Antioxidant Potential of the Shell of Razor Clams (Solen spp) in Antidiabetic Mellitus Type II Therapy

Mirwa Adiprahara Anggarani¹, Erlix Rakhmad Purnama² dan Jihan Shofwatul Islam Dalilah Aziz³

¹Departement of Chemistry, Faculty of Mathematics of Natural Sciences, Surabaya State University
²Departement of Biology, Faculty of Mathematics of Natural Sciences, Surabaya State University
Jl. Ketintang Timur PTT Gg. V, Ketintang, Kec. Gayungan, Kota Surabaya, Jawa Timur 60231
jihanshofwatul@gmail.com

Abstract. Razor clams (Solen spp) are one type of bivalves found in Madura waters and suggested to have antioxidant bioactive compounds. This research was conducted to determine the chemical compounds and antioxidant activity as well as testing blood sugar levels on mice that are conditioned to suffer from diabetes mellitus. The extraction method used is stratified by using three solvents which have different polarity. The test being done was phytochemistry tests to determine the bioactive compounds contained in the shell of razor clams, DPPH method to determine antioxidant activity, and blood sugar test in mice. The crude extracts of the shell of razor clams contained alkaloid and saponins. The highest antioxidant activity (IC₅₀) was found from the shell of razor clams extracted with ethanol was 489.56 ppm. The results of the blood sugar test showed that the addition/consumption of antioxidant extract of razor clams of 0.0063g/20g body weight (BW) was able to reduce blood sugar levels of mice with diabetes mellitus type 2.

Key words: Antioxidant activity, razor clams (Solen spp), bioactive compound, blood sugar levels

1. Introduction

From ancient times, it was not noticed that the people of Indonesia relied heavily on their surroundings to provide for life, such as food, clothing, furniture, and even beauty. Additionally, the medicinal use of natural substances has long been known and is being used in addressing health issues. Knowledge of the medicinal benefits of nature was obtained on the basis of the experience and legacy of ancestors who were still handed down from one generation to the next. In recent years, people in Indonesia have a tendency to return to nature and thus make more use of traditional ingredients/drugs, even though new modern drugs are emerging in the market [1].

Diabetes Mellitus (DM) is a syndrome characterized by high blood sugar (hyperglycemia) endured because of production difficulties, the secretion of insulin or insulin resistance. The disease is significant because of the increasing number of sufferers, currently projected globally to be 200 million [2]. DM is distinguishable for DM type 1 (DM-1) or insulin-insulation diabetes mellitus (IDDM) and DM type 2 (DM-2) or noninsulin-supported diabetes mellitus (NIDDM). Research shows DM-1 frequency of roughly 10-20% and DM-2 is 80-90% of all diabetics. In DM-2 besides insulin deficiency, along with insulin resistance, insulin cannot regulate blood sugar levels for the body's optimal needs, contributing to the increase in blood sugar levels. DM-2 usually appears after 30 to 40 years of age, even at 50 or 60 [3].

This hyperglycemia in diabetics leads to an imbalance between antioxidants and free radicals. Antioxidant activity is a parameters that can illustrate the extent of the percentage of a material's ability in free radicals. The antioxidant and free radical imbalance in diabetes sufferers may result from molecular modification in various tissues. This is the first cause of oxidative stress.
that will result in oxidative damage in complications of diabetes that will leave the condition for diabetics worse [4]. Such complications include macrovascular disease (cardiovascular disease and hypertension) and microvascular disease (diabetes nephropatik, retinopathy and neuropathy). And therefore, antioxidant intake has a protective role to the progress of diabetes [5].

Increased antioxidial supply may help in the prevention of clinical complications of diabetes mellitus. This is because antioxidants can prevent oxidative stress in diabetics reaching mellitus. Many of the antioxidant products produced today, some of which are carcinogenic to the reproductive system, making them dangerous to consume by some with mellitus diabetes that is incompatible with synthetic antioxidants. In natural antioxidants, most people are familiar with the source of the antioxidant, although Marine life also has little known value as an antioxidant.

Antioxidants are found naturally in all foodstuffs, both land and water. Most Marine biographies contain bioactive components that act as antioxidants. The traditional Marine biota contains antioxidants are Amusium pleuronectes [6], Cerithidea obtuse [7], Anodonta woodiana Lea. [8], Cymodocea sp. [9], Fasciolaria salmo [10], Discodoris sp. [11], Atactodea striata [12].

Mechanisms of various natural substances potentially containing antioxidants in lower blood sugar levels in diabetes mellitus: 1) Have the ability to protect the intestines by forming a layer that can inhibit glucose intake and thus prevent blood glucose from overharvesting. 2) Speeding up blood circulation and speeding out kidney filtration and excretion so that urine production increases and glucose levels decrease [13].

