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

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**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

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hypoglycemic agents (Gabbia and DeMartin, 2020). Some *Sargassum* species that have been studied as hypoglycemic agents include *Sargassum hystrix*, *Sargassum yezoense*, *Sargassum polycystum*, *Sargassum hemiphyllum*, *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 to obtain the extract. High performance liquid chromatography (HPLC) grade of aquadest, acetonitrile, and formic acid was used to identify the bioactive compounds of *S. olygocystum*. The structure of compounds identified from *S. olygocystum* in SDF format was downloaded from the PubMed database. The HPLC-high resolution mass spectrometry (HRMS) Thermo Scientific Dionex Ultimate 3000 RSLCnano using a Hypersil GOLD aQ column (50 × 1 mm × 1.9 µ particle size) was used to identify the active compounds of *S. olygocystum*. An HP Intel® Core TMi3-5005U with a Microsoft Windows 10 operating system was used for the in silico method. Open Babel GUI version 2.4.1, PyMOL 1.7.4 Edu (Schrödinger), BIOVIA Discovery Studio 2019 (Dassault Systèmes BIOVIA Corp.), and PyRx 0.8 (The Scripps Research Institute) were used for the docking analysis (Firdaus *et al*., 2020). The materials used in the *in vivo* study were male *Rattus norvegicus* aged 2–3 months, pioglitazone (Dexa Medica), streptozotocin (BioWorld), rat *i*nsulin 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.

### 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 diabetogen 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,

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 ($AUC_{glu}$)

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 $AUC_{glu}$ 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 $AUC_{glu}$ formula is as follows:

$AUC = 0.25 \times A + 0.5 \times B + 0.75 \times C + 0.5 \times D$ ($A$, $B$, $C$, 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):

$$\text{HOMA-IR} = \text{insulin (mU/l)} \times \frac{\text{glucose}\left(\frac{\text{mg}}{\text{dL}}\right)}{405}.$$

### 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 $\alpha = 5\%$.

**Table 1.** The binding affinity value of pioglitazone and bioactive compounds of *S. olygocystum*.

| Compounds | Binding affinity (kkal/mol) |
|---|---|
| *D-(-)*-Glutamine | −5.1 ± 0.21 |
| Betaine | −3.8 ± 0.16 |
| DL-Carnitine | −4.6 ± 0.21 |
| *L*-Glutamic acid | −4.9 ± 0.13 |
| N-Methyl-*D*-aspartic acid | −5.0 ± 0.08 |
| Acetylcholine | −5.0 ± 0.21 |
| Valine | −4.4 ± 0.19 |
| *L*-Pyroglutamic acid | −4.6 ± 0.21 |
| Adenine | −5.5 ± 0.15 |
| N6-Acetyl-*L*-lysine | −4.7 ± 0.16 |
| Acetyl-β-methylcholine | −4.3 ± 0.21 |
| Guanine | −5.8 ± 0.18 |
| 3,4-Dihydroxyphenylpropionic acid | −5.9 ± 0.22 |
| Adenosine | −6.8 ± 0.29 |
| 2′-Deoxyadenosine | −6.4 ± 0.31 |
| *L*-Norleucine | −4.5 ± 0.22 |
| Acetophenone | −5.1 ± 0.21 |
| Rhamnetin | **−8.4 ± 0.14** |
| *L*-Phenylalanine | −5.3 ± 0.26 |
| δ-Valerolactam | −4.1 ± 0.18 |
| *trans*-3-Indoleacrylic acid | −6.0 ± 0.23 |
| Caprolactam | −4.4 ± 0.21 |
| 2-Hydroxybenzothiazole | −5.2 ± 0.41 |
| Ageratriol | −6.8 ± 0.27 |
| DEET | −5.6 ± 0.24 |
| Nootkatone | −6.7 ± 0.19 |
| *D*-(+)-Camphor | −5.8 ± 0.32 |
| N1-Methylidenebenzene-1-sulfonamide | −7.5 ± 0.27 |
| 1-Tetradecylamine | −4.2 ± 0.16 |
| 3,5-di-*tert*-Butyl-4-hydroxybenzaldehyde | −5.0 ± 0.33 |
| α-Eleostearic acid | −5.3 ± 0.23 |
| Dibutyl phthalate | −5.4 ± 0.41 |
| 4-Methoxycinnamic acid | −5.7 ± 0.12 |
| Choline | −3.4 ± 0.17 |
| Pioglitazone | **−7.6 ± 0.18** |

Bold values show that the binding energy of rhamnetin to glucosidase is higher than pioglitazone and others bioactive in *S. olygocystum* extract.

