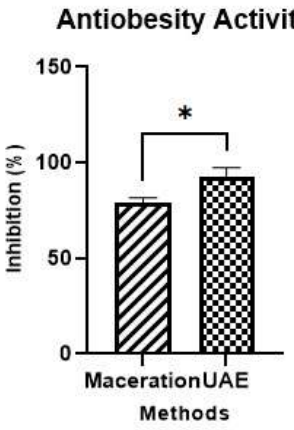

Figure 4. Antiobesity Activity

According to Ado et al. (2013), a sample is said to have weak pancreatic lipase inhibitory activity (<40%), moderate (41%-80%), strong (>80%). Based on the study'sresults,Sargassum polycystum extract with the maceration extraction method is included in the moderate category, while ~~with~~ the ultrasonic extraction method ~~it~~ is included in the strong category.

IV. CONCLUSION

The results of the study of Sargassum polycystumextract were obtained using two extraction methods, fast maceration and ultrasonic, each for 20 minutes. The best results were obtained with the ultrasonic extraction method because the

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yield was 1.30 ± 0.11%, antioxidant activity IC50 79.65 ± 0.42 µg / mL and anti-obesity activity with pancreatic lipase inhibition 92.35 ± 4.02%.

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