The razor clams (Solen spp) is a member of the solenidae family. These shells have a valve that opens to one another, thin and elongated [14]. The shellfish habitat is on the muddy sand with weak seawater currents. The razor clams is one of many bivalves found in Madura waters but has not been used optimally. Most communities use knife shells as food because they contain complete amino acids, which are 9 essential amino acids and 8 nonessential amino acids [15].

In view of the importance of the antioxidant's function for the human body, the razor clams may be used as a source of the antioxidant based in the natural world of Marine life. Further, they can be used as a supplement to diabetes mellitus by lowering blood sugar levels in sufferers. Based on the established background, it is necessary to study the potential for antioxidant extract of the shell of razor clams in antidiabetes type 2 therapy, as well as the concentration of the crude extract of antioxidant from the most effective mistress of blood sugar. The results of the study are expected to add to the public's information on the benefits of the shell of razor clams and as an alternative to antidiabetic type 2 therapy.

2. Materials and Methods
2.1 Materials and Tools

The substance used in this study is the scallop, aquades, chloroform p.a, ethyl acetate p.a, methanol p.a., crystals 1.1-diphenil-2pixyzil (DPPH), fragmentation antioxidants BHT, sulfuric acid 2 N, Wagner reduction, Meyer reduction, Dragendroff reduction, chloroform, acetate anhidra, high sulfuric acid, magnesium, alcohol amyl, hot water, HCl 2 N, ethanol 70%, FeCl₃ 5%.

An instrument used is the analtic scales, digital scales, knives, porcelain cups, blender, desikator, reaction tube, Erlenmeyer’s glass, soxhlet, heater, destilator, burettor, foaker, orbital shaker, rotary vacuum evaporator, micropipet, spectrocopys UV-Vis.

2.2 Sample Preparations

The sample that would be prepared first is the razor clams (Solen spp) obtained from the Pamekasan Madura waters. The razor clams used in this study averages 3-4 cm in length and 0.5-1 cm in width. The first step taken was the razor clams's shellfish separated from the shell. The meat is then washed clean with running water, and then sun dried for 35 days until dry
meat is less than 12%. The dry razor clams that is then smoothed until obtained a dry powder, which is done to facilitate storage and extraction process. The number of grains of dry razor clams shells needed in the study is as much as 500 grams.

2.3 Extraction Active Material

Extraction of active material is done according to quinn procedure (1988) in darusman et al., (1995). Extraction uses three solvents based on chloroform p.a. (non polar), etil acetate p.a. and methanol p.a. (polar). The first step taken was a 25 g dry powdered knife clam at 100 mL of chloroform p.a. Then the results of the mase ration were filtered using whatman's paper so it got filtrat and the residue. The next step is that an earlier chloroform residue was reproduced in 100 mL of ethyl asetate p.a. the process takes place in an orbital shaker at 8 rpm for 48 hours. Then the results of the mase ration were filtered using whatman's paper so it got filtrat and the residue. The next step is the ectile acetate residue which had been recovered earlier in 100 mL of methanol p.a. this process takes place in an orbital shaker at 8 rpm for 48 hours. Then the results of the mase ration were filtered using whatman's paper so it got filtrat and the residue. The final step of filtrate obtained from individual solvents is evaporated by using the vacuum vacuum rotary evaporator at a temperature of 50°C evaporators to the solvent splits with extract.

2.4 Chemical Compound Test

A chemical compound test was made to detect components of bioactive at the rough extract of razor clams shells that had antioxidant activity. Phytogenic tests include alkaloid, steroid/triterpenoid, flavonoid, saponin, phenolic and tannin. In an alkaloid test, a number of samples were dissolved in a few drops of sulfuric acid 2 N and then tested with dragendorff reducer, meyer's and Wagner reduction. Tests tested positive for alkaloid deposits in the form of a yellowish white deposit with the processing of meyer, a brown deposit with wagner and a red to orange deposit with a dragendorff derivative. In a steroid/triterpenoid test, as much as 0.5 grams of sample is dissolved by 2 mL of chloroform in a dry reaction tube. Then add 10 drops of anhidra and 3 drops of sulfuric acid. Tests tested positive for steroids/triterpenoid if the originally red solution turned green and blue [16].

