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Submission date: 03-Jun-2020 02:33PM (UTC+0700)

Submission ID: 1336960242

File name: Artikel_pharmacognosy_Journal-Ace_Baehaki.pdf (571.36K)

Word count: 2294

Character count: 13020

Antidiabetic Activity with N-Hexane, Ethyl-Acetate and Ethanol Extract of *Halodule uninervis* Seagrass

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History

- Submission Date: 02-04-2020;
- Review completed: 28-04-2020;
- Accepted Date: 11-05-2020

DOI : 10.5530/pj.2020.12.

Article Available online

<http://www.phcogj.com/v12/i4>

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ABSTRACT

The purpose of this research was to observe the antidiabetic activity from N-hexane, ethyl-acetate and ethanol extract of *Halodule uninervis* seagrass. The antidiabetic activity with extract concentrations of 125 ppm, 250 ppm, 500 ppm, 1000 ppm and 2000 ppm from seagrass extract with ethanol solvent showed that it had the ability as an anti-diabetic agent stronger than seagrass extract with n-hexane and ethyl acetate solvents. Calculation of IC₅₀ of *Halodule uninervis* seagrass with ethanol extract was 74.99 ppm and ethyl acetate was 1,517,05 ppm. Glukobay as control was 0.27 ppm.

Key words: Antidiabetic, Extract, *Halodule uninervis*, Seagrass.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and a body imbalance in the metabolism of carbohydrates, fats and proteins. Treatment of diabetes mellitus can be done by giving synthetic drugs, but the use of synthetic drugs can have side effects on users. So the treatment of diabetes mellitus shifts to the use of herbal medicines from plants which are believed to be an α -glucosidase inhibitors.

α -Glucosidase enzyme released from the intestinal chorion epithelium is responsible for carbohydrate degradation. α -Glucosidase inhibitors fall into the third category of oral hypoglycemic agents.¹ Some α -glucosidase inhibitors, such as acarbose and voglibose obtained from natural sources, can effectively control blood glucose levels after food intake and have been used clinically in the treatment of diabetes mellitus.² So far, around 800 plants worldwide have demonstrated antidiabetic potential.³ Plants have traditionally been used for the treatment of diabetes mellitus, and they provide a great source of drugs and biological compounds.⁴

Seagrass is a group of flowering plants in the marine environment. Seagrasses have rhizomes, leaves, and true roots and live immersed in the sea which adapt in waters with high salinity.⁵ Habitat of seagrass habitats is rather sandy shallow waters and often found on coral reefs. The same seagrass species can grow in different habitats by showing different forms of growth and groups of seagrass species forming clear stand zones, whether pure or associated with several species.⁶

Seagrasses found in Indonesia have 7 genera, namely *Enhalus*, *Thalassia*, *Halophila*, *Halodule*, *Cymodocea*, *Syringodium*, and *Thalassodendrom*.⁷ *Halodule* seagrass is found from mid tidal to a depth of 20 m, generally at depths between 0-3 m in sublittoral lagoons and near coral reefs.⁸ Seagrass *Halodule* sp. the same as plants generally contain

bioactive compounds. bioactive compounds are active chemical compounds produced by organisms through the biosynthetic pathway secondary metabolites namely alkaloids, phenols, flavonoids, saponins, terpenoids, steroids, tannins, plobatamine and coumarin.⁹ According to Widuri¹⁰, flavonoids, steroids, tannins and saponins in ingredients can inhibit α -glucosidase activity.

There are many medicinal plants that have been known to be beneficial and can be used as antidiabetic agents.¹¹ One plant that has not been widely studied scientifically is used as an antidiabetic agent, namely seagrass *Halodule uninervis*. Therefore it is necessary to conduct further studies regarding the testing of antidiabetic activity with n-hexane, ethyl acetate and ethanol extract in *Halodule uninervis*.

MATERIALS AND METHODS

Preparation before extraction

Seagrass plants before extraction are prepared first: Seagrass washed with running water to remove foreign objects (rocks, sand, shells and so on). Then, it is dried using an oven with a temperature of 31.3°C for 3 x 24 h.

Extraction

Seagrass plants that have been prepared will be carried out with the following extraction process: seagrass powder is extracted by multilevel maceration using n hexane (nonpolar), ethyl acetate (semipolar), and 70% ethanol (polar) solvents respectively for 2x24 h. Searching is done three times. The extract obtained from the three types of solvents was concentrated with rotavapor, except for ethyl acetate solvents. Ethyl acetate extract was dried in a fume hood.

