The glucose uptake of type 2 diabetic rats by *Sargassum olygocystum* extract: In silico and *in vivo* studies

Muhamad Firdaus* 1, Rahmi Nurdiani 1, Bachtiar Rivai, Windy Hapsari Hemassonida, Aqilatul Badzliyah, Nur Khasanah Sugiat
Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia.

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Brown seaweed extract, glucose uptake, PI3K/Akt, PTP1B, type 2 diabetic rats.

**ABSTRACT**
This study was to improve the glucose uptake in type 2 diabetic rats by *Sargassum olygocystum* extract. The identity of *S. olygocystum* metabolome was determined by high performance liquid chromatography-high resolution mass spectrometry. The value of the energy binding interactions between metabolomes of *S. olygocystum* and pioglitazone against protein tyrosine phosphatase 1B (PTP1B) was determined by the docking method. Male *Rattus norvegicus* weighing between 180 and 200 g was used as an experimental animal model for type 2 diabetes mellitus. This experiment consisted of six groups, i.e., normal, diabetes mellitus type 2 (DM2), DM2 + pioglitazone, DM2 + administered with *S. olygocystum* extract once, twice, and thrice per day, consecutively. The treatment was carried out for 45 days. The parameters were blood glucose, the area under the curve, insulin, homeostasis model assessment-insulin resistance, and expression of phosphatidylinositol-3-kinase (PI3K) and Akt. The data were stated as mean and standard deviation, and the differences between the treatments were determined by the Duncan test. The significance level used in this study was 5%. This study showed that *S. olygocystum* extract capable of reducing the blood glucose and rhamnetin of this seaweed extract enhances the glucose uptake in type 2 diabetic via inhibition of PTP1B activity and inducing PI3K/Akt expression.

**INTRODUCTION**
Type 2 diabetes mellitus is a metabolic disorder in which the body cells become resistant to insulin. This is possibly due to the increased activity of protein tyrosine phosphatase 1B (PTP1B) (Abdelsalam *et al.*, 2019) and reduced activation of phosphatidylinositol-3-kinase (PI3K) and Akt (Huang *et al.*, 2018). These activities reduce the glucose transporter 4 (Glut 4) translocation from the cytoplasm to the cell membrane. The blood glucose uptake into the cells also decreases (Afzalpoura *et al.*, 2016). Increased blood glucose uptake is one mechanism for controlling the blood glucose level in type 2 diabetics (Natali and Ferrannini, 2006).

PI3K is an enzyme that catalyzes the formation of phosphatidylinositol-3,4,5-triphosphate in the cell membrane (Abdelsalam *et al.*, 2019). The formation of this phosphate compound can activate Akt, which then plays a role in controlling the most important cellular processes in metabolism, including the Glut translocation (Abdelsalam *et al.*, 2019; Natali and Ferrannini, 2006). Previous studies have shown the increase of Glut 4 translocation in 3T3-L1 cells and diabetic type 2 mice via the PI3K and Akt signaling pathways (Jang *et al.*, 2020; Ramachandran and Saravanan, 2015) and the increase of glucose uptake in experimental animals and type 2 diabetics through the accumulative presence of Gluts (Różańska and RegulskaIlow, 2018).

The protein tyrosine PTP1B is an enzyme that catalyzes the dephosphorylation of tyrosine-phosphorylated proteins and plays a role in the state of insulin resistance. This enzyme is widespread in the muscles, liver, adipose tissue, and brain (Cho, 2013; Valverde and González-Rodríguez, 2011). Previous studies have shown that inhibiting PTP1B activity can increase the translocation of Glut 4 and further improve blood sugar levels in type 2 diabetes test animals (Chen *et al.*, 1997; Yang *et al.*, 2018; Zhang *et al.*, 2014).

It is known that brown algae contain many bioactive substances that are beneficial to human health and may include...
hypoglycemic agents (Gabbia and DeMartin, 2020). Some Sargassum species that have been studied as hypoglycemic agents include Sargassum hystrix, Sargassum yeozaene, Sargassum polycystum, Sargassum hemiphylum, Sargassum serratifolium, and Sargassum echinocarpum. The active ingredients in Sargassum spp. that are known to act as hypoglycemic agents include plastoquinones, polyphenols, and phlorotannins, although their bioavailability is low. The mechanisms of these compounds as hypoglycemic agents include α-amylase and α-glucosidase inhibitors, insulin secretion enhancers, insulin sensitivity enhancers, and PTP1B activity inhibitors (Ali et al., 2017; Corona et al., 2016; Firdaus and Chamidah, 2018; Gotama et al., 2018; Soliman et al., 2020; Hwang et al., 2015; Motshakeri et al., 2013).

Decocting refers to a method of extracting active ingredients using water and heat. This method is used because there are many active ingredients, it is cheap, and the extracts are free from toxic solvents (Yang et al., 2020). Previous studies showed that decoction of Syzygium cumini (Perera et al., 2017) and traditional Chinese medicine (Qi et al., 2019) had the ability to lower blood sugar in rats and people with type 2 diabetes. Most of the active ingredients dissolved in it are organic acid derivatives and polyphenols (Akhtar et al., 2019).

Sargassum sp. is known to contain bioactive substances that play a role in lowering blood glucose in animals with diabetes mellitus induced by alloxan and streptozotocin. However, the study of the active substance in Sargassum olygocystum obtained by decoction in lowering blood glucose based on glucose uptake in a type 2 diabetes animal model has not been explored. Therefore, the purpose of this study was to obtain the active ingredient from an S. olygocystum decoction, which plays a role in the glucose uptake in type 2 diabetes rats.

**MATERIALS AND METHODS**

**Materials**

Sargassum olygocystum was collected in February–March 2021 from Talango waters, Sumenep, Madura. Seaweed was authenticated by the Research Centre of Oceanography, Indonesian Institute of Sciences (1368/IPK.2/KS). Sargassum olygocystum was boiled for 23 min in aquadest (1/6.5: w/v) at a temperature of about 90°C, cooled at room temperature, then filtered with Whatman No. 40 paper. The filtrate was then diluted with aquadest containing 0.1% formic acid, vortexed at 2,000 rpm for 2 minutes, and spun down at 6,000 rpm for 2 minutes. Afterward, the supernatant was filtered with a 0.22 μm filter syringe and then 1 ml of supernatant injected into the HPLC-HRMS autosampler (Thermo Scientific™) for untargeted metabolome identification. This analysis used an aquadest with 0.1% formic acid as solvent A and acetonitrile with 0.1% formic acid as solvent B. The flow rate of the mobile phase was 40 μl/minute. The gradient ratios of solvents A and B were 95:5 at minutes 0–15, 40:60 at minutes 15–22, and 5:95 at minutes 22–25. The column temperature was 30°C. The metabolome identification was based on the similarity of detected compounds and compounds information contained in the Compound Discoverer, mzCloud MS/MS Library.

**Docking methods**

The 3D ligand structures of S. olygocystum compounds and pioglitazone in the form of SDF format were changed to PDB form using Open Babel. Before the docking process, the energy of these ligands was then minimized to optimize their conformation with Open Babel. The minimization results were then formatted in pdbqt and were finally made ready for the docking process. The macromolecule was PTP1B (ID: 2hnp), which was downloaded from http://www.rcsb.org/ (Huang et al., 2018). PTP1B as a macromolecule in *.pdb format was converted into *.pdbqt format using PyRx. Each ligand was in a flexible state that interacted with the macromolecule under rigid conditions. AutoDock Vina was used to simulate the test ligands’ docking and comparison ligand against PTP1B (Hwang et al., 2015). All calculations were executed via a grid-box size of x = 66.77 Å, y = 49.04 Å, z = 40.19 Å, with a grid center of x = 43.42 Å, y = 15.89 Å, z = 14.73 Å. An exhaustiveness search parameter of eight was used to predict the binding affinities due to the probability of finding the global minimum of the scoring functions. The docking results were evaluated, and the best value (ΔG was the most negative) was observed in the area of the ligands attached to the macromolecule. Interactions in the form of hydrogen bonds, hydrophobic bonds, and electrostatic bonds and bond distances were visualized in 2D and 3D with Discovery Studio and PyMOL with an interaction radius of 5 Å (Firdaus et al., 2020).

**Animal model**

Two- to three-month-old male Wistar rats weighing 200–250 g were acclimatized in individual cages for 1 week by feeding and drinking ad libitum. A type 2 diabetic rat model was obtained by high-fat feeding and diabetes injection in the normal rats. After the acclimatization phase, the treated group of rats was administered a high-calorie diet until hypercholesterolemia. The rats were then injected intraperitoneally with streptozotocin (stz) at a dose of 30 mg/kg body weight. Ten days after the injection, the blood glucose levels were determined. If the glucose level of rats was >200 mg/dl, rat PK3 kit (BT-Lab E0707Ra), rat PI3K kit (BT-Lab E0438Ra), and rat Akt kit (BT-Lab E0201Ra).