On the flavonoid test, 0.5 grams of sample is added 0.10 milligrams of magnesium powder and 0.40 mL of chloric acid (37% and ethanol 95% by the same volume). Then another 4 mL of alcohol and a shake. Tests tested positive for flavonoid when the red, yellow or orange lining an alcohol amyl was formed. At the saponin test, the sample was mixed with hot water and shaken. Test positive for saponin if a firm foam develops for 30 minutes and is not lost in a 1 drop of HCL 2 N [16].

In the saponin test, 1 mL of sample is put into a reaction tube, then 5 mL of hot water is added and 2 drops of 2N HCl are added and shaken vigorously. After that it was observed the formation of foam after settling for 10 minutes. Positive samples contain saponins if there is foam with a lot of intensity and is consistent for 1 minute.

2.5 Antioxidant Activity Test

Test of the antioxidant extract of razor clams was done by DPPH (Blois, 1985 in Hanani et al., 2005). Extract of coarse razor clams is dissolved in methanol p.a. to be obtained concentrations of 200, 400, 600 and 800 ppm. Synthetic antioxidants BHT is used as compared and positive controls are diluted in methanol p.a. vents with concentrations of 2, 4, 6 and 8 ppm. DPPH solution is created by dissolving DPPH crystals in methanol p.a., with a 1 mM concentrating. DPPH 1 mM solution is administered in a low temperature and is protected from sunlight.
The antioxidant activity test, the extract and antioxidant solution of BHT are each taken 4.50 mL and inserted into 2 different test-tubes. Then add 500 µL of DPPH 1 mM to each vial. The solution is allowed to react for 30 minutes at temperature 37°C nutrients then measured its absorbers using UV-Vis spectrophotometer on a wavelength of 517 nm. In addition, blanko absorption absorption (absorption) by applying between 4.50 ml [4.50 mL] of methanol solvents and 500 µL of DPPH 1 mM should also be measured for use in inhibisi percent counts. The results of antioxidant activity are expressed in percent of inhibisi, which is calculated in formula:

\[ \text{% inhibisi} = \frac{\text{blanko absorption} - \text{sample absorption}}{\text{blanko absorption}} \times 100\% \]

2.6 Blood Sugar Test

Testing for blood sugar levels was performed in vivo on a male white mouse of approximately 2-3 months at a weight of 100-200 g. Treatment began with the white mouse was cured for eight hours and measured early glucose levels. Then the white mouse is induced by aloxins with 110 mg/kg of body weight in an intraperitoneal manner. In the next step the mice were divided into 4 groups with different treatments. In the first group, mice were given 1% Na-CMC. In the second group, mice were given a knife shell shell extract of 0.0063g / 20g BW. In the third group, mice were given extracts of shells by 0.0126g / 20g. In the fourth group, mice were given an extract of a knife shell of 0.0252 g / 20g BW. During the study, all mice were observed for their blood sugar levels. Post-treatment blood glucose levels were measured every 7 days, namely the 7th day and the 14th day after treatment.

3. Result and Discussion

3.1 Chemical Compounds

The results of the extraction of multi-level knife shells with chloroform, ethyl acetate and methanol solvents showed that the polar methanol solvent produced the highest extract yield of 12.786%. While non-polar chloroform produces extract yield of 3.65%, and ethyl acetate which is semi-polar produces extract yield of 0.58%.

Testing of chemical compounds in razor clams (Solen spp) was carried out to determine bioactive compounds in materials that have potential as antioxidants. Screening of the bioactive component in each extract was carried out by phytochemical test methods which included testing of alkaloids, steroids/triterpenoids, flavonoids, saponins and phenol hydroquinone. The results of the chemical tests of crude extracts of the shell of razor clams (Solen spp) can be seen in Table 1

| Phytochemical Test | Type of Solvent | Chloroform | Ethyl Acetate | Ethanol | Standart (color) |
|--------------------|----------------|------------|---------------|--------|-----------------|
| Alkaloid:          |                |            |               |        |                 |
| a. Dragendorff     |                | +++        | +++           | +++    | Red or orange deposits |
| b. Meyer           |                | -          | -             | -      | Yellowish white deposits |
| c. Wagner          |                | +++        | +++           | +++    | Brown sediment |
| Steroid/triterpenoid|               | +          | +             | +      | Change color from red to blue/green |
| Flavonoid          |                | -          | -             | -      | The amyl alcohol layer is red/yellow/green |
| Saponin            |                | +++        | +++           | +++    | Foam formed |
| Phenolic           |                | +          | +             | +      | Green or blue color |

Table 1. The results of the chemical tests of crude extracts of the shell of razor clams (Solen spp)
Antioxidant-rich compounds are found in alkaloids, steroids, flavonoids and saponins (Kannan et al., 2009). Based on Table 1 it can be seen that the bioactive compounds of alkaloids and saponins are found in the three solvents used. This shows that the shell of razor clams extract has the antioxidant activity which is relatively weak. Generally alkaloids are often used in the field of medicine [16]. According to Hanani, E., A. Mun’im (2005), alkaloids can function as antioxidants.