## 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, acetylcholine, valine, *L*-pyroglutamic acid, adenine, N6-acetyl-*L*-lysine, acetyl-β-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,

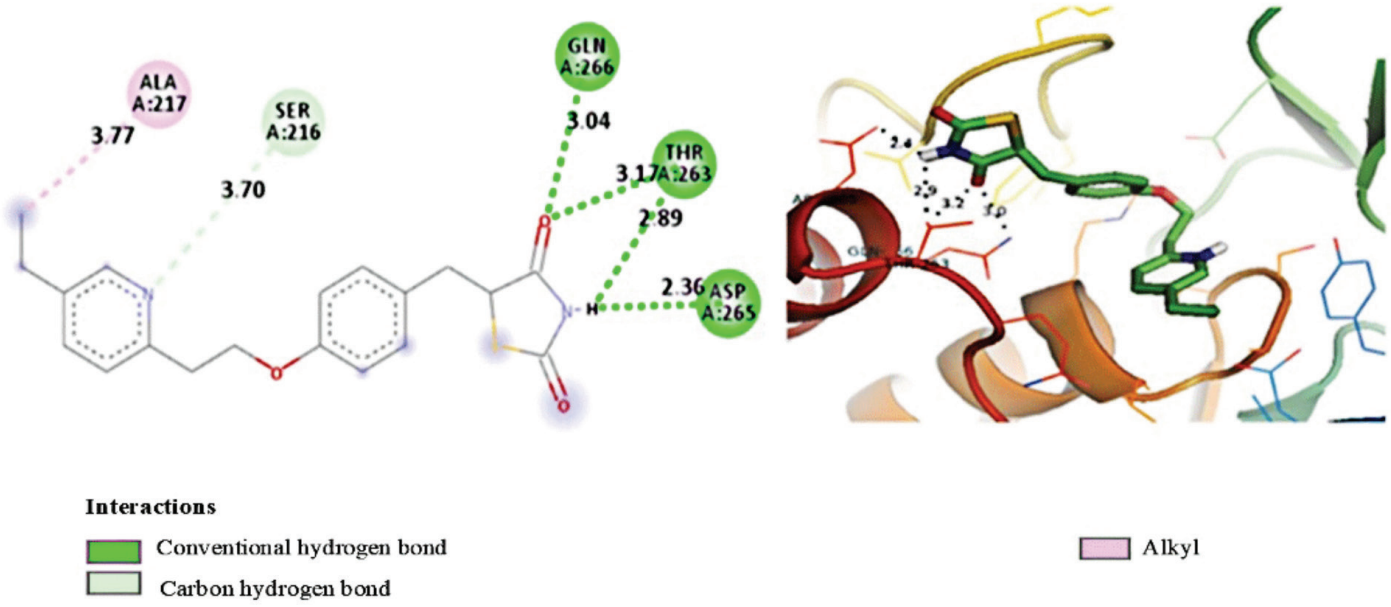


**Figure 1.** 2D and 3D visualization of interaction between pioglitazone and PTP1B.

ageratriol, DEET, nootkatone, D-*(+)*-camphor, 1-tetradecylamine, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, α-eleostearic acid, dibutyl phthalate, 4-methoxycinnamic acid, choline, and rhamnetin.

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.

Figure 1 and Table 2 show the hydrogen bond between pioglitazone and PTP1B residue on aspartate 265, threonine 263, and glycine 266, with pioglitazone acting as a proton donor for aspartate 265 but as a proton acceptor in threonine 263 and glycine 266. The interaction of pioglitazone and threonine 263 is also in the form of hydrogen bonds, but pioglitazone acts as a proton donor. Pioglitazone also acts as a proton acceptor in the hydrogen bond of carbon with serine 216. The alkyl bond occurs between pioglitazone and alanine 217.