**HPLC-HRMS analysis**

Sargassum olygocystum was decocted in water (1:6.7: w/v) for 23 minutes at around 90°C, cooled at room temperature, and then filtered with Whatman No. 40 paper. The filtrate was then diluted with aquadest containing 0.1% formic acid, vortexed at 2,000 rpm for 2 minutes, and spun down at 6,000 rpm for 2 minutes. Afterward, the supernatant was filtered with a 0.22 μm filter syringe and then 1 ml of supernatant injected into the HPLC-HRMS autosampler (Thermo Scientific™) for untargeted metabolome identification. This analysis used an aquadest with 0.1% formic acid as solvent A and acetonitrile with 0.1% formic acid as solvent B. The flow rate of the mobile phase was 40 μl/minute. The gradient ratios of solvents A and B were 95:5 at minutes 0–15, 40:60 at minutes 15–22, and 5:95 at minutes 22–25. The column temperature was 30°C. The metabolome identification was based on the similarity of detected compounds and compounds information contained in the Compound Discoverer, mzCloud MS/MS Library.
it was declared diabetes, while those who had lower glucose levels were excluded from the study (Firdaus and Chamidah, 2018). This study group included six groups, namely, normal (A), DM (B), DM + pioglitazone at a dose of 2 mg/kg (C), DM + administration once with 4 ml/kg of S. olygocystum extract (D), DM + administration twice with 4 ml/kg of S. olygocystum extract (E), and DM + administration thrice with 4 ml/kg of S. olygocystum extract (F).

**Blood glucose and area-under-curve glucose (AUC<sub>glu</sub>)**

The measurement of blood glucose in rats was carried out by taking blood samples from the tail. On day 45 of the animal experiment, overnight fasting and then instantaneous glucose levels were measured. Blood glucose was measured with a glucometer (GlucoDr AGM-2100) and expressed in mg/dl. The AUC<sub>glu</sub> determination was carried out on rats based on an oral glucose tolerance test whose blood glucose levels were observed at 0, 30, 60, and 120 minutes after administering 5 ml/kg body weight of a 10% glucose solution (Cai et al., 2016). This assay was determined in rats that had been fasted overnight. The AUC<sub>glu</sub> formula is as follows:

\[
AUC = 0.25 \times A + 0.5 \times B + 0.75 \times C + 0.5 \times D
\]

where A, B, C, and D represent blood glucose levels at 0, 30, 60, and 120 minutes, respectively.

**Homeostasis model assessment-insulin resistance (HOMA-IR)**

HOMA-IR was determined based on glucose and insulin level and was measured using the following formula (Esteghamati et al., 2010):

\[
\text{HOMA-IR} = \frac{\text{insulin} \, (\text{mU/l}) \times \text{glucose} \, (\text{mg/dL})}{405}
\]

**Biochemical determination**

The insulin, PI3K, and Akt levels of rats were measured based on the enzyme-linked immunosorbent assay method. The measurement was based on the guidelines listed in each kit. Blood was drawn from the heart for insulin determination, whereas the liver was taken for PI3K and Akt determination. These organs were centrifuged at 3,000 rpm for 20 minutes to obtain serum and supernatant. The serum and supernatant were stored at −20°C until they were used. Approximately 50 μl of a standard solution was inserted into the standard well, while 40 μl of sample and 10 μl of insulin or kinases antibody were added to the sample well. Fifty μl of streptavidin-horseradish peroxidase was then added to the two wells and homogenized. The solution was incubated for 60 minutes at 37°C. After that, the well was washed with a washing buffer five times and soaked in 0.35 ml of the buffer for 1 minute. The wells were then dried, and 50 μl of substrate A and 50 μl of substrate B were added. The well plates were incubated for 10 minutes at 37°C in the dark, and finally, 50 μl of a stopping solution was added. The optical density of the color change of the reaction was measured 30 minutes after administering the stopping solution. The absorption measurement of the reaction result was carried out on a microplate reader (Bio-Rad Model 550) with a wavelength of 450 nm.

**Data analysis**

The data were expressed as the mean and standard deviation. The difference in treatments was analyzed using a fully randomized design method. The significance level used in this study was α = 5%.