3.2 Antioxidant Activity

Antioxidant activity testing uses the DPPH method which will produce an IC$_{50}$ (inhibitor concentration 50%) value of each solvent and the concentration used. This IC$_{50}$ value states the amount of concentration of extracts and types of solvents needed to reduce free radicals (DPPH) by 50%. The results of antioxidant activity tests on the shell of razor clams (Solen spp) can be seen in Table 2.

| Sample              | % Inhibition     | IC$_{50}$ (ppm) |
|---------------------|------------------|------------------|
| BHT                 |                  |                 |
| 2 ppm               | 12.55            |                 |
| 4 ppm               | 23.67            |                 |
| 6 ppm               | 79.37            |                 |
| 8 ppm               | 89.45            | 4.91            |
| Chloroform Extract  |                  |                 |
| 200 ppm             | 20.68            | 1119.37         |
| 400 ppm             | 29.87            |                 |
| 600 ppm             | 34.79            |                 |
| 800 ppm             | 40.15            |                 |
| Ethyl Acetate Extract|               |                 |
| 9.85                | 27.24            | 916.43          |
|                    | 24.95            |                 |
|                    | 47.70            |                 |
| Ethanol Extract     |                  |                 |
| 34.79               | 37.31            | 489.56          |
|                    | 67.71            |                 |
|                    | 70.90            |                 |

The results of antioxidant activity testing using the DPPH method showed that the shell of razor clams extract using chloroform solvent had an IC$_{50}$ value of 1119.37 ppm, while ethyl acetate extract was 916.43 ppm and ethanol extract was 489.56 ppm. When compared with the antioxidant activity of BHT which has an IC$_{50}$ value of 4.91 ppm, the results obtained from the three solvents include having weak antioxidant activity because it has a value greater than 200 ppm. A compound is said to be a very strong antioxidant if the IC$_{50}$ value is less than 50 ppm, strong if the IC$_{50}$ value is between 50-100 ppm, while if the IC$_{50}$ value is around 100-150 ppm, and is weak if the IC$_{50}$ value is around 150-200 ppm [18].

Antioxidant activity of the three solvents used, the shell of razor clams extract using ethanol solvent has a high antioxidant activity which is characterized by a small IC$_{50}$ value. This is because the extract tested is still in the form of a crude extract which is thought to contain other active compounds which do not have antioxidant activity, so it needs to be purified. Based on Table 2 it can also be seen that the higher the concentration of an ingredient, the percent inhibition in inhibiting free radical activity is also greater [18].

3.3 Blood Sugar Level

Blood sugar level testing was carried out to determine the effect of adding crude extracts of razor clams (Solen spp) to the blood sugar levels of mice that had been treated to suffer from diabetes mellitus. The test results obtained are decreased blood sugar levels by adding the shell of razor clams extract using ethanol solvent. Bioactive compounds contained in the extract using ethanol solvents are alkaloids and saponins. These alkaloids compounds play a role in reducing blood sugar levels of mice with diabetes mellitus. The result of the blood sugar levels of mice that received treatment can be seen in table 3.
Table 3. Blood sugar levels before and after the induction using extract of razor clams

| Dose               | Blood sugar (mg/dl) Before induction | Blood sugar (mg/dl) After induction | Decrease blood sugar |
|--------------------|--------------------------------------|-------------------------------------|----------------------|
| 0.0063g/20gBW      | 522                                  | 105                                 | 417                  |
| 0.0126g/20gBW      | 255                                  | 242                                 | 13                   |
| 0.0252g/20gBW      | 466                                  | 266                                 | 200                  |

From table 3, it seen that the best decrease in blood sugar levels at dose 0.0063g/20g body weight, while negative control, ie mice with alloxan inductoin has increase blood sugar level by 291 mg/dl. Mices treated with alloxan will experience pancreatic cell damage and cause persistent hyperglycemia. Alkaloids work from outside the pancreas by stimulating the utilization of peripheral glucose. Alkaloids work outside the pancreas, alkaloids stimulate the utilization of peripheral glucose, by increasing the glycolytic and glycogenic pathways, which simultaneously suppress the glycogenolysis and gluconeogenesis pathways. Through this mechanism, alkaloids can control blood glucose, so that rat blood glucose levels decrease.