Figure 2 and Table 2 show the hydrogen bonds between rhamnetin and the PTP1B residue of glutamate 115, aspartate 181, cysteine 215, arginine 221, aspartate 265, and glycine 266, while rhamnetin acts as a proton donor for glutamate 181, aspartate 181, and aspartate 265. Rhamnetin acts as a proton acceptor in cysteine 215, arginine 221, and glycine 266. The interaction of rhamnetin with threonine 263 is the pi orbital hydrogen bond with threonine as a proton donor.

Insulin resistance is a metabolic disorder characteristic in people with diabetes mellitus 2. Blood glucose levels in type 2 diabetics are still high, despite the high insulin level in the blood. The low sensitivity of the cells to insulin leads to the body's low glucose uptake. Pioglitazone is one of the sensitizers of fat, liver, and muscle cells to the presence of insulin. An in silico study showed that pioglitazone inhibits PTP1B but does not anchor on this protein's active site. In this study, a glitazone derivative can replace pioglitazone because the barrier is located directly on the enzyme's active site, namely, Cys215 and Arg221, at a distance of 4–5 Å (Bhattarai *et al*., 2010). Rhamnetin from brown seaweed also showed a docking on the protein's active site, and even the interaction was a residue only 3.5–4 Å apart. It means that this bioactive compound has tremendous potential as an inhibitor of PTP1B compared to the glitazone derivative. This ability is possible due to the conformation of the rhamnetin hydroxyl

