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An In Vitro Study of Antidiabetic Activity of Sargassum Duplicatum and Turbinaria Decurens Seaweed

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ABSTRACT: *The whole brown seaweed has been known for its antidiabetic properties, but the antidiabetic activity of its fractionation results has not yet been known. This research was performed to investigate the ability of laminaran, fucoidan and alginic fractions from Sargassum duplicatum and Turbinaria decurens brown seaweed as an antidiabetic agent. On the first stage of this research, extraction and fractionation processes were performed to obtain laminaran, fucoidan and alginic fractions. On the second stage, characterization of the fractions and in vitro antidiabetic activity were performed using α-glucosidase enzyme. The results showed that the fractions from Sargassum duplicatum and Turbinaria decurens seaweed had similar FTIR (Fourier Transformation Infra Red) characteristics, but had different characteristic in λ-max absorption. For the inhibition activity toward α-glucosidase enzyme, laminaran fraction of brown seaweed showed higher activity compared to fucoidan fraction, whereas the alginic fraction showed no inhibition activity. Based on IC_{50} inhibition toward α-glucosidase, the fraction which had the highest potential as a type 2 antidiabetic agent was laminaran fraction from Sargassum (IC_{50} = 36.13 ppm), followed by laminaran of Turbinaria (IC_{50} = 44.48 ppm), fucoidan of Turbinaria (IC_{50} = 63.39 ppm) and fucoidan of Sargassum (IC_{50} = 75.10 ppm), respectively.*

KEYWORDS: *antidiabetic, brown seaweed, fractions, inhibition*

I. INTRODUCTION

Diabetes is a condition in which blood sugar levels are seen hyperglycemia. Hyperglycemia is caused by a decrease in insulin secretion and insulin action. According to the Ministry of health of Indonesia[1], hyperglycemia can be treated in two ways, namely by using injections of insulin and oral antidiabetic medications such as sulfonylureas, biguanide, thiazolidinedion, and to supplement or functional food. Functional foods are like a drug and there are also an inhibiting the enzyme glucosidase and amylase work so that it can inhibit the absorption of glucose for patients with diabetic type two [2].

One of the many functional foodstuffs belonging to Indonesia's seaweed that reach over 70% of the natural wealth of the sea. Brown seaweed is one of a class of seaweed in Indonesia and still not optimally utilized by the people of Indonesia[3]. Some type of Brown seaweed, *Cystoseirra sp*., *Dictyopteris sp*., *Dictyota*, *Hormophysa*, *Hydroclathrus*, *Padina*, *Sargassum* and *Turbinaria*.

Hardoko [4] has reported that seaweed may reduce blood sugar levels in the blood for invivo. According Limantara and Heryanto [5],in the brown seaweed found fucoxanthine can inhibit the accumulation of fat in preventing obesity and can be used as antidiabet. In addition, brown seaweed containing polysaccharides such as laminaran, fucoidan, and alginate [6].Therefore, it is necessary to do research on brown seaweed commonly found in Indonesia, such as *Sargassumduplicatum* and *Turbinaria decurens* to determine the activity of laminaran, fucoidan and alginate as antidiabet. If the results are found antidiabet fraction, then the brown seaweed will be developed further in the food processing industry, such as the manufacture of food supplements antidiabetic.

II. MATERIAL AND METHODS

2.1 Materials and Equipment

Materials studied were brown seaweed *Sargassum duplicatum* and *Turbinariadecurens* obtained from Pari Island, Seribu Islands, Jakarta. In addition, the required supporting material used for the extraction and fractionation: ethanol 85%, 2% $CaCl_2$, HCl, and 3% Na_2CO_3 and materials used for such analysis α-glucosidase enzyme, phosphate-buffer (pH 7), serum albumin, *p*-nitrophenyil α-D-glucopyranose, dimethyl sulfoksid (DMSO), and water demineralisation.

The equipments used for extraction are a rotary evaporator, double beam UV -Vis spectrophotometer, pH meter, water bath, sterile glass bottles, analytical balance, test tubes, measuring cup, volumetric pipette, elenmeyer, Whatman filter paper # 4, round gourd, stir, centrifuge apparatus, funnel, vacuum filter. The tools used for analysis are waterbath, elenmeyer, micropipette, test tubes, measuring cup, volumetric pipette, micro pipette, beaker glass.