**RESULTS AND DISCUSSION**

**Compounds identity of S. olygocystum**

HPLC-HRMS analysis shows that S. olygocystum extract consisted of 34 compounds, i.e., D-(-)-glutamine, betaine, DL-carnitine, L-glutamic acid, N-methyl-D-aspartic acid, acetycholine, valine, L-pyroglutamic acid, adenine, N6-acetyl-L-lysine, acetyl-L-methylcholine, guanine, 3,4-dihydroxyphenylpropionic acid, adenosine, 2′-deoxyadenosine, L-norleucine, acetophenone, L-phenylalanine, δ-valerolactam, N-butylbenzenesulfonamide, trans-3-indoleacrylic acid, caprolactam, 2-hydroxybenzothiazole,
The metabolites of the *S. olygocystum* extract consist of essential amino acids, nonessential amino acids, amino acid derivatives, terpenes, terpenoids, indoles, caprolactam, sulfonamides, nucleotides and their derivatives, carboxylic acid derivatives, cinnamic acid derivatives, flavonoid derivatives, and polyphenols. Previous studies have reported that this type of algae also contains phenols and flavonoids (Kanimozhi *et al.*, 2015; Mehdinezhad *et al.*, 2016). Meanwhile, another study showed that *Cystoseira barbata* contains rhamnetin, a derivate of flavonoid (Ibrahim and Abdel-Tawab, 2020). The presence of these two metabolites in the algae genera is possible because there is a synthesis process. Its synthesis needs precursor compounds, namely, phenylalanine and cinnamic acid, the two precursor compounds found in this algae extract (Koes *et al.*, 2005; Milke *et al.*, 2018).

**Docking analysis**

The interaction analysis results of the bioactive compounds of the *S. olygocystum* extract against PTP1B showed that rhamnetin had the strongest binding affinity among the active substances of *S. olygocystum* and a greater affinity than pioglitazone. The binding affinity value of pioglitazone is −7.6 kcal/mol, while the binding affinity value of rhamnetin is −8.4 kcal/mol. Table 1 displays the binding affinity value of pioglitazone and the bioactive compounds of the *S. olygocystum* extract. Table 2 exhibits the interaction and binding affinity of pioglitazone and rhamnetin. Figures 1 and 2 show the visualization of 2D and 3D interactions between pioglitazone and rhamnetin with PTP1B.
group, which readily accepts protons from the two residues on the enzyme’s active site (Lopez et al., 2017).

Glucose, insulin, HOMA-IR, and AUC$_{glu}$

The results of blood glucose, insulin, HOMA-IR, and AUC$_{glu}$ determination showed that treatment with the S. olygocystum extract resulted in lower parameter levels than in the diabetic rats, although the levels were higher compared with those in the animals treated with pioglitazone. Table 3 and Figure 3 presents the blood glucose, insulin, HOMA-IR, and AUC$_{glu}$ levels of diabetic rats treated with the S. olygocystum extract.

The cells of type 2 diabetics have low insulin sensitivity, and glucose entering the blood circulation cannot enter directly into the body’s cells. This study also obtained HOMA-IR and AUC$_{glu}$ values of the experimental animals. The administration of pioglitazone provides improved insulin sensitivity through decreased blood glucose levels and hyperinsulinemia. Similar results were also reported for the use of pioglitazone in people with type 2 diabetes (Rajagopalan et al., 2015). Pioglitazone is a hypoglycemic agent

Table 2. Interaction and binding affinity of pioglitazone and rhamnetin.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Interaction*</th>
<th>Chemistry bond</th>
<th>Types</th>
<th>Binding affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N:UNL1:H–A:THR263:OG1</td>
<td>Hydrogen bond</td>
<td>Conventional hydrogen bond</td>
<td>$-7.6 \pm 0.18$</td>
</tr>
<tr>
<td>Rhamnetin</td>
<td>A:ALA217–N:UNL1:C</td>
<td>Hydrophobic</td>
<td>Alkyl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A:GLN266:NE2–N:UNL1:O</td>
<td>Hydrogen bond</td>
<td>Conventional hydrogen bond</td>
<td>$-8.4 \pm 0.14$</td>
</tr>
<tr>
<td></td>
<td>A:THR263:OG1–N:UNL1</td>
<td>Hydrogen bond</td>
<td>Pi-donor hydrogen bond</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. 2D and 3D visualization of interaction between rhamnetin and PTP1B.
that increases insulin sensitivity in the liver, muscle, and fat tissue. Glitazone, besides working by activating peroxisome proliferator-activated receptor-γ, is also able to inhibit PTP1B.

_Sargassum oligocystum_ extract treatment can improve these metabolic disorders. Improvements in insulin sensitivity in type 2 diabetic rats can be attributed to the _S. polycystum_ and _Sargassum coreanum_ extracts (Motshakeri et al., 2013; Park et al., 2016). The administration of the _S. serratifolium_ extract showed improvement through the inhibition of PTP1B. Plastoquinones from _S. serratifolium_ can perform competitive and noncompetitive inhibition against these enzymes by binding to an enzyme’s allosteric site or the substrate-enzyme complex (Ali et al., 2017). In this study, the improvement of insulin resistance due to _S. oligocystum_ decoction was possible due to the presence of rhamnetin, a quercetin derivative. Quercetin is known to control blood glucose levels by increasing blood glucose uptake in muscles. The enhancement of glucose uptake is induced by the activation of AMPK and PI3K/Akt expressions. The increase in the expression of these kinase enzymes can be caused by the inhibition of PTP1B activity (Shi et al., 2019).