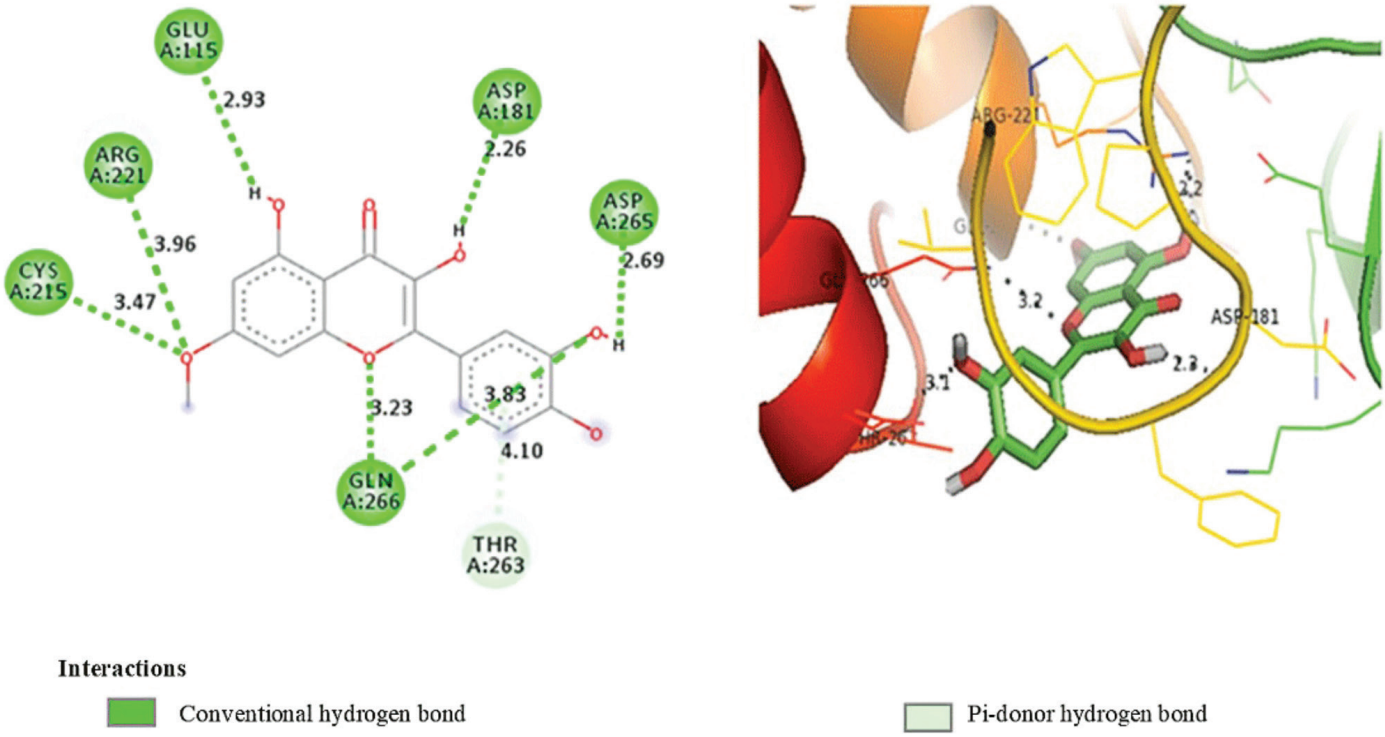


**Figure 2.** 2D and 3D visualization of interaction between rhamnetin and PTP1B.

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

| Compound | Interaction* | Chemistry bond | Types | Binding affinity (kkal/mol) |
|---|---|---|---|---|
| Pioglitazone | A:THR263:OG1–N:UNL1:O | Hydrogen bond | Conventional hydrogen bond | −7.6 ± 0.18 |
| | A:GLN266:NE2–N:UNL1:O | Hydrogen bond | Conventional hydrogen bond | |
| | N:UNL1:H–A:THR263:OG1 | Hydrogen bond | Conventional hydrogen bond | |
| | N:UNL1:H–A:ASP265:OD2 | Hydrogen bond | Conventional hydrogen bond | |
| | A:SER216:CB–N:UNL1:N | Hydrogen bond | Carbon hydrogen bond | |
| | A:ALA217–N:UNL1:C | Hydrophobic | Alkyl | |
| Rhamnetin | A:CYS215:SG–N:UNL1:O | Hydrogen bond | Conventional hydrogen bond | −8.4 ± 0.14 |
| | A:ARG221:N–N:UNL1:O | Hydrogen bond | Conventional hydrogen bond | |
| | A:GLN266:N–N:UNL1:O | Hydrogen bond | Conventional hydrogen bond | |
| | A:GLN266:NE2–N:UNL1:O | Hydrogen bond | Conventional hydrogen bond | |
| | N:UNL1:H–A:ASP265:OD2 | Hydrogen bond | Conventional hydrogen bond | |
| | N:UNL1:H–A:ASP181:O | Hydrogen bond | Conventional hydrogen bond | |
| | N:UNL1:H–A:GLU115:OE1 | Hydrogen bond | Conventional hydrogen bond | |
| | A:THR263:OG1–N:UNL1 | Hydrogen bond | Pi-donor hydrogen bond | |

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 3.** Glucose, insulin, HOMA-IR, and $AUC_{glu}$ levels of diabetic rats treated with *S. olygocystum* extract.

| Groups | Glucose (mg/dl) | Insulin (µU/ml) | HOMA-IR | $AUC_{glu}$ |
|---|---|---|---|---|
| A | 123.6 ± 6.6† | 4.21 ± 0.1† | 1.3 ± 0.1† | 295.35 |
| B | 342.2 ± 15.3* | 7.57 ± 0.3* | 6.4 ± 0.5* | 763.00 |
| C | 152.4 ± 6.2*† | 4.52 ± 0.2*† | 1.7 ± 0.1*† | 372.15 |
| D | 337.5 ± 14.8*† | 7.09 ± 0.4*† | 5.9 ± 0.3*† | 642.00 |
| E | 236.4 ± 10.6*† | 6.13 ± 0.3*† | 3.6 ± 0.2*† | 499.80 |
| F | 180.2 ± 12.1*† | 5.16 ± 0.4*† | 2.3 ± 0.3*† | 433.15 |

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

**Table 4** Expression levels of PI3K and Akt of diabetic rats treated with *S. olygocystum* extract.

| Groups | PI3K (ng/ml) | Akt (ng/ml) |
|---|---|---|
| A | 7.45 ± 0.03† | 40.07 ± 0.8† |
| B | 3.67 ± 0.02* | 16.87 ± 0.4* |
| C | 6.72 ± 0.03*† | 36.08 ± 2.6*† |
| D | 4.20 ± 0.02*† | 22.08 ± 1.4*† |
| E | 5.55 ± 0.01*† | 28.89 ± 1.2*† |
| F | 6.19 ± 0.04*† | 32.14 ± 1.8*† |

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

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 olygocystum* 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. olygocystum* 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. olygocystum* 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.