2.2 Research Methods

The method used experimental methods fraction kinds of brown algae on antidiabet activity in vitro using α-glucosidase enzyme. This experiment is divided into two stages namely fractionation stage and phase of in vitro antidiabet activity assay. Brown algae fractionation stage based on the method of Rioux *et al*. [6] to obtain laminaran, fucoidan and alginate fractions. Parameters observed seaweed proximate [7],the fraction of water content, yield fraction, the fraction of the maximum wavelength (UV - Vis), FTIR spectrum of the fraction, and α-glucosidase inhibitory activity [8].

2.3 Brown Seaweed Fractionations Procedure

Sample of brown seaweed (*Sargassum duplicatum*and *Turbinaria decurens*) were extracted by using the method of Rioux *et al*. [6]. Seaweed is reduced in size to be extracted with a blender and weighed then performed using a maceration with 85% ethanol (ethanol and seaweed ratio is 1:4) [9] at a temperature of 23^{o}C for 2 times of 12 hours (after the first 12 hours and then replaced solvent extracted samples again for 12 hours) and at 70°C for 2 times of 5 hour.

After that, the solution is filtered using a vacuum filter and using a Whatman # 1 filter paper, and obtained filtrate and residue. The seaweed residue was extracted again using 2% $CaCl_2$ solution with a 1: 4 ratio on temperature of 70°C for 3 times of 3 hours. The solution filtered using a vacuum filter with a Whatman # 1 filter paper. The obtained filtrate then was evaporated using a rotary evaporator with a temperature of 60°C to get extract as laminaran (fraction A), and then calculated moisture content and yield of the extracts as laminaran. The seaweed residue was extracted again by using HCl pH 2 with a ratio of 1: 4 at a temperature of 70°C for 3times of 3 hours. After that, the solution filtered using a vacuum filter and using a Whatman # 1 filter paper and obtained filtrate and residue. The filtrate then is evaporated using a temperature of 60°C to get extract fucoidan (fraction B), and then calculated moisture content and yield of extract fucoidan. While the residue was extracted by using 3% Na_2CO_3 again with 1: 4 ratio on temperature of 70°C for 3 times 3 hours. After that, the solution is filtered using a vacuum filter and using a Whatman # 1 filter paper. The filtrate evaporated using a temperature of 60°C to get extract alginate (fraction C) is then calculated moisture content and yield of extract alginate. All fractions obtained were performed a test using FTIR to know the functional group isomers of the faction and invitro tested by using α-glucosidase enzyme.

2.4 Method of Invitro Antidiabet Test

Antidiabet activity in vitro was tested using the method of α-glucosidase enzyme inhibition by [7] with modified. Glucosidase enzyme solution made by dissolving 1 mg α-glucosidase enzymes in 100 mL of phosphate buffer (pH 7.0) containing 200 mg of albumin serum. Before use, the enzyme solution 1 ml diluted as much as 25 times with phosphate buffer (pH 7.0) wich containing 200 mg of albumin serum.

Each the obtained faction (fraction A = laminaran, B = fucoidan, and aginate = C fraction) was dissolved into the solution of DMSO, then added 250 µl of 20 mM p-nitrofenil α-D-glucopyranose is used as a substrate and 490 µl 100 mM phosphate buffer (pH 7.0). After that, the mixture is incubated in a waterbath at 37° C for 5 minutes. Once incubated, mix added 250 µl of enzyme solution and stored in the waterbath for 15 minutes, then add 100 µl 200 mM solution of sodium carbonate. Results of p-nitrophenol is then tested using a Double Beam Spectrophotometer with a wavelength of 440 nm. Doses to the concentration of the fraction used was 6.25 ppm, 12.50 ppm, 25.00 ppm, and 50 ppm.

Absorbance is then measured using a Double-beam spectrophotometer. Phosphate buffer solution was added into the spectrophotometer to calibrate (make zero absorbance value), then inserted a blank front arrangement is blank of enzyme, and the rear is the control and the numbers will appear on the screen which is the absorbance value of the blank (C). After that, do the same for sample testing by injecting samples into the spectrophotometer and the absorbance obtained a sample absorbance (S). Percent inhibition can be calculated by the equation:

$$\%\ inhibition = \frac{C - S}{C}\ x\ 100\%$$

Where: C = absorbance control; S = sample absorbance.

IC_{50} value calculation used was obtained from the calculation of the concentration of the samples used on the activity of enzyme inhibition of 50 percent. Done after getting a plot of the data in the form % inhibition (Y) against concentration sample (X) using linear regression. It was created out of the equation Y = 50% value so as to get the value of X which is the value of the IC_{50} in the sample.