Antidiabetic activity test (Inhibition of α -Glucosidase Enzymes)

Inhibition of alpha glucosidase activity was tested according to Sancheti¹², the reaction mixture

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consisted of 50 μL 0.1 M phosphate buffer (pH 7), 25 μL 0.5 mM 4-nitrophenyl α -D-glucopyranoside (dissolved in 0.1 M buffer phosphate pH 7), 10 μL test sample (concentration of 500 mg mL⁻¹) and 25 μL α -glucosidase solution (stock solution of 1 mg mL⁻¹ in 0.01 M phosphate buffer pH 7 diluted 0.04 units mL⁻¹ with buffer phosphate pH 7). This reaction mixture is then incubated at 37 °C for 30 min. Then, the reaction is added 100 μL sodium carbonate 0.2 M solution, then the reaction is calculated absorbance using uv-vis spectrophotometry 410 nm. As a comparison, glucobay solution is used. The inhibition of α -glucosidase enzyme activity is determined by the formula:

$$\text{Inhibition (\%)} = ((\text{Abt}-\text{Ast})) / \text{Abt} \times 100\%$$

Where :

Abt = corrected absorbance of blanks

Ast = absorbance of the corrected sample

RESULTS AND DISCUSSION

Antidiabetic activity test (Inhibition of α -Glucosidase Enzymes)

The testing of antidiabetic activity is using the in-vitro inhibition of the α -glucosidase enzyme with p-nitrophenol- α -glucosidase as the substrate using seagrass of *Halodule uninervis* extract with n-hexane (nonpolar), ethyl acetate (semi-polar) and ethanol (polar) solvents as a test sample and glucobay solution as a positive control. Tests were carried out with various extract concentrations in order to determine the effect of various extract concentrations on enzyme inhibition. The concentrations of extracts used were 125 ppm, 250 ppm, 500 ppm, 1,000 ppm and 2,000 ppm.

The results of the percentage of inhibition of antidiabetic activity in samples of *Halodule uninervis* with ethanol solvents can be seen in Figure 1.

In Figure 1 it can be seen that the *Halodule uninervis* sample with ethanol solvent shows the smallest percentage inhibition value found at a concentration of 125 ppm which was 58.78% and the largest percentage inhibition value was at a concentration of 2,000 ppm which is 94.69%. This shows that the sample using ethanol solvent was an antidiabetic agent. Biocative compounds that play a role in the antidiabetic activity of flavonoids, saponins, steroids, and triterpenoids in phytochemical testing showed positive results found on seagrass. The results of the antidiabetic activity from extract of *Halodule uninervis* using ethyl acetate solvents are shown in Figure 2.

In Figure 2 it can be seen that the *Halodule uninervis* extract with ethyl acetate solvent shows that the sample with a concentration of 2,000 ppm has an antidiabetic inhibition of 18.08%. This shows that seagrass extract using ethyl acetate solvents has a weak inhibition in antidiabetic activity. This can be seen in percent inhibition, the sample can inhibit α -glucosidase > 2,000 ppm. Biocative compounds were identified in *Halodule uninervis* extract with ethyl acetate solvent extraction namely flavonoids, steroids, and triterpenoids while saponin compounds showed negative results. The results of the antidiabetic activity from glucobay (control) are shown in Figure 2.

Glucobay is a commercial antidiabetic drug that can inhibit α -glucosidase activity and is often recommended in diabetic patients. Testing of α -glucosidase activity using glucobay as a positive control with concentrations of 0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm and 10 ppm. Figure 4 can be seen that glucobay samples showed the smallest percentage inhibition value at a concentration of 0.1 ppm, which was 26.32%. For IC₅₀ values each solvent is found in Figure 4.

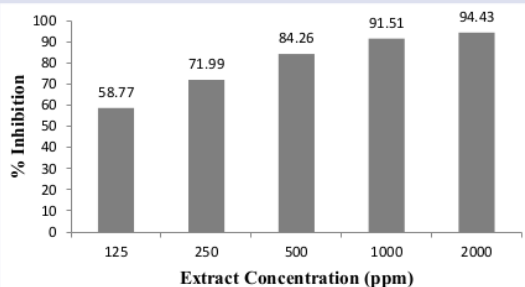


Figure 1: Antidiabetic activity of *Halodule Uninervis* extract with ethanol solvent.