**PI3K and Akt expression**

The results showed that treatment with _S. oligocystum_ extract increased the PI3K and Akt expression levels in the liver of diabetic rats, although the value was lower than in the diabetic rats treated with pioglitazone. Table 4 presents the PI3K and Akt expression levels in the liver of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PI3K (ng/ml)</th>
<th>Akt (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.45 ± 0.03†</td>
<td>40.07 ± 0.8†</td>
</tr>
<tr>
<td>B</td>
<td>3.67 ± 0.02*</td>
<td>16.87 ± 0.4*</td>
</tr>
<tr>
<td>C</td>
<td>6.72 ± 0.03*†</td>
<td>36.08 ± 2.6*†</td>
</tr>
<tr>
<td>D</td>
<td>4.20 ± 0.02*†</td>
<td>22.08 ± 1.4*†</td>
</tr>
<tr>
<td>E</td>
<td>5.55 ± 0.01*†</td>
<td>28.89 ± 1.2*†</td>
</tr>
<tr>
<td>F</td>
<td>6.19 ± 0.04*†</td>
<td>32.14 ± 1.8*†</td>
</tr>
</tbody>
</table>

*p < 0.05 versus A.
†p < 0.05 versus B.

Table 4. Expression levels of PI3K and Akt of diabetic rats treated with _S. oligocystum_ extract.

![Figure 3. Oral glucose tolerance test values of diabetic rats treated with _S. oligocystum_ extract.](image-url)
PI3K and Akt are kinases that play essential roles in various metabolic activities, which include controlling blood glucose levels. The activity of these kinases was decreased in diabetic animals but increased in the group given pioglitazone and the S. olygocystum extract. It has been shown that the translocation of Glut 4 in diabetic animals is due to PI3K and Akt’s low activity (Pinent et al., 2004). Pioglitazone treatment in people with type 2 diabetes can increase glucose uptake (Rajagopalan et al., 2015). Glitazone can increase glucose uptake due to its ability to inhibit PTP1B activity (Bhattarai et al., 2010). The Glut is a transporter that is responsible for the entry of glucose into cells. These transporters are transferred from the cytoplasm to the membrane as a result of the presence of insulin. Glut 4 is a type of Glut that is most abundant in muscle and fat tissue. Glut 4 translocation to muscle and fat cell membranes occurs due to a series of reactions triggered by the presence of insulin through the PI3K/Akt pathway. Through this route, many flavonoids are involved in glucose uptake. Procyanidins, the polymers of flavan-3-ol catechins and epicatechins, increase glucose uptake in 3T3-L1 adipose cells and myotubes L6E9 with Akt activity (Afzalpoura et al., 2016), while flavanone eriodictyol and flavonoids 7-O-methylaromadendrin increase glucose uptake via the PI3K/Akt pathway in liver cells and fat cells (Zhang et al., 2010; Zhang et al., 2012).

CONCLUSION

This study found that the S. olygocystum extract lowered blood sugar levels and increased PI3K and Akt expression in the liver in rats with type 2 diabetes. HPLC-HRMS analysis identified alternative bioactive compounds contained in the S. olygocystum extract. The docking analysis of the identified active substances showed that rhamnetin was the most effective compound for inhibiting PTP1B. In summary, rhamnetin from the S. olygocystum extract is a natural ingredient that plays an important role in lowering blood sugar levels in rats with type 2 diabetes through the mechanism of inhibiting PTP1B activity and activating PI3K/Akt expression. However, an in vivo study of the ability of rhamnetin to control blood sugar levels in type 2 diabetes needs to be performed.

ACKNOWLEDGMENTS

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CONFLICT OF INTERESTS

The authors state no conflicts of interest.

ETHICAL APPROVAL

The study of this animal has received the approval of the Feasibility Study to Treat Laboratory Animals from the Ethics Committee of Univesitas Brawijaya (096-KEP-UB-2021), dated: July 30, 2021.

AUTHORS’ CONTRIBUTION

Muhamad Firdaus and Rahmi Nurdiani conceptualized the study; Bachtiar Rivai, Windy Hapsari Hemassonida, Aqilatul Badzliyah, and Nur Khasanah Agustika Sugiat conducted the experiment; Muhamad Firdaus and Rahmi Nurdiani analyzed the results. All authors reviewed the manuscript.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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