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Anti-hyperglycemia Test of Extract *Gelidium* sp. using the *In-vivo* Method in Male Mice (*Mus musculus L*)

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ABSTRACT

Background: *According to the International Diabetes Federation (IDF) and the World Health Organization (WHO) data there are 382 million people living with diabetes in the world in 2013. 2035 this number will increase to 592 million people. One of the natural resources that has the potential as anti-diabetic but has not been used optimally is Gelidium sp. which contains polysaccharides and polyphenols which can play a role in increasing HDL (High Density Lipoprotein) levels.*

Purpose: *The purpose of this study was to determine the polysaccharide activity against the reduction in blood of male mice (c L). The sample used is Gelidium sp. extracted to obtain polysaccharide compounds and polyphenols which will test anti-hyperglycemia activity and in 4 treatment groups namely positive control group using Akarbose, negative control given 1% NaCMC and each treatment given dose variation of Agarose Dosage I 1.25 mg/20 grams BB Mice and Agarose Dosage II 2.5 mg/20 grams BB Mice.*

Result: *The results of the two-way ANOVA statistical test on fasting blood glucose levels between the negative control group to positive control and agarose dose of 2.5 mg/kg BW mice showed a significant difference with a value of P <0.05. Whereas between negative groups with other treatment groups P value> 0.05 means that there is no significant difference.*

Keywords: *Gelidium sp.*, Polysaccharide compounds, Polyphenol compounds, Anti-hyperglycemia

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INTRODUCTION

The phenomenon of diabetes very worrying for the world population. According to the International Diabetes Federation (IDF) and the World Health Organization (WHO) data there are 382 million people living with diabetes in 2013. 2035 this number will increase to 592 million people. An estimated 382 million people, 175 million of whom have not been diagnosed. Whereas in Indonesia in 2013 diabetics had reached 8,554,115 people and made Indonesia the 7th country with the largest diabetes population in the world after China, India, the United States, Brazil, Russia and Mexico [1]. In Indonesia has reached 9.1 million people making position of Indonesia rose from 7^{th} place to the 5^{th} highest rank among the countries with the highest number of diabetics. Indonesia is one of the countries that is famous for natural resources both on land and in the ocean that has potential as anti-diabetic but not been able to known and used optimally. A potential natural product is derived from marine organisms which are also useful as anti-diabetic. Anti-diabetic from marine organisms can be whole, crude extract, or extract of polysaccharides. Extracts of marine organisms reported as potential anti-diabetic *in vitro* include water alone by the Society of Endocrinologists (PERKENI) states of diabetics in Indonesia.

The existence of various discoveries in the development of research and technology in all fields, especially regarding the use of seaweed for humans is not limited to raw materials for making agar, making paper agar, and making candy jelly [2]. Dewi, [3] stated that the methanol extract of brown seaweed *Sargassum prismaticum* can improve pancreatic tissue of diabetic induced streptosotocin mice. Red seaweed extract (Rhodophyta) type of *Gracilarria, Euchema, Gelidium, Hypnea* turned out to have bromophenol compounds which are known to act as anti-diabetic compounds. Marine algae contain polysaccharides, minerals, certain vitamins, proteins, fats and polyphenols, including as antibacterial, antiviral and antifungal. This provides a large potential for marine algae as a supplement in functional foods or for the extraction of compounds [4].

Utilization of *Gelidium sp.* has been widely used in various countries as a food source, but now with the development of technology utilization has also spread in the health sector because *Gelidium sp*. also has compound components bioactive alkaloids, saponins, phytosterols, phenolic compounds, and flavonoids [5]. The content of polyphenols has pharmacological effects as antioxidants, radiation protection, antibiotics, anti-inflammatory, anti-allergic, antibacterial and anti-diabetic [5]. Bromophenol is a part of polyphenol compounds as a powerful antioxidant that can also contribute to the prevention of type 2 diabetes through anti-inflammatory, antimicrobial and immunomodulatory mechanisms [6]. Research conducted by Jo Sung-Hoond et al., 2016 that the content of polyfenol is able to inhibit the activity of α-glucosidase enzyme so that it has the extracts from brown, red and green seaweed [7]. Activity of agarose extract and polyphenols from *Gelidium* sp. may enable it to pave the

way for the discovery and development of alternative treatments specifically for the treatment of diabetes originating from nature. Therefore, this study aims to look at the anti-hyperglycemic activity of agarose extract and polyphenols from *Gelidium* sp. *in-vivo* in male white mice (*Mus musculus*) induced by sucrose.