III. RESULT AND DISCUSSIONS

3.1 The characteristics of physico-chemical fractions of Brown Seaweed

Chemical and physical characteristics of each fraction of the Brown seaweeds *Sargassum duplicatum* and *Turbinaria decurens* can be seen in Table 1.

Table 1. Characteristics of physico-chemical fractions from the brown seaweed

Parameters		*Sargassum duplicatum*			*Turbinaria decurrens*		
		Laminaran	**Fucoidan**	**Alginate**	**Laminaran**	**Fucoidan**	**Alginate**
Yield (%)		3.42	2.09	33.13	3.92	1.45	16.53
Moisture content (%)		67.65	84.61	90.05	62.84	83.75	81.87
λmax (nm)		262.8	583.4	600.5	282.0	579.8	596.3
FTIR (cm^{-1})	C=O	1641.42	1610.56	1647.21	1641.42	1622.73	1641.42
	S=O	1192.01	1215.15	1224.8	1315.45	1220.94	1367.53
	C-O	1028.06	1010.70	1028.06	1055.36	1010.70	1029.99
	O-H	3466.08	3468.01	3452.58	3458.37	3429.43	3462.22

Based on Table 1 of the largest proportion of seaweed Sargasum and Turbinaria are alginate, but in quantity seen no difference in the amount of alginate between both types of seaweed.Another character that looks different is at a wavelength of maximum (λ_{max}) as laminaran and fucoidan. This characteristic differences can be caused by various factors. Factors that may affect differences in the characteristics of polysaccharides is a variation of species, molecular weight, molecular structure, and composition [10], purity, and the source, as well as the extraction method. [11-13].

Turbinaria decurens of fucoidan fractions have a yield of 1.45% extract, whereas from *Sargassum duplicatum* 2.09%. According to [14], fucoidan extract crude results was of 2.7%, while the Fitton [10] stated that yield fucoidan in general is 2-10%. This shows that the yield obtained is not much different and possible illicit can be caused by the existence of different types of seaweed, where different types of seaweed affecting the amount of fucoidan yield generated. Besides the factors age and local climate also affects the yield and the structure of fucoidan [15].

FTIR characteristics of crude fraction as laminaran, alginate, and both types of fucoidan from the seaweed is similar to that reported by [16, 17 and [18] so that it can be said that the crude fraction each contain a alginate as laminaran, and fucoidan. Moisture content of each faction is quite high. This corresponds with the solvent used is polar and the nature of the polysaccharide is to absorb water. Rioux, *et al* [19] said that fucoidan is also included into compounds that are water soluble so that it can generate high water levels. According [20], that alginate is hydrophilic hydrocolloid, so easily bind water.

3.2 Inhibition activity of Fractions of Brown Seaweed

Enzymes α-glucosidase was included in the group of hydrolase or often called maltase. These enzymes can hydrolyze maltose into glucose. In addition the enzyme α-glucosidase work break down branches α-(1.6). Polysaccharides such as amylopectin, glycogen, and the limit of dextrin can be broken down into glucose and maltose by small amounts of amylase enzyme and inhibition of the enzyme α-glucosidase, so the α-glucosidase can prevent the increase of glucose in the blood [21]. Substances that can inhibit the enzyme α-glucosidase work is commonly used as a type 2 anti diabetic drugs work by inhibiting the digestion of carbohydrates such as starch and sugar [22].

Test results of enzyme inhibition α-glucosidase from the fraction as laminaran, fucoidan, and alginate from Brown algae *Turbinarium decurens* and *Sargasum duplicatum* presented in Figure 1.