4. Conclusion

The results of extraction using multilevel maceration method with three different types of solvents whose polarity results in different IC$_{50}$ values. The highest antioxidant activity was found in the extract of the shell of razor clams using ethanol solvent that is equal to 489.56 ppm with the content of alkanoid and saponins bioactive compounds. Alkaloids contained in the extract of the shells of razor clams act as antioxidants that can reduce blood sugar levels in mice with diabetes mellitus. The best reduction in blood levels is induction extract using dose 0.0063g/20g body weight.

5. Reference

[1] S. Pramono, “Kontribusi Bahan Obat Alam dalam Mengatasi Krisis Bahan Obat di Indonesia,” J. Bahan Alam Indonesia, no. 1, pp. 18–20, 2002.
[2] S. H. K. Kariadi, Peranan Radikal Bebas dan Antioksidan pada Penyakit Degeneratif Khususnya Diabetes Mellitus. Bandung: Bagian Penyakit dalam. Fakultas Kedokteran/RS Hasan Sadikin, 2001.
[3] J. M. R. Tiwari, A.K., “Diabetes Mellitus and Multiple Therapeutic Approaches of Phytochemicals: Present Status and Future Prospect,” Curr. Sci., vol. 83, no. 1, pp. 30–38, 2002.
[4] K. M. dan M. U. Nuttal SL, Dunne F, “Age-Independent Oxidative Stress in Elderly Patients with Non-Insulin Dependent Diabetes Mellitus,” Q J Med, no. 92, pp. 33–38, 1999.
[5] M. Franz, Medical Nutrition Therapy for Diabetes Mellitus and Hypoglicemia of Nondiabetic Origin. Philadelphia: WB Saunders Company, 2012.
[6] P. Suptijah and O. Yanuarizki, “AKTIVITAS ANTIOKSIDAN DAN KOMPONEN BIOAKTIF KERANG SIMPING (Amusium pleuronectes) Antioxidant Activity and Bioactive Compounds of Scallop (Amusium pleuronectes),” vol. 16, pp. 242–248, 2014.
[7] S. Purwaningsih, D. Teknologi, H. Perairan, and F. Perikanan, “Aktivitas Antioksidan dan Komposisi Kimia Keong Matah Merah (Cerithidea obtusa),” vol. 17, no. 1, pp. 39–48, 2012.
[8] E. Salamah, “Penapisan Awal Komponen Bioaktif dari Kijing Taiwan (Anodonta woodiana Lea) sebagai Senyawa Antioksidan,” vol. XI, no. 0251, pp. 119–128, 2008.
[9] E. Lamun and C. Sp, “Antioxidant Activity and Toxicity of Seagrass Cymodocea sp. Extracts,” no. January, 2018, doi: 10.21776/ub.jtp.2016.017.01.5.
[10] A. A. & A. A. Nurjanah, “Aktivitas Antioksidan dan Komponen Bioaktif pada Keong Ipong-Ipong (Fasciolaria salmo),” J. Pengolah. Has. Perikan. Indonesia, vol. 16, no. 1, pp. 22–29, 2011.
[11] B. M. & D. R. A. Nurjanah, L. Hardjito, D. Monintya, “Aktivitas Antioksidan Lintah Laut dari Perairan Pulau Buton Sulawesi Tenggara,” in Prosiding Seminar Nasional Pengolahan Produk dan Bioteknologi Kelautan dan Perikanan, 2009.

[12] A. Mutaqin, “Pengujuan Toksisitas Kerang Mas (Atactodea striata),” Institut Pertanian Bogor, 2009.

[13] W. Widowati, “Potensi Antioksidan sebagai Antidiabetes,” Jkm, vol. 7, no. 2, pp. 1–11, 2008.

[14] M. B. Jacobs, The Chemistry and Technology of Food. Jakarta, 1951.

[15] K. & S. R. Nurjanah, “Karacteristik Gizi dan Potensi Pengembangan Kerang Pisau (Solen spp) di Perairan Kabupaten Pamekasan, Madura,” J. Perikan. dan Kelaut., vol. 13, no. 1, pp. 41–51, 2008.

[16] J. Harborne, Metode Fitokimia Edisi kedua. Bandung: ITB, 1987.

[17] R. S. Hanani, E., A. Mun’im, “Identifikasi Senyawa Antioksidan dalam Spons Callyspongia sp. dari Kepulauan Seribu,” Maj. Ilmu Kefarmasian, vol. 2, no. 3, pp. 127–133, 2005.

[18] P. Molyneux, “The Use of The Stable Free Radical Dyhenylpicrylhydrazil (DPPH) for Estimating Antioxidant Activity,” Journals Sci. Technol., vol. 26, pp. 211–219, 2004.