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Research Article

In vitro Antidiabetic Activity of *Sargassum hystrix* and *Eucheuma denticulatum* from Yogyakarta Beach of Indonesia

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Abstract: Marine algae are a potential bioactive source that began to be developed as a new pharmaceutical agent, including antidiabetic. The objective of this research was to determine the potential of polyphenols and phlorotannins extract from *Sargassum hystrix* (J. Agardh, 1847) and *Eucheuma denticulatum* [(N. L. Burman) F. S. Collins & Hervey, 1917] in inhibiting *α*-amylase and *α*-glucosidase. Polyphenols were extracted using 50 % methanol, and phlorotannins were extracted using methanol, and the non-lipid layer was separated by using distilled water, methanol and chloroform, and then partitioned using ethyl acetate twice. The total content of polyphenols and phlorotannins were analyzed. Both types of the compounds were tested to determine their ability to inhibit *α*-amylase and *α*-glucosidase activity. Total phenols content of *S. hystrix* and *E. denticulatum* were observed to be 3.17 g GAE. 100 g^{-1} extract and 0.33 g GAE. 100 g^{-1} extract, respectively. Total phlorotannin content of *S. hystrix* and *E. denticulatum* were obtained 0.02 g PGE. 100 g^{-1} extract and 0.02 g PGE. 100 g^{-1} extract, respectively. The results showed that polyphenols *S. hystrix* (IC_{50} = 0.58±0.01 mg.mL^{-1}) can inhibit *α*-amylase, similar to acarbose (IC_{50} = 0.53±0.00 mg.mL^{-1}) and phloroglucinol (IC_{50} = 0.56±0.01 mg·mL^{-1}), but inhibiton activity of polyphenol and phlorotannin from *E. denticulatum* was lower (IC_{50} = 1.43±0.19 and 1.92±0.14 mg.mL^{-1}, respectively). Inhibitory activity of polyphenols from *S. hystrix* (IC_{50} = 0.59±0.02 mg.mL^{-1}) in inhibiting *α*-glucosidase was also similar to acarbose (IC_{50} = 0.61±0.01 mg.mL^{-1}) and phloroglucinol (IC_{50} = 0.56±0.05 mg.mL^{-1}), but inhibiton activity of polyphenol and phlorotannin from *E. denticulatum* was also lower (IC_{50} = 1.43±0.19 and 0.86±0.06 mg. mL^{-1}, respectively). So, *S. hystrix* had more potential as an antidiabetic substance compared to *E. denticulatum*.

Keywords: *α*-amylase, *α*-glucosidase, Antidiabetic activity, *Eucheuma denticulatum*, *Sargassum hystrix*

1. INTRODUCTION

Diabetes mellitus (DM) is a group of a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action [1]. Chronic hyperglycemia in diabetes is related to long-term damage and dysfunctioning of several organs of the body [2]. WHO [3] has reported approximately 346 × 10^6 people in the world to suffer from DM. The International Diabetes Federation [4] estimated that in 2030, people with diabetes would rise to 438 × 10^6.

Until now, marine resources in Indonesia have not been widely used as a source of active ingredients for the food and pharmaceutical industries. One source of marine organisms, that was found in Indonesia but has not been used either as a source of food and a source of bioactive ingredients, is seaweed. Gunung Kidul Beach was a potential area for producing both bioactive compounds from seaweed and sponges [5, 6].

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Bioactive compounds from marine brown algae are potential as an antitumor, antifungal, antiviral, antioxidant, antihypertensive and antidiabetic [7, 8]. The polyphenol content of marine algae has pharmacological effects as antioxidants, antibiotics, anti-inflammatory, hypo-allergenic, antibacterial, and antidiabetic [9]. Nwosu et al. [10] stated that phenol extract of seaweed *Palmaria palmate* and *Ascophyllum nodosum* have potential as an antidiabetic agent by inhibiting α-amylase and α-glucosidase activity. Marine algae also have a high content of antioxidants and can be used to stave off free radicals that arise due to the condition of hyperglycemia in diabetic people [11]. The objective of this research was to analyze the activity of *Sargassum hystrix* J. Agardh, 1847 and *Eucheuma denticulatum* N. L. Burman F. S. Collins & Hervey, 1917 extracts obtained from Gunung Kidul Beach of Yogyakarta in inhibiting the activity of α-amylase and α-glucosidase.