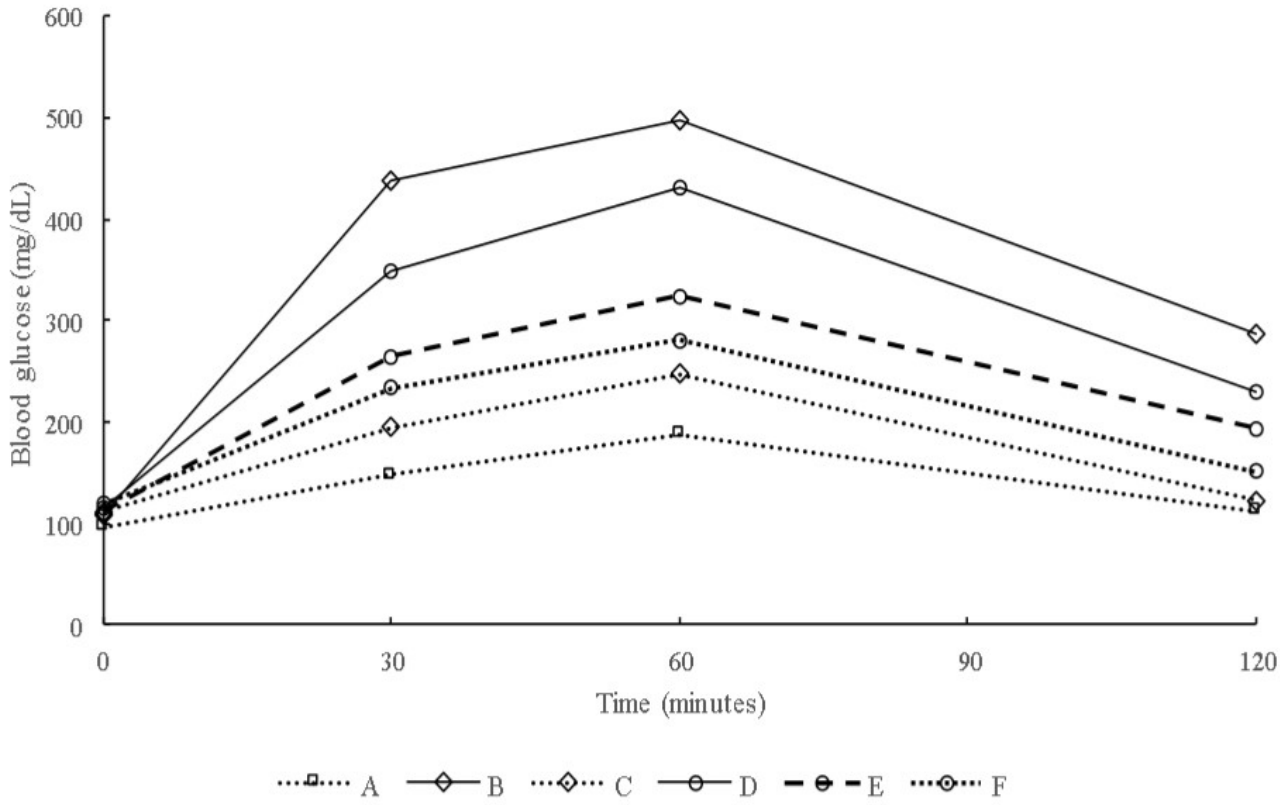


**Figure 3.** Oral glucose tolerance test values of diabetic rats treated with *S. olygocystum* extract.

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.

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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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