MATERIALS AND METHODS

Gelidium sp., Aquadest, Glucose, Akarbose, Na. CMC, physiological Nacl 0.9%, NaOH, 95% Ethanol, DMSO, 50% Methanol, $FeCl_3$. Hanscoon, mask, oral sonde, syringe, beakeglas, stirring rod, measuring flask, measuring cup, analytic balance, electric stove, vaporizer, glucometer, test tube, drop pipette.

Research procedure

This research was carried out with the actual experimental method (true experimental) using the Randomized Posttest Only Control Group Design design [8].

Sample preparation fresh

Gelidium sp. obtained in the Indonesian Ocean from Yogyakarta Indonesia, washed and separated with other impurities then dried in an oven and sieved using 8/12 no mesh sieve.

Polysaccharide extraction (Agarose)

Weigh 50 grams of seaweed powder into the Erlenmeyer add distilled water until submerged, add a solution of sodium hydroxide (NaOH) 0.1 N to pH 8.5 and proceed with heating with an electric heater (heater) to a temperature of 80°C, while occasionally stirring until formed solution. Strain while hot using Whatman number 41 filter paper vacuum to get the filtrate. Furthermore, 300 ml of 95% ethanol was added to the filtrate then allowed to stand for 24 hours at room temperature (25-27°C) then filter using ordinary filter paper. The precipitate formed was separated by adding 95% ethanol as much as 200 ml and allowed to stand again for 24 hours then strain. The precipitate with filter paper is placed in a desiccator for several hours until it reaches a constant weight. The deposits obtained are gelatinous extract. Gelatin extract was added to DMSO solution to a concentration of 1% (w/v), heat at 70°C for 2 hours while stirring. The cold solution was centrifuged (356G, 20 minutes at 4°C) to separate agarose and agaropectin. The obtained deposits are agaropectin, while the supernatant is agarose. Agarosa (supernatant) was washed with aquadestilation then filtered with vacuum [9].

Polyphenol extraction

Seaweed *Gelidium sp.* dried in aquadest for 12 hours then smoothed with a blender and dried in an oven at 40°C for 24 hours. Weigh 10 grams dissolved in 50% methanol as much as 200 ml of extraction for 1 hour at room temperature (25-27°C), using a shaker with a speed of 150 rpm then centrifuged at 4°C for 20 minutes at 7000 rpm. Steam using vacuum rotary evaporator [10].

Total phenol identification

Weigh the sample as much as 300 mg then heat with 10 ml distilled water for 20 minutes. After cold plus 3 drops of $FeCl_3$. If there is a green color shows the presence of polyphenols.

EXPERIMENTAL PROCEDURE

Preliminary tests are carried out every 10 minutes. Mice were satisfied for 8-10 hours but were still given ad libitum before treatment, then blood samples were taken from the tip of the tail which was injured and attached to Dr. Glucometer strip. After a few seconds the KGD number will appear on the device. This process is carried out to determine fasting KGD, while fasting for 8-10 hours is not needed. Mice are divided into 6 groups with 5 mice each. Group A: Negative control (only given Na.CMC 1%), Group B: Positive control of antihiperglycemia (Rootbond 0.364 mg/20 g BB Mice), Group C: Agarose Dosage I 1.25 mg/20 grams BB Mice, Group D :Agarose Dosage II 2.5 mg/20 grams BB Mice, Group E: Polyphenols Dosage I 1.25 mg/20 grams BB Mice, Group F: Polyphenols Dosage II 2.5 mg/20 grams BB Mice.

The *in vivo* effect testing method was carried out using the Oral Glucose Tolerance Test (OGTT) method which is the standard method in effect testinganti-hyperglycemic [11]. Baseline blood glucose values were obtained through the tail end using Easy Touch®GCU and immediately after that, a glucose solution of 0.14 grams/20 grams of BB mice orally was given to all mice to induce hyperglycemia. About 30 minutes after entering the blood test is done to confirm hyperglycemia.

$$\text{Percentage of Decrease in Blood Glucose} = = \frac{AUC\ GL\ 0\ \text{negatif} - AUC\ GL\ n\ positif}{AUC\ GL\ 0\ negatif} \times 100\%$$

STATISTICAL ANALYSIS

Differences in blood glucose levels (mean ± SD) were analyzed using Statistical Package for the Social Sciences (SPSS) 18one-way analysis of variance (ANOVA) measurements. Then proceed with Duncan and LSD tests with a 95% confidence level.

RESULTS AND DISCUSSION

The yield of agarose extract from *Gelidium sp.* can be seen in Table 1.

Table 1: Results of agarose extract and polyphenols *Gelidium sp.*

Weight of Simplicia	Solvent	Result of Rendement	Result (%)
Agarosa :100 grams	1000 ml	3,7 grams	37%
Polyphenols: 250 grams	1800 ml	8,82 grams	3,53%

According to Armisen and Galatas states that extracts generally have a moisture content of about 12-35%, so that in this study agarose extract from *Gelidium* sp. has a 37% yield which is slightly higher than the FAO criteria. According to Sur and Guven and Balkan et al. agar extract generally has CH_2 functional group at 2960 cm^{-1} uptake, 3,6 anhydrogalactose group at 1070 cm^{-1} uptake, 1,3 β-D group galactosepiranosil 897 cm^{-1} uptake, and cluster sulfate ester at an absorption of 1180 cm^{-1}; 1250 cm^{-1}; 1370 cm^{-1}. The number of 3,6 anhydrogalactose bonds in agarose is higher than the agaropectin fraction.

The agaropectin fraction will have a 3.6-anhydrogalactose group at peak absorption of 1070 cm^{-1}, and peak absorption of 1250 cm^{-1} and 850 cm^{-1} which indicates the presence of sulfate groups. This is what affects the yield value of agarose extract from *Gelidium sp.* has a value slightly higher than the standard set. The resulting high yield value indicates more extract value. But it is inversely proportional to the quality of the extract produced. The higher the extract value produced, the lower the quality of the extract. Good yield is <8%. Comparative control of the drug used is rootbose. Akarbose is one of the blood sugar-lowering drugs that work to inhibit the action of the enzyme a-glucosidase which works to inhibit the absorption of carbohydrates by inhibiting disaccharide enzymes in the intestine. Where rootbose acts as an inhibitor of the enzyme sukrase, the action of this enzyme will be reversibly inhibited competitively, so that not all sucrose can be hydrolyzed to glucose and fructose and a decrease in glucose absorption [12].

Gelidium sp. is one type of macro algae that has been studied for its bioactive content. The results of Elsie and Dhanarajan showed that *Gelidium* sp. has components of bioactive compounds alkaloids, saponins, phytosterols, phenolic compounds, and flavonoids. Another mechanism that may be caused by epikatekin, flavonoid compounds which belong to a group of collective compounds called catechins has been reported to have insulin-like properties [13]. Flavonoids are the largest group of polyphenol

compounds. Flavonoids are very effective for use as antioxidants. Flavonoid compounds can prevent cardiovascular disease by reducing the rate of fat oxidation. Some studies also state that flavonoids can reduce hyperlipidemia [14]. Based on previous research, the active substance content which has hypoglycemic effects is flavonoids, alkaloids and saponins. Alkaloids, flavonoids and saponins increase antioxidant enzyme activity and are able to regenerate damaged pancreatic β cells. Insulin deficiency can be overcome. In addition, alkaloids, flavonoids and saponins can also work on insulin receptor sensitivity which has beneficial effects for people with diabetes mellitus [10].

Polyphenols are able to act as natural inhibitors in hydrolyzing carbohydrates from enzymes so they can help inhibit increased blood glucose levels [3]. Positive results of the presence of phenol compounds are indicated by the formation of green. Phytochemical test results showed that the extract contained phenol compounds. Extracts obtained were then tested for identification of polyphenols by adding $FeCl_3$. Total phenol identification results can be seen in Figure 1 and Table 2.

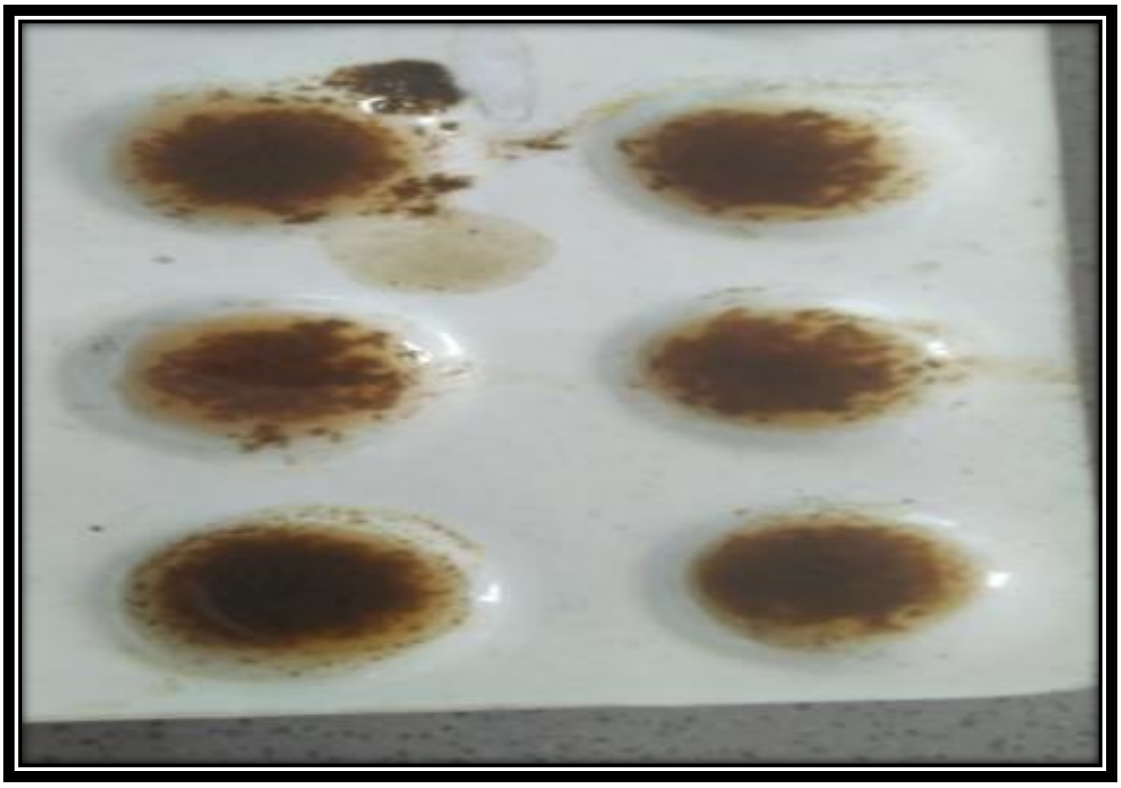

Figure 1: Identification of total phenol with $FeCl_3$ reagent.

Table 2: Effect of *Gelidium* extract on glucose tolerance activity in mice.

Treatment Group	Blood glucose level (Mean ± SD) (mmol/L) At every minute							
	0	**5**	**10**	**20**	**30**	**60**	**90**	**120**
Negative Control	93.2 ± 2.658	146.6 ± 9.19	164.6 ± 17.56	145.8 ± 15. 37	126.6 ± 8.37	111.6 ± 5.54	89.2 ± 0.59	113.4 ± 10.5
Agarose 1.25 mg/20 grams BB Mice	117.2± 8.07	121 ± 2.26	123.6 ± 1.72	122.4 ± 4.92	111.8 ± 1. 75	91 ± 3. 67	94.6 ± 1. 82	83.2 ± 3.0
Agarose 2.5 mg/20 grams BB Mice	92.2± 3.10	102.4 ± 10. 57	103.6 ± 9. 71	75 ± 16. 28	89.8 ± 8. 08	103 ± 1.7	86 ± 2. 02	81.2 ± 3. 9
Polyphenols 1.25 mg/20 grams BB Mice	96.6 ± 1.138	122 ± 1. 812	124.8 ± 0. 323	114 ± 1. 153	92.4 ± 6. 92	92.4 ± 3.045	98.8 ± 3. 69	96.2 ± 2. 80
Polifenol 2.5 mg/20 gram BB Mencit	97 ± 0.95	144 ± 8.02	132 ± 2.98	113 ± 0.70	160 ± 23.30	99 ± 0.093	90 ± 0.24	87 ± 1.32
Positive Control	98.67 ± 0.212	120.3 ± 2.57	103.3 ± 9.85	98.3 ± 3.63	66.7 ± 4.28	98.3 ± 0.406	84.6 ± 2.65	78.6 ± 5.07

All groups showed baseline blood glucose levels that were almost the same (0 minutes) and experienced an increase in blood glucose levels in the 5th minute. Then decreased in the 20th minute determination of sampling schedule is needed in the study in order to obtain a good profile of blood glucose levels. This test aims to ensure that in sampling the researchers will not lose critical points in the study. Blood glucose levels of the group treated with all extract doses were significantly reduced after 20 minutes compared to each time from the control group. Meanwhile, the group Akarbose group showed a significant decrease in blood glucose after administration of glucose. The results are expressed as an average ± standard deviation of seven mice. The values in parentheses show the percentage of blood glucose reduction relative to 0 minutes of each treatment group. The area under the glucose curve (AUC Glucose) for each group was calculated to determine the increase in blood glucose concentrations from 0 minutes to 120 minutes (Table 3).

Table 3: AUC data and percent effectiveness.

Treatment Group	AUC	% Effectiveness
Negative Control	13875.5	0
Positive Control	10610	23.53%
Agarose 1,25 mg/20 grams BB Mice	12101	12.79%
Agarose 2,5 mg/20 grams BB Mice	10953.5	21.06%
Polyphenols 1,25 mg/20 grams BB Mice	11954.5	13.84%
Polyphenols 2.5 mg/20 grams BB Mice	11306	18.52%

In this study, the administration of agarose doses of 2.5 mg/20 grams of BB mice was able to cause a decrease in blood glucose levels of mice which was seen from the value of% effectiveness that was 21.06. Whereas in the polyphenol dose group that is 2.5 mg/20 grams BB mice are able to reduce blood glucose levels with a% effectiveness value of 18.52%. On the positive control using rootbose has a effectiveness of 23.53%, this indicates that the administration of agarose doses of 2.5 mg/20 grams and a dose of polyphenols that is 2.5 mg/20 grams BB to the positive control has a% effectiveness of glucose reduction blood that is not so much different. So that it can be said of content both from agarose and polyphenols can reduce blood glucose levels. That the results of the measurement of blood sugar levels in white male mice (*Mus musculus*) using *Gelidium sp.* extract with various variation (Figure 2).

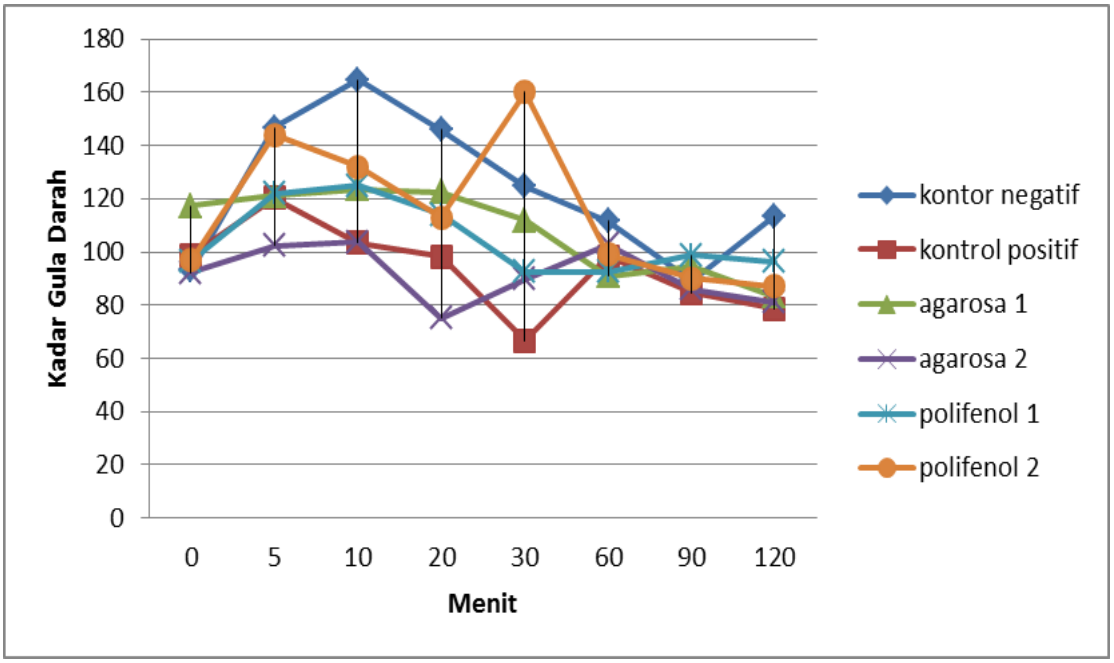

Figure 2: Anti-hyperglycemia test graph.

The results of the reduction in blood glucose levels in glucose induced mice after 8 hours of treatment were continued to the SPSS 18 statistical test two-way ANOVA on fasting blood glucose levels between each group. As for the homogeneity and normality test results using One-Sample Kolmogorov-Smirnov, the p value> 0.05 means that the data is homogeneous and normal. From the results of the SPSS test, negative control (Na.CMC) does not have a hypoglycemic effect, while positive control has the most effect of reducing optimal blood glucose levels. There was a significant or significant difference between group control of negative controls on positive control and agarose 2 (dose of agarose 2.5 mg/20 kg BW) marked by a significance

value of p <0.05. However, there were no significant or meaningful differences when compared between negative control of agarose 1 (dose of agarose 1.25 mg/20 kg BW) and dose of polyphenols 1 and 2 with a significant value of p>0.05.

CONCLUSION

Gelidium sp. extract has the potential as antihyperglycemia, because it is statistically different in meaning (p> 0.05). The best extract that can reduce blood glucose levels in mice induced by glucose is agarose extract dose 2 is 2.5 mg/20grBB with a higher percentage of effectiveness than polyphenol extract.

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