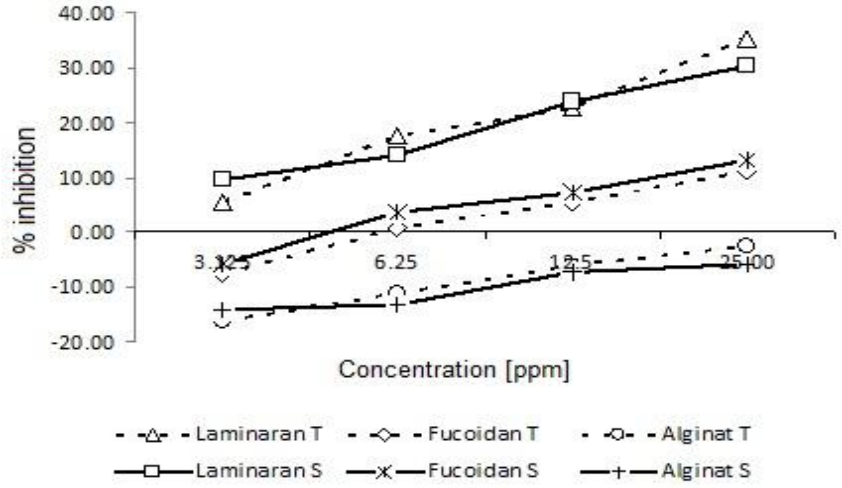

Figure 1. Inhibitory activity of the brown algae fractions on α-glucosidase
Description : T = Turbinaria ; S= Sargassum

Based on Figure 1 it can be seen that there is a noticeable difference between the fraction and dosage of enzyme inhibition of α-glucosidase. In this case up to the dose or concentration of 25 ppm alginate faction of *Sargassum duplicatum* and *Turbinaria decurens* unable to inhibit α-glucosidase enzyme activity. If a alginate doses are known to want to start inhibit the α-glucosidase enzyme regression can be carried out and replace the Y values, such as Figure 2. For example, if Y = 0 estimated alginate *Turbinaria decurens* will start to impede on the concentration of 37.89 ppm and if Y = 1 then a alginate could hamper already at concentrations 40.46 ppm and so on. By invivo reported [23], [24 and 25], that a alginate salt was able to lower blood sugar. This may indicate that the inability of the alginate in inhibiting α-glucosidase enzyme is because less dose factors or doe the nature of alginate in lowering blood glucose instead of the other way is not working inhibit α-glucosidase enzymes.

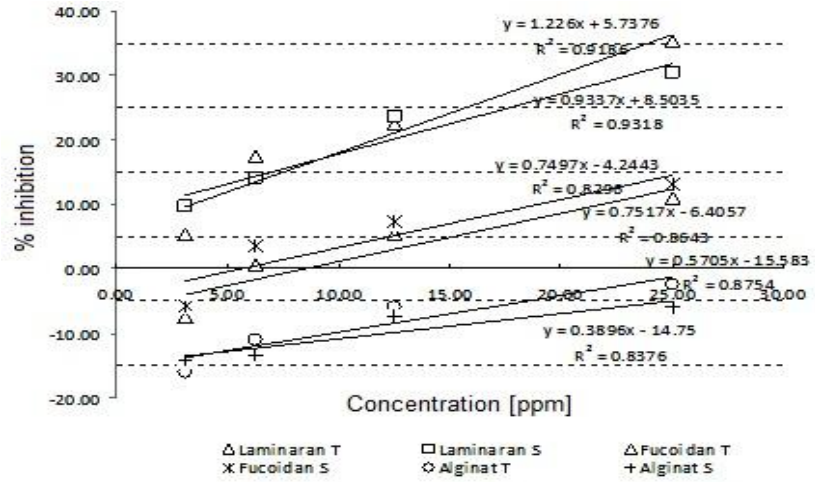

Figure 2. Correlation of dose fractions with inhibitory activity of brown seaweed α-glucosidase enzyme
Description: T = Turbinaria ; S= Sargassum

Based on the type of brown seaweed (Figure 2) shows that the inhibitory activity of each faction is almost the same, but when examined seen a change at some point (intersection of the equation) between laminaran fractions from *Turbinaria decurens* with *Sargassum duplicatum* and between fractions of alginate. These changes are thought to be related to the differences as well as alginate polymer structure laminaran of both types of seaweed or other factors that have been mentioned by [10-13] related characteristics.

In general, based on Figure 1 and 2 shows that the inhibitory activity of fractions of brown seaweed (*Sargassum* and *Turbinaria*) are highest in laminaran and followed fucoidan fraction. Inhibitory ability of laminaran and fucoidan can be shown that both the potential as drug antidabetic type 2 that could lower blood

glucose in vivo. This is supported by [10, 26 and 27],who showed that in vivo laminaran and fucoidan can lower blood glucose of type 2 diabetes and even type 1 diabetes.