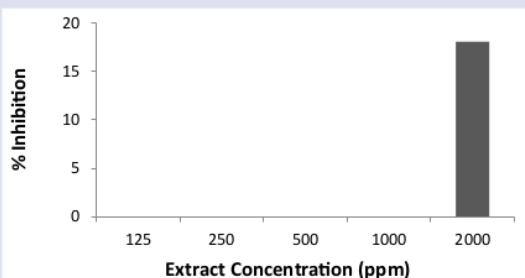


Figure 2: Antidiabetic activity of *Halodule uninervis* extract with ethyl acetate solvents.

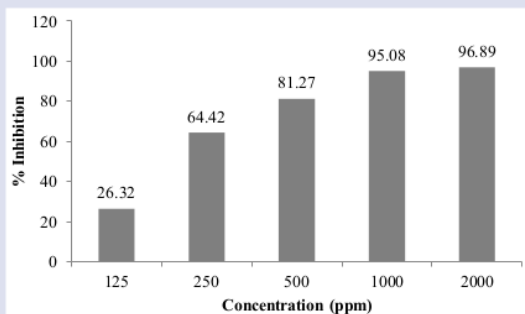


Figure 3: Inhibition of α -glucosidase enzymes from glucobay.

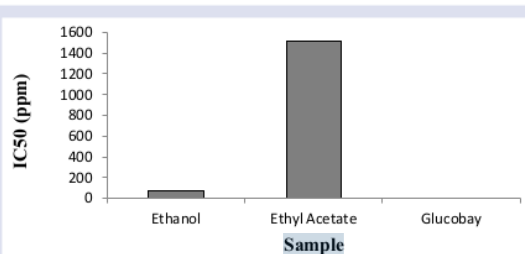


Figure 4: IC₅₀ value of *Halodule uninervis* extract with ethanol and ethyl acetate solvents and glucobay samples.

The activity test was carried out to determine the inhibitory activity of sample extracts against α -glucosidase from various extract concentrations by looking at the percent inhibition value and knowing the inhibitory strength of the enzyme by looking at the IC_{50} value. The IC_{50} value was determined by making a curve between percent inhibition and the concentration of mining samples so that the regression equation was obtained. From the regression equation can be determined the amount of extract concentration which has an inhibitory ability to α -glucosidase enzyme activity by 50%.¹³ Calculation of IC_{50} of *Halodule uninervis* seagrass with ethanol extract was 74.99 ppm, ethyl acetate extract was 1,517.05 ppm and glukobay as control was 0.27 ppm.

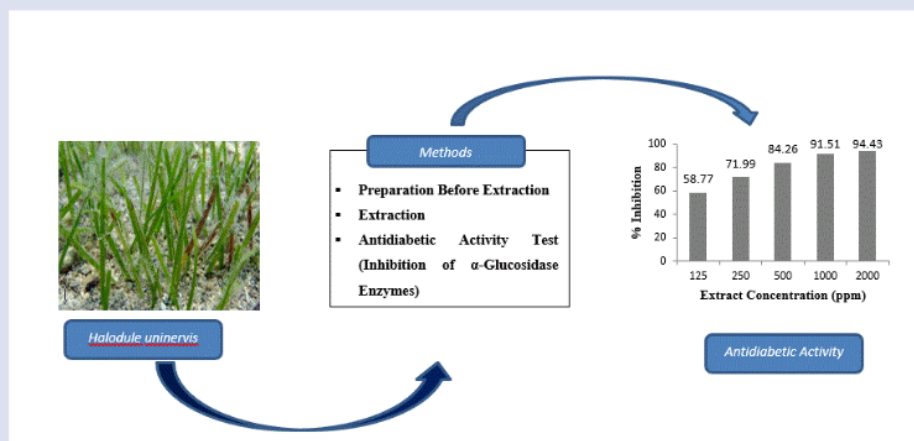
CONCLUSION

The content of an antidiabetic activity with extract concentrations of 125 ppm, 250 ppm, 500 ppm, 1,000 ppm and 2,000 ppm from seagrass extract with ethanol solvent showed that it had the ability as an antidiabetic agent stronger than seagrass extract with n-hexane and ethyl acetate solvents. Calculation of IC_{50} of *Halodule uninervis* seagrass was 74.99 ppm, which means that as much as 74.99 ppm the sample can inhibit 50% of α -glucosidase activity.

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GRAPHICAL ABSTRACT



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Dr. Ace Baehaki, S.Pi, M.Si, Associate Professor, Department of Fisheries Product Technology, Faculty of Agriculture, Sriwijaya University since 2001. His research area focuses on biological screening and bioactive activity of natural products from aquatic resources.



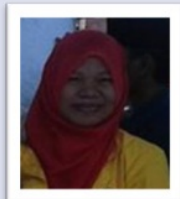
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