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Inhibitory Activity of α-Glucosidase by the Extract and Fraction of Marine Sponge-Derived Fungus *Penicillium citrinum* Xt6

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Abstract

BACKGROUND: Diabetes mellitus is a metabolic condition characterized by high blood glucose levels due to insufficient insulin secretion or activity. Diabetes treatment may include inhibiting carbohydrate breakdown enzymes like α-glucosidase. Chemical compounds of the marine-derived fungus have the potential to inhibit α-glucosidase and, thus, could be used in therapy. Marine sponge-derived fungus lives in a colony on the tissues of living things. In the marine sponge *Xestospongia testudinaria* DD-01, there is a colony of the fungus *Penicillium citrinum* Xt6. *P. citrinum* Xt6 has been reported to reduce blood glucose levels in alloxan-induced diabetic mice.

AIM: This study aimed to investigate the inhibitory activity of α-glucosidase by the extracts and fractions of marine-derived fungus *P. citrinum* Xt6.

MATERIALS AND METHODS: The study was carried out *in vitro* using p-nitrophenyl-α-D-glucopyranoside (PNPG) substrate and α-glucosidase enzyme from *Saccharomyces cerevisiae*. ELISA was used to measuring the enzyme's inhibition activity at the wavelength of 405 nm. Acarbose was used as the standard drug, which inhibits the activity of α-glucosidase.

RESULTS: Inhibitory concentration (IC_{50}) value of ethyl acetate extract was 37.39 μg/mL, methanol fraction was 60.01 μg/mL, n-hexane fraction was 75.45 μg/mL, and acarbose was 124.39 g/mL.

CONCLUSION: It can be concluded that the extract and fraction of marine-derived fungus *P. citrinum* inhibit α-glucosidase activity. *P. citrinum* could be developed into an antidiabetic agent.

Introduction

Diabetes mellitus (DM) is a long-term metabolic disorder that requires continuing medical care to prevent severe complications and lower the risk of long-term complications [1]. DM sufferers are increased along with the increasing welfare and human lifestyle. Global study data indicates that people with DM in 2014 reached 387 million. International Diabetes Federation (IDF) estimates that it will increase to 592 million people in 2035. Indonesia is the fifth country with the highest number of DM sufferers, with 9.1 million people, following China, India, the U.S.A, and Brazil [2].

Several classes of antidiabetic drugs are used in the treatment of DM with several mechanisms, that is, suppressing hepatic glucose production (biguanides); stimulating insulin secretion (sulfonylureas and glinides); delaying digestion and absorption of carbohydrates to maintain postprandial glucose levels (inhibitors of α-glucosidase and α-amylase); increasing insulin receptor sensitivity; and glucose uptake in peripheral tissues (thiazolidinedione and metformin) or insulin [3]. In type 2 diabetes, the treatment with an α-glucosidase inhibitor is beneficial in delaying postprandial glucose uptake. The enzyme α-glucosidase contributes to the conversion of carbohydrates to glucose. Glucose levels in the blood can be reduced back to normal by blocking the enzyme α-glucosidase [4].

In recent years, many attempts have been made to discover effective and natural α-glucosidase inhibitors to develop as antidiabetic drugs. Secondary metabolites such as alkaloids, phenolics, flavonoids, terpenoids, and glycosides isolated from natural ingredients have been reported to inhibit the α-glucosidase enzyme [5]. Bioactive compounds derived from marine biota are known to have the potential as a treatment for diabetes [6]. Sponges are marine biota that produces secondary metabolites with pharmacological activities [7]. One of the sponges with drug potential is *Xestospongia testudinaria*. It produces various metabolites such as alkaloids, quinones,

sterols, terpenoids, and fatty acids [8]. A previous study reported a species of fungus obtained from the *X. testudinaria*, namely, *Penicillium citrinum* [9].

Studies on the ethyl acetate extract of the marine sponge-derived fungus *P. citrinum* from *X. testudinaria* showed its antimicrobial, cytotoxic, and antidiabetic activity [9], [10]. In addition to fungus sourced from the sea, *Penicillium* spp. from plants inhibits α-amylase, α-glucosidase, and dipeptidyl peptidase-4 (DPP-4). Another study on the endophytic fungus *P. citrinum* extract isolated from *Boswellia sacra* showed high activity against α-glucosidase enzyme inhibition [11].