2. MATERIALS AND METHODS

2.1 Materials

The main materials used in this study were seaweed, *S. hystrix* and *E. denticulatum* obtained from the coastal of Gunung Kidul, Yogyakarta Indonesia. The sample of seaweed was identified by expert of Plant Taxonomy Laboratory, Faculty of Biology, Universitas Gadjah Mada. Methanol, sodium carbonate, Folin-Ciocalteu's reagent, essential oils and acid gallate were obtained from E. Merck. The *α*-amylase from *Bacillus* sp. type II-A, *α*-glucosidase from *Saccharomyces cerevisiae* type I, p-nitrofenil-*α*-d-glucophiranoside and 3,5-dinitrosalisilat (DNS) acid were purchased from Sigma-Aldrich.

2.2 Marine Algae Extractions

In this study, the extraction of seaweed has been made to obtain an extract containing polyphenols and phlorotannin. The extraction of the seaweed *S. hystrix* and *E. denticulatum* to get polyphenols uses a modification of the method Zhang et al. [12].

Phlorotannin extract was obtained by following a modified method of Chowdhury et al. [13]. Polyphenol powder of 5 g was added to Erlenmeyer flask wrapped with aluminum foil. Afterward, 40 mL of methanol was added and stirred for 2 h, and then allowed to stand for 24 h, and then 20 mL of chloroform was added while stirring for 20 min. The mixture was centrifuged at 3500 revolutions per minute (rpm) for 20 min and the supernatant was separated (1 rpm = 1/60 Hz). A volume of 15 mL aquabidest was added to the supernatant with constant stirring for 10 mins and lipid and non-lipid layers were formed. Non-lipid layer that floats over the lipid layer was separated with the addition of 25 mL ethyl acetate and stirred for 30 min. Subsequently, the mixture was evaporated, freeze-dried and stored at -20 °C before use for further analysis.

2.3 Measurement of Total Polyphenol Content

The total phenols content in seaweed was analyzed using a modified method of Zhang et al. [12]. Gallic acid was used as a standard with concentrations ranging from 0 $mg.mL^{-1}$ to 400 $mg.mL^{-1}$. Serial dilutions of polyphenol extract with concentrations of 3.125 $mg.mL^{-1}$ to 200 $mg.mL^{-1}$ were made. An Aliquot of 200 mL from each solution was added to the test tube, followed by the addition of 1000 mL of Folin-Ciocalteu reagent and incubated for 5 min. After the incubation, 800 mL of 20 % Na_2CO_3 solution was added to the mixture and incubated in dark at 27 °C temperature for 75 min. The absorbance of the incubated mixture was observed at 750 nm wavelength.

2.4 Measurement of Total Phlorotannin Content

The total content of phlorotannin of seaweed was analyzed using a modified method of Koivikko et al. [14]. Phlorotannin extract of 0.1 g was macerated with 200 mL of 85 % ethanol (1:2) in the dark for 8 hours. Phloroglucinol standard solution were made at various concentrations, i.e. 6.25; 12.5; 25; 50; 100 μg . mL^{-1}. Phlorotannin extract was made in a concentration of 1 mg · mL^{-1} following the serial dilution series of concentration, 125 $mg.mL^{-1}$ to 200 $mg.mL^{-1}$. Then, the solution of each sample of 500 mL was pipetted and put into a test tube. Then, 500 mL of Folin-Ciocalteu reagent and 1 mL 20 % Na_2CO_3 were added, and then the mixture was left for 3 min. Furthermore, the solution in a test tube was incubated in the dark room temperature of 27 °C for 45 min, then centrifuged for 10 min at a speed of 3500 rpm. The

absorbance of supernatant was recorded at 730 nm wavelength. A total Phlorotannin content was expressed as phloroglucinol equivalents (PGE) in mg. mg^{-1} extract.

2.5 Inhibition of α-Amylase Activity

Inhibitory activity of *α*-amylase was decided by calculating changes in 3.5-dinitrosalicylate acid into nitro-aminosalicylate utilizing spectrophotometry [15]. A volume of test solution was made from 25 mL of sample extract at differing concentrations and 25 mL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 13 U mL^{-1} of *α*-amylase. The test solution was mixed using vortex mixer and incubated at 37 °C for 10 min. After that, 25 mL of soluble starch 1 % in 0.02 M sodium phosphate buffer was added into the test solution and it was incubated at 37 °C for 10 min. Then, it was further handled with the addition of 50 mL 96 mM 3, 5-dinitrosalisilat acid (DNS) and incubated for 5 min in water bath. The solution was cooled at room temperature and the absorbance of the solution was recorded at a wavelength of 550 nm. Absorbance values of sample were obtained and used to calculate the percentage (%) inhibition of the enzyme.

$$\%\,inhibition = \frac{K - (S1 - S0)}{K} x100\ \% \quad (1)$$

where:
K= Absorbance of control-blank
S_1= Absorbance of sample with enzyme
S_0= Absorbance of sample without enzyme

2.6 Inhibition of α-Glucosidase Activity

Inhibitory activity of *α*-glucosidase was performed as stated by the method of Mayur et al. [16]. Test solution consists of 50 mL 0.1 M phosphate buffer (KH_2PO_4) pH 7; about 25 mL substrate 0.5 mM p-nitrophenyl-α-D-glucopyranoside (PNP-G), 10 mL of sample at various concentrations and 25 mL of 0.2 U · mL^{-1} *α*-glucosidase. The test solution was combined and incubated at 37 °C for 30 min. The reaction was finished by the addition of 100 mL of 0.2 M Na_2CO_3. Inhibition of enzyme activity was analyzed by the amount of p-nitrophenyl formed by measurement of the absorbance utilizing a microplate reader at a wavelength of 405 nm. Absorbance values of sample were obtained then used to calculate the percentage (%) inhibition of the enzyme as shown in Equation 1.

2.7 Statistical Analysis

The data of the extract concentration versus percent inhibition of the enzyme were plotted to obtain the regression equation. The IC_{50} activity value of *S. hystrix* and *E. denticulatum* extracts against *α*-amylase and *α*-glucosidase were achieved from the regression equation. The IC_{50} values were analyzed statistically with one-way analysis of variance using the SPSS (Statistical Package for Social Sciences, IBM, USA) at 95 %.

3. RESULTS AND DISCUSSION

3.1 Total Polyphenol and Phlorotannin Contents

The data of polyphenols and phlorotannins extracts along with the content of each compound was shown in Table 1. The total phenol content of *S. hystrix* and *E. denticulatum* was 3.17 g GAE per 100 g extract and 0.33 g GAE per 100 g extract, respectively. These results were consistent with the previous reports of Kumar et al. [17]. The research of Cox et al. [18] showed that the brown algae (*Himanthalia elongata*) has a higher total phenol, which was (151.33±6.75) mg $GAE.g^{-1}$ extract. Damongilala et al. [19] showed that *P. pavonica* extracted using 60 % methanol resulted in the total phenols content of 4.98 mg $PGE.g^{-1}$ extract to 5.87 mg $GAE.g^{-1}$ extract. The differences in total phenol content could be influenced by intrinsic factors, such as age, type, etc., and extrinsic factors namely tidal cycles, salinity, etc. [20].

Table 1 showed that total phlorotannin content of *S. hystrix* and *E. denticulatum* calculated as 0.02 g PGE per 100 g extract and 0.02 g PGE per 100 g extract, respectively. According to Koivikko et al. [21], only brown algae contained phlorotannin. However, based on this study, phlorotannin content of the red alga *E. denticulatum* was detected although it was not higher than *S. hystrix*. Phlorotannin content is specific to each species [22], factors. i.e. age, species, and environment (location, season, waves, presence or absence of light, salinity, UV radiation, the presence or absence of herbivores and nutrients) could be the reason of the Phlorotannin content. Likewise, Gunung Kidul coastal area is a

Table 1. Yield and total content of polyphenols and phlorotannin extracts from *S. hystrix* and *E. denticulatum*

Sample		Extracts	
		Polyphenols	**Phlorotannin**
Sargassum hystrix	Yield	15.47 %	2.36 %
	Total content	3.17 g GAE per 100g	0.021 g PGE per 100g
Eucheuma denticulatum	Yield	12.40 %	8.24 %
	Total content	0.33 g GAE per 100g	0.018 g PGE per 100g

rocky shore with longer emersion cycle; therefore *Sargassum* species at Gunung Kidul has longer exposed to solar radiation and generally has higher phenolic content. Moreover, Gunung Kidul beach has a high waves so that the seaweed that grows there tends to have high phlorotannin content [23].

3.2 Inhibition of α-Amylase Activity

Based on Fig. 1. the highest inhibitory activity was observed in polyphenols of *S. hystrix* (94.09 %). On the other hand acarbose, polyphenols and phloroglucinol of *E. denticulatum* showed a higher inhibitory activity (88.81 %, 85.84 %, and 85.92 %, respectively) compared to phlorotannin of *S. hystrix* (68.39 %) and phlorotannin of *E. denticulatum* (69.02 %). Polyphenols of *S. hystrix* at these concentrations showed higher inhibitory activity compared to polyphenols of *E. denticulate*, phlorotannin of *S. hystrix* and phlorotannin of *E. denticulatum*. According to Kunyanga et al. [24], phenolic compounds were able to bind to the active site of *a*-amylase. Bioactive components group of phenolics such as anthocyanins, flavonols, flavones, flavanones, gallic acid, vanillic acid, quercetin and trans-cinnamic have been reported to have inhibitory activity against the activity of *α*-amylase [25, 26]. The ability of both seaweeds in inhibiting *α*-amylase was supported by Firdaus et al. [11]. In addition, Lamela et al. [27] reported hypoglycemic activity of *Eucheuma* sp.

Table 2 exhibited the IC_{50} of inhibition activity of *α*-amylase by *S. hystrix* and *E. denticulatum* extracts, acarbose, and phloroglucinol. There were no significant differences between IC_{50} values of acarbose, phloroglucinol and polyphenol of *S. hystrix*. IC_{50} values displayed higher activity compared to other marine algae, for example chloroform extract of *Chaetomorpha aerea* (IC_{50} = 408.9 μg.mL^{-1}) and methanol extract of *Chlorodesmis* (IC_{50} = 147.6 μg.mL^{-1}) [28]. However, it was relatively lower than *Ascophyllum nodosum*. Senthil Kumar & Sudha [29] informed that IC_{50} value of water extract of marine algae (*S. policystum, R. corticata* and *G. lactuca*) for *α*-amylase inhibtion was 60 μg.mL^{-1}, 67 μg.mL^{-1}, and 82 μg.mL^{-1}, respectively. Nwosu et al. [10] reported that methanol extract of *A. nodosum* has a smaller IC_{50} value (0.1 μg.mL^{-1}).

3.3 Inhibition of α-Glucosidase Activity

Inhibition activity of *α*-glucosidase is shown in Fig. 2. These results displayed similar results for acarbose and polyphenols of *S. hystrix* in inhibiting *α*-glucosidase activity, followed by phloroglucinol, polyphenols of *E. denticulatum*, phlorotannin of *E. denticulatum*, and phlorotannin of *S. hystrix*. The ability of polyphenols to inhibit α-glucosidase in the digestive tract and activation of glucose uptake lowered blood glucose [12, 15]. Anthocyanin, especially polyphenols, flavonols, proanthocyanins, and phenolic acids, significantly suppress the elevated blood glucose and reduce the rate of digestion of sucrose and glucose absorption in the intestine [30]. The workings of a polyphenol are similar to acarbose, which extends the time revamp of carbohydrates and inhibit the absorption of glucose [31].

Alpha-amylase and alpha-glucosidase are enzymes that are closely associated with diabetes mellitus. Phenols are one of the bioactive components that can inhibit the action of *α*-amylase and *α*-glucosidase [32, 33]. Polyphenols can inhibit the enzyme in the breakdown of carbohydrates into glucose. The content of phenol has an inhibitory effect on *α*-amylase through bond hydroxylation and ring substitution on *β*. The principle is similar to acarbose inhibition, i.e., by generating delays and disaccharide carbohydrate hydrolysis and absorption of glucose and inhibiting the metabolism of sucrose into glucose and fructose [34]. Besides, that phlorotannin is one of the phenolic components which can also inhibit the

work of the α-amylase and α-glucosidase [35, 36]. As polyphenols, phlorotannin inhibits enzymes work in the breakdown of carbohydrates into glucose. The principle was also similar to acarbose inhibition; that was, to produce a delay, hydrolysis and absorption of carbohydrates and disaccharides inhibit the metabolism of glucose and sucrose into glucose and fructose [34].

4. CONCLUSION

Seaweed *S. hystrix* has more potential as an antidiabetic substance compared to *E. denticulatum*. The results displayed that polyphenols *S. hystrix* [IC_{50} = (0.58 ± 0.01) mg.mL^{-1}] can inhibit α-amylase similar to like acarbose [IC_{50} = (0.53 ± 0.00) mg.mL^{-1}] and phloroglucinol [IC_{50} = (0.56 ± 0.01)

Table 2. Inhibitory activity (IC_{50}) of polyphenol and phlorotannin extracts from *S. hystrix, E. denticulatum*, acarbose, and phloroglucinol against α-amylase and α-glucosidase.

Inhibitors	$IC_{50}$of α-amylase (mg · mL^{-1})	$IC_{50}$of α-glucosidase (mg · mL^{-1})
Acarbose	0.53 ± 0.00[a]	0.61 ± 0.01[a]
Phloroglucinol	0.56 ± 0.01[a]	0.56 ± 0.05[a]
Polyphenols *S. hystrix*	0.58 ± 0.01[a]	0.59 ± 0.02[a]
Polyphenols *E. denticulatum*	1.43 ± 0.19[b]	1.43 ± 0.19[d]
Phlorotannin *S. hystrix*	3.29 ± 0.12[d]	0.78 ± 0.04[b]
Phlorotannin *E. denticulatum*	1.92 ± 0.14[c]	0.86 ± 0.06[c]

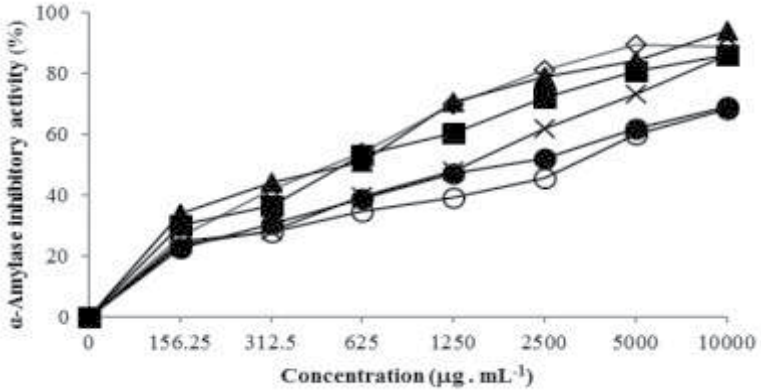

Fig. 1 Effect of sample concentration (▲: polyphenol *S. hystrix*, ´: polyphenol *E. denticulatum*, ○: phlorotannin *S. hystrix*, ●: phlorotannin *E. denticulatum*) and control (◊: acarbose, ■: phloroglucinol) on inhibition activity of α-amylase.

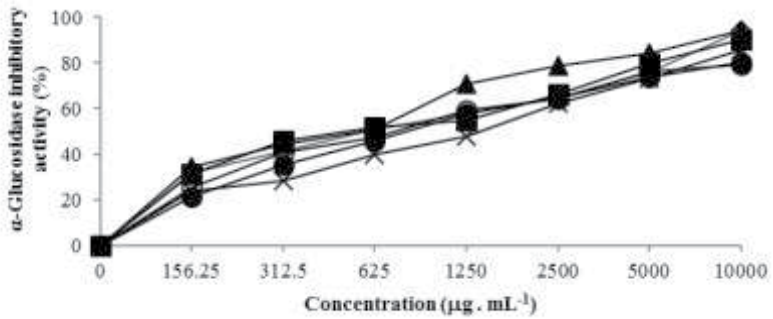

Fig. 2 Effect sample concentration (▲: polyphenol *S. hystrix*, ´: polyphenol *E. denticulatum*, ○: Phlorotannin *S. hystrix*, ●: Phlorotannin *E. denticulatum*) and control (◊: acarbose, ■: phloroglucinol) on inhibition activity of α-glucosidase.

mg. mL^{-1}]. Inhibitory activity of polyphenols *S. hystrix* [IC_{50} = (0.59 ± 0.02) mg.mL^{-1}] in inhibiting α-glucosidase is also similar to acarbose [IC_{50} = (0.61 ± 0.01) mg.mL^{-1}] and phloroglucinol [IC_{50} = (0.56 ± 0.05) mg.mL^{-1}].

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