3.3 IC_{50} Value Fractions of Brown Seaweed

In order to compare the inhibitory activity on the same basis, so it can use their respective IC_{50} values of fraction of Brown seaweed. In this case the value of capability was indicated the IC_{50} (amount of) fractions of the brown seaweed *Sargassum duplicatum* and *Turbinaria decurens* in inhibiting the α-glucosidase activity as much as 50%. The lower of IC_{50} values showed inhibitory activity of α-glucosidase activity is higher. This activity can be compared with the principle it works antidiabet drugs (acarbose) inhibit the enzyme activity of α-glucosidase, which is shown in Figure 3.

Figure 3 shows that the ability of α-glucosidase inhibitory each fraction of one type of seaweed is different, so too for the same fraction of seaweed. This is also demonstrated by the difference of the wavelength of maximum absorption characteristics (Table 1). It can be caused by factors: species variation, molecular weight, composition, and molecular structure, purity, and source [10-13].

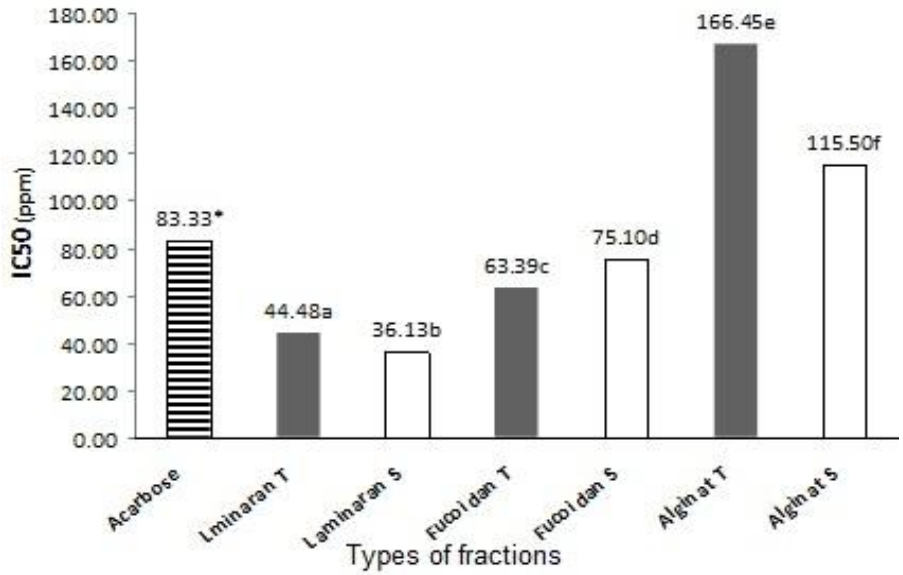

Figure 3. IC_{50} values of fractions of brown seaweed against α-glucosidase

Description: T = Turbinaria S = Sargassum, * Source: [28]; the notation of fraction showed significant difference at α 00:05

Based on IC_{50} values, the most inhibiting α-glucosidase enzyme is a laminaran fraction of*Sargassum duplicatum*, followed by laminaran of *Turbinaria decurens*, fucoidan of *Turbinaria decurens*, fucoidan of *Sargassum duplicatum* and alginate of*Sargassum* and *Turbinaria*. However, when compared with the acarbose so much better as laminaran faction and fucoidan fractions equivalent or slightly better in inhibiting the α-glucosidase enzyme.

According to Sugiwati [2 and 22], that acarbose is one of the antidiabetic drugs that can be and has the ability as an inhibitor of the α-glucosidase enzyme, and included into the competitive inhibitor because it has a high affinity with α-glucosidase. Thus a alginate faction of the Brown algae have no potential as a functional food or anti diabetic drugs, while another faction of the second fraction as laminaran and fucoidan still has potential as an alternative medicine anti diabetic.

IV. CONCLUSION

The fraction as laminaran, fucoidan, and alginate from *Sargassum duplicatum*and *Turbinaria decurens* had characteristics that are similar in FTIR, but different in his λ_{maks} absorption.

Inhibitory activity of α-glucosidase of Brown seaweed as laminaran faction is highest, followed by the fraction of fucoidan, but the fraction of alginate had no inhibitory activity of α-glucosidase.

The fraction of Brown seaweed that is potentially as antidiabet type 2 is as laminaran of *Sargassum duplicatum* (IC_{50} = 36.13 ppm), laminaran of *Turbinaria decurens* (IC_{50} = 43.65 ppm), fucoidan of *Turbinaria decurens* (IC_{50} = 63.39 ppm), and fucoidan of *Sargassum duplicatum* (IC_{50} = 75.10 ppm).

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