According to Bakhtra *et al.*, the extract of *P. citrinum* from *X. testudinaria* showed the presence of secondary metabolites of alkaloids and phenolics [9]. Alkaloids and phenol compounds have been shown to have therapeutic potential on blood glucose pathogenesis by inhibiting or stimulating various mechanisms, including the α-glucosidase enzyme and protein tyrosine phosphatase 1B (PTP-1B) inhibition; DPP-4 deactivation; enhanced insulin sensitivity; and modulation of oxidative stress [12], [13]. The present study investigated the antidiabetic effect of *P. citrinum*, a marine-derived fungus isolated from the marine sponge *X. testudinaria*, focusing on α-glucosidase inhibition by the extract and fraction of *P. citrinum*.

Materials and Methods

Sample preparation

Marine sponge-derived fungus was obtained from the fungus *P. citrinum* Xt6 from *X. testudinaria* DD-01 [9]. It was rejuvenated with Sabouraud Dextrose Agar (SDA) (Merck) and incubated for 5–7 days at 25°C. The fungus was cultured on 240 g of rice media and incubated for 4–6 weeks at 25°C [14]. The results of fungal cultures were extracted with ethyl acetate for 3–5 days with three repetitions. The extract was separated from the culture medium by filtration. The ethyl acetate was evaporated to obtain ethyl acetate extract by a rotary evaporator (Heidolph).

A separating funnel was used to fractionate approximately 3 g of ethyl acetate extract using methanol and n-hexane as solvent. The methanol and n-hexane fractions were evaporated using a rotary evaporator. Furthermore, the extract and the fractionation results were used to investigate the inhibitory activity of α-glucosidase.

Secondary metabolite screening

The secondary metabolite contents of *P. citrinum* extract and fraction were determined. The assay was conducted to determine the alkaloids, phenolics, terpenoids, and steroids compounds [15], [16].

Alkaloids detection

1 mL extract/fraction was dissolved in 0.5 mL of 10% hydrochloric acid before being filtered. In addition, four drops of Mayer reagent were added to the 1 mL filtrate. The yellow color of the filtrate indicates positive alkaloids.

Phenolics detection

1 mL extract/fraction was dissolved in 0.5 mL of 10% hydrochloric acid before being filtered. Then, four drops of ferric chloride solution were added to the filtrate. The blue color of the filtrate indicates positive phenolic.

Terpenoids and steroids detection

1 mL extract/fraction was dissolved in chloroform and then filtered. Then, one drop of 99% acetic anhydride and H_2SO_4 was added to the filtrate. The blue or purple color of the filtrate indicates positive steroids, while the red color indicates terpenoids.

α-glucosidase inhibition activity assay

The inhibitory activity of the α-glucosidase enzyme was determined by modifying the α-glucosidase assay [4]. Acarbose as standard, fungus extract, methanol, and n-hexane fractions was dissolved with 40, 60, 80, 100, and 120 μg/mL in DMSO, respectively. 30 μL of each solution added 36 μL of phosphate buffer pH 6.8 and 17 μL of p-nitrophenyl-D-glucopyranoside (PNPG) (Sigma Aldrich) 5 mM substrate, incubated for 5 min at 37°C. Then, add 17 μL of α-glucosidase enzyme derived from *Saccharomyces cerevisiae* recombinant (Sigma Aldrich) 0.045 U/mL in 2% bovine serum albumin (BSA) (Sigma Aldrich), incubation for 15 min. After incubation, 100 μL sodium carbonate (Merck) 200 mM was added. The standard and sample control solutions added sodium carbonate before adding the enzyme. DMSO was used as a blank. Then, the absorbance was measured with an ELISA reader (Sunostik) at a wavelength of 405 nm. This study was carried out with three repetitions.

The following formula was used to determine the percent inhibition of the α-glucosidase enzyme [17]:

$$\%\ \text{inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where: A_0 = Absorbance blank, A_1 = Absorbance sample

Based on the inhibition concentration, the inhibitory activity of the α-glucosidase enzyme was determined (IC_{50}). It was determined using a regression equation involving each sample concentration and percent inhibition.

Results and Discussion

Marine-derived fungi can be defined as microorganisms that live in colonies or a collection of microorganisms in the tissues of organisