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Antidiabetic potential and secondary metabolites screening of mangrove gastropod *Cerithidea obtusa*

Reni Tri Cahyani[1*], Sri Purwaningsih[1], Azrifitria[2]

[1]*Department of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Dramaga, Bogor 16680, Indonesia*

[2]*Study Programme of Pharmacy, Faculty of Medicine and Health Sciences Universitas Islam Negeri Syarif Hidayatullah, Jakarta 15412, Indonesia*

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ABSTRACT

Objective: To study the possible effects of *Cerithidea obtusa* extract as antidiabetic and to screen the secondary metabolites presence.
Methods: Antidiabetic activity of *Cerithidea obtusa* extract was measured *in vitro* using α-glucosidase inhibition method. Whereas, secondary metabolites screening was measured qualitatively.
Results: The methanol extract had antidiabetic activity (IC_{50} = 36.40 mg/mL). However, the control drug acarbose had significantly higher antidiabetic activity (IC_{50} = 0.32 mg/mL). Secondary metabolites screening showed the presence of alkaloids, flavonoids, triterpenoids and saponins.
Conclusions: The methanol extract had antidiabetic activity and the presence of alkaloids, flavonoids and triterpenoids might contribute to the activity.

1. Introduction

Diabetes mellitus is a chronic progressive metabolic disorder characterized by hyperglycemia condition due to defects in insulin secretion, insulin action, or both. World Health Organization estimates that more than 346 million people worldwide have diabetes. This number is likely to more than double by 2030 without any intervention. Almost 80% of diabetes deaths occur in low and middle-income countries[1]. Type 2 diabetes is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes. Type 2 diabetes is a heterogenous disorder caused by a combination of genetic factors related to impaired insulin secretion and insulin resistance and environmental factors such as obesity, over eating, lack of exercise, and stress as well as aging[2]. One of the established therapeutics to treat type 2 diabetes is to control blood glucose levels after eating. Decreased blood glucose levels after eating can be done with delaying the absorption of glucose by inhibiting the enzyme α-glucosidase activity. With the presence of these inhibitors, digestion time becomes longer and absorption of glucose in the body can be slowed so very high glucose levels after eating can be controlled.

Marine gastropods are one of the marine organisms of the phylum Mollusca which is widely spread. These organisms are able to adapt well to changes in environmental factors caused by the ocean tides, temperature and salinity[3]. Gastropods are molluscs having many defense mechanisms against their predators. The chemical defenses of these sessile organisms are built through the secretion of strongly acidic substances, glandular secretions or inbuilt bioactive compounds of their metabolism[4]. They are regarded as one of the important sources to obtain bioactive

*Corresponding author: Reni Tri Cahyani, Department of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Dramaga, Bogor 16680, Indonesia.
Tel: +6281288365637
E-mail: renitri_c@yahoo.com
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compounds such as antitumor, antimicrobial, anti-cytotoxic, antiinflammatory, antileukemia, antineoplastic and antioxidant[5]. Some of marine gastropods are commercially exploited for food. Certain species of the genus *Cerithidea* are commonly sold as seafood in Vietnam and other Asian countries[6]. They also have a high nutritional value, thus they are good to be used as a source of food. In addition to consumption, they have long been used as a medicinal ingredient, found to have antioxidant activity and anticancer activity[7-9]. The investigation of antidiabetic activity of *Cerithidea obtusa* (*C. obtusa*) extract was conducted *in vitro*. Ingredients from marine sources are expected to be used as a therapeutic alternative to control blood glucose levels after eating. This research aimed to study the possible effects of *C. obtusa* extract as antidiabetic and to screen the secondary metabolites presence.

2. Materials and methods

2.1. Collection of samples

Raw materials used in this study were *C. obtusa* obtained from Muara Angke market, Jakarta, Indonesia. The raw materials were brought to the laboratory alive, taken using cool box. The raw materials were then washed with clean water and separated for the shell, flesh and offal parts. Flesh part was then cut into small pieces to maximize the extraction process.

2.2. Extraction

Extraction was conducted by maceration method using single solvent, *i.e.* methanol (polar), ethyl acetate (semi-polar) and hexane (non-polar). The flesh of *C. obtusa* was weighed (75 g), then cut into small pieces, put in the Erlenmeyer flask. Afterward, the sample was added with 225 mL of solvent, then the Erlenmeyer flask was covered with aluminium foil. The sample was macerated and stirred using a shaker for 24 h. Extract solution obtained was filtered with filter paper to separate the filtrate and the residue. The residue was resoaked with 225 mL of solvent for 24 h and filtered using filter paper, then the same process repeated. The filtrate was evaporated with rotary evaporator at 40 ℃.

2.3. Antidiabetic assay

Antidiabetic activity was measured *in vitro* using α-glucosidase inhibition method[10]. About 1 mg of α-glucosidase enzyme was dissolved in 100 mL of phosphate buffer (pH 7.0) containing 200 mg of bovine serum albumin. Before use, 1 mL of enzyme solution was diluted 25 times with phosphate buffer (pH 7.0) containing 200 mg of bovine serum albumin. The stock solution of extract was prepared by dissolving 50 mg extract in 1 mL of dimethyl sulfoxide with ultrasonic bath sonicator. A total of 10 μL extract was then added to a test tube containing 25 μL of *p*-nitrophenyl α-D-glucopyranoside (P-NPG) (20 mmol/L) and 50 μL of phosphate buffer (pH 7.0). The reaction was started by an addition of 25 μL of enzyme solution and 25 μL of phosphate buffer followed by incubation for 30 min at 37 ℃. The reaction was stopped by an addition of 100 μL of Na_2CO_3 (200 mmol/L). The solution absorbance was measured using ELISA reader at 410 nm. The percentage of α-glucosidase inhibition was determined using this equation:

Alpha-glucosidase inhibition (%) = [(B1 - B0) / (S1 - S0)] × 100

Where B1 is the absorbance of the blank, B0 is the absorbance of the blank control, S1 is the absorbance of the sample and S0 is the absorbance of the sample control. The reaction system of α-glucosidase inhibition in this study was presented in Table 1.

Table 1

The reaction system of α-glucosidase inhibition.

Solution	B1 (μL)	B0 (μL)	S1 (μL)	S0 (μL)
Dimethyl sulfoxide	10	10	-	-
Sample	-	-	10	10
Phosphate buffer	50	50	50	50
P-NPG	25	25	25	25
Enzyme 0.04 units/mL	25	-	25	-
Phosphate buffer	-	25	-	25
Incubated at 37 ℃ for 30 min				
Na_2CO_3 200 mmol/L	100	100	100	100

2.4. Secondary metabolites screening

2.4.1. Detection of alkaloids

About 0.1 g of sample was added with 3 drops of 10% ammonia and 1.5 mL of chloroform, then shaken. Chloroform layer was obtained and dissolved in 1 mL of 2 Eq/L sulfuric acid, then shaken. The extract was tested with three reagents *i.e.* Meyer, Wagner and Dragendorff. Positive test was characterized by the formation of yellowish white in Meyer's test, brown precipitate in Wagner's test and orange red in Dragendorff's test[11].

2.4.2. Detection of flavonoids

About 0.1 g of sample was added with 10 mL of hot water, boiled for 5 min, then filtered. About 10 mL filtrate was added with 0.5 g of Mg powder, 1 mL of concentrated HCl, and 1 mL of amyl alcohol. The mixture was vigorously shaken. Positive test was characterized by the emergence of red, yellow, or orange colors in the amyl alcohol layer[11].

2.4.3. Detection of phenol hydroquinone

About 0.1 g of sample was extracted with 20 mL of 70% ethanol. About 1 mL resulting solution was pipetted and added with 2 drops of 5% $FeCl_3$ solution. The formation of green or blue-green color indicates the presence of phenolic compounds in

the sample[11].

2.4.4. Detection of steroids and triterpenoids

About 0.1 g of sample was added with 2 mL of chloroform in a test tube, then dripped into the drip plate, and allowed to dry. About 1 drop of Liebermann-Burchard reagent was added. The formation of red color indicates the presence of triterpenoid compounds and the formation of blue or purple color indicates the presence of steroids[11].

2.4.5. Detection of saponins

About 0.1 g of sample was added with 20 mL of distilled water, and then heated for 5 min. The solution was poured into a test tube while hot. A total of 10 mL of solution was pipetted, then shaken vigorously in vertical for 10 seconds. The presence of saponins is characterized by the formation of stable foam at 1-10 cm height for 10 min and the foam is not faded when added with 1 drop of 2 Eq/L HCl[11].

2.5. Statistical analysis

The experimental design was conducted using completely randomized design with three replications. Data were analyzed by ANOVA *F* test at 95% confidence level. If the ANOVA *F* test gave different effects, then the analysis would be continued with Duncan's test[12].

3. Results

3.1. Screening of antidiabetic activity

The screening result of antidiabetic activity was presented in Figure 1. All samples were demonstrated antidiabetic activity with different levels at a concentration of 25 mg/mL. The methanol extract of *C. obtusa* had the highest percentage of α-glucosidase inhibition at 40.10%, while hexane and ethyl acetate extracts had a very low percentage of α-glucosidase inhibition at 2.01% and 0.64%, respectively.

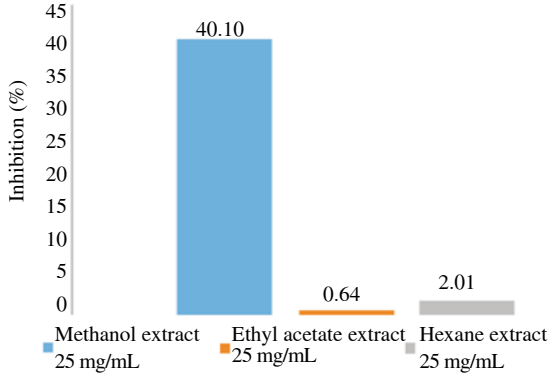

Figure 1. Antidiabetic activity of *C. obtusa* extract.

3.2. Antidiabetic activity

According to the ANOVA *F* test on the methanol extract of *C. obtusa*, significant value of 0.000 ($P < 0.05$) was obtained, which indicated that the difference in the extract concentrations (10, 20, 30, 40 and 50 mg/mL) affected the α-glucosidase inhibition with the percentages of 12.19%, 33.97%, 43.25%, 54.48% and 57.91%, respectively. The IC_{50} value was 36.40 mg/mL. A further Duncan's test result showed that there was significant difference in the α-glucosidase inhibition at various concentrations of the extract.

According to the result from the ANOVA *F* test in acarbose, significant value of 0.000 ($P < 0.05$) was obtained, which indicated that the difference in acarbosa concentrations (0.1, 0.5, 1, 5 and 10 mg/mL) affected the α-glucosidase inhibition with the percentages of 23.83%, 63.20%, 75.98%, 92.36% and 96.05%, respectively. The IC_{50} value was 0.32 mg/mL. A further Duncan's test result showed that there was significant difference in the α-glucosidase inhibition at various concentrations of acarbose.

The correlation between the concentration of the methanol extract of *C. obtusa* with the percentage of α-glucosidase inhibition and the correlation between the concentration of acarbose with the percentage of α-glucosidase inhibition were presented in Figures 2 and 3. Based on Figure 2, there was a correlation between the concentration of the extract with the percentage of inhibition, where an increased concentration of extract was followed by an increased α-glucosidase inhibition. The curve was formed by $y = 28.821 \ln(x) - 53.599$ equation, generated IC_{50} value of 36.40 mg/mL. Acarbose as a positive control, the curve was formed by $y = 15.306 \ln(x) + 67.478$ equation which generated IC_{50} value of 0.32 mg/mL (Figure 3).

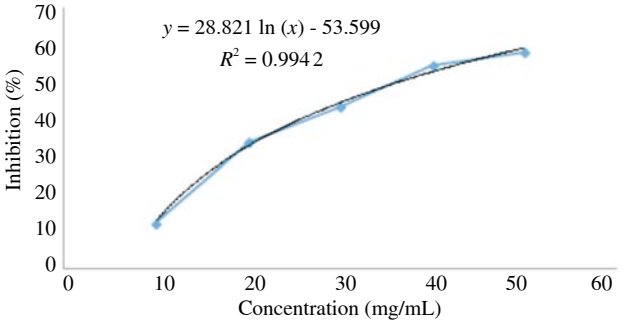

Figure 2. The relationship between the concentration of the methanol extract of *C. obtusa* with the percentage of inhibition of α-glucosidase.

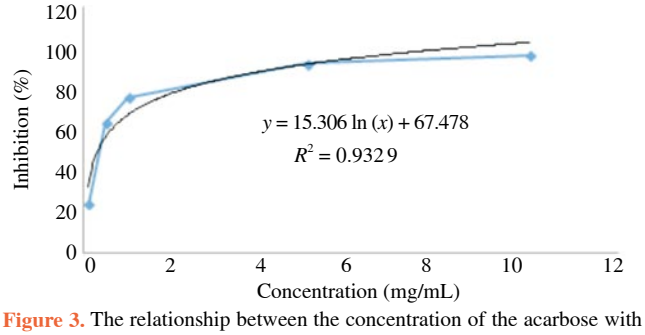

Figure 3. The relationship between the concentration of the acarbose with the percentage of inhibition of α-glucosidase.

3.3. Secondary metabolites screening

Secondary metabolites screening of the methanol extract of *C. obtusa* was presented in Table 2. Based on Table 2, the methanol extract of *C. obtusa* contained alkaloids, flavonoids, triterpenoids and saponins.

Table 2

Secondary metabolites screening of methanol extract of *C. obtusa*.

Compounds	Presence
Alkaloids	+
Flavonoids	+
Phenol hydroquinones	-
Triterpenoids	+
Steroids	-
Saponins	+

+: Present; -: Absent.

4. Discussion

Diversity of living marine resources provide new hope for drug discovery from natural sources. Marine organisms living continuously in the marine environment will experience a variety of pressures, causing organisms produce bioactive compounds for self-defense of the environment and predation. Research of bioactive compounds from marine organisms has now been growing rapidly and producing a variety of compounds for potential new drug substance. Molluscs are one of the marine organisms, which potential as natural sources produce many bioactive coumponds such as antioxidant[13], antidiabetic[4,14], antiinflammatory[14] and antibacterial[14,15].

Antidiabetic activity can be measured *in vitro* by α-glucosidase inhibition method. Alpha-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) is an enzyme that catalyzes the breakdown of 1.4 α-glycoside bond on the non-reducing ends of maltooligosaccharide by releasing β-D-glucose. This enzyme can also hydrolyze 1,6-α-D-glucosidic bond slowly, thus it can continue α-amylase work, which is further hydrolyze α-limit dextrins to glucose[16]. Inhibition of α-glucosidase work is based on the substrate breakdown to produce a colored product, in which its absorbance is measured during a specific time period. After being hydrolyzed, P-NPG substrate will turn into α-D-glucose and *p*-nitrophenol which has a yellow color. The resulting yellow color is an indicator of the inhibitor ability to inhibit the reaction. When the ability of inhibitor to inhibit α-glucosidase work is greater, the resulting yellow color of the solution will be paler compared to the solution without inhibitor[17]. Inhibition of carbohydrate hydrolysis enzymes such as α-glucosidase become an important strategy in the control of blood glucose levels in the therapeutics of type 2 diabetes.

Based on the screening of antidiabetic activity of *C. obtusa* extract, the methanol extract of *C. obtusa* had the highest percentage of α-glucosidase inhibition compared with ethyl acetate extract and hexane extract of *C. obtusa*. This was supported with the research by Sadhasivam *et al*.[4], which stated that the methanol extract of *Aplysia* sp. showed the highest α-amylase inhibitory activity at 93%, *Bursatella leachii* at 70.60% and *Kalinga ornata* at 49.03%, while on the acetone extract, α-amylase inhibitory activity showed the percentage below 10%. This suggested that α-glucosidase inhibitory activity of *C. obtusa* was active on polar compounds. Methanol is a polar compound that is widely used in the isolation of organic compounds from natural materials as it can dissolve the entire class of secondary metabolites[18]. So, the methanol extract was the best extract and can be used in the next step compared with acarbose as a positive control.

Antidiabetic activity of methanol extracts of *C. obtusa* was measured with various concentration and generated IC_{50} value of 36.40 mg/mL. While, acarbose generated IC_{50} value of 0.32 mg/mL. When compared to the IC_{50} value of acarbose, the inhibition rate on methanol extract of *C. obtusa* was still very low. This was supported with the research by Ravi *et al*.[14], which stated that the methanol extracts of marine gastropods (*Hemifusus pugilinus* and *Natica didyma*) showed low α-glucosidase inhibitory activity with IC_{50} values on the methanol extract of *Hemifusus pugilinus* at 20.27 mg/mL while on the methanol extract of *Natica didyma* at 56.44 mg/mL.

In this study, acarbose as a α-glucosidase inhibitor was tested *in vitro* to inhibit the activity of α-glucosidase with the inhibition percentage at nearly 100% at a concentration of 10 mg/mL. Acarbose is used to inhibit the enzyme that works to break down carbohydrates into glucose. Acarbose binds the enzyme reversibly and competitively. Acarbose's task is to inhibit the enzyme that works to hydrolyze polysaccharides in the small intestine. Acarbose does not stimulate insulin secretion by pancreatic β cells, thus it does not cause hypoglycemia unless it is given together with other oral hypoglycaemic drugs or with insulin[19].

The compounds produced in *C. obtusa* extract were the yield of secondary metabolites. Secondary metabolites are compounds that are synthesized by living organisms not to meet their basic needs, but to withstand extreme environmental conditions. Several metabolites, in particular their structures and biological activities have been isolated from marine animals. These metabolite compounds have potential as a drug. Bioactive compounds that are interesting to be studied are generally isolated from marine sponges, jellyfish, coral reefs, mollusks, echinoderms and crustaceans. Bioactive compounds that have been isolated from marine animals are steroids, terpenoids, isoprenoid, nonisoprenoid, quinone and nitrogen heterocycles[20].

Several studies have reported the antidiabetic activity from secondary metabolites. Alkaloids may decrease the activity of transaminases and creatinine production in diabetic mice[21]. Flavonoids are compounds known as α-glucosidase inhibitors. The

mechanism of flavonoids inhibition against α-glucosidase enzyme is through bond hydroxylation and substitution on the ring β. This inhibitory principle produces hydrolysis of carbohydrates and delays glucose absorption and inhibits the metabolism of sucrose into glucose[22]. Flavonoids have been reported to have antiplatelet aggregation and aldose reductase inhibitory activities, which may have helped to reduce the severity of the diabetic syndrome[23]. Flavonoid and triterpenoid could be responsible for the good clinical effects on type 2 diabetes through targeting oxidative stress and postprandial hyperglycemia[24]. It can be concluded that the presence of alkaloids, flavonoids and triterpenoids in methanol extract of *C. obtusa* might contribute to antidiabetic activity though its activity is still low when compared with acarbose.

Conflict of interest statement

We declare that we have no conflict of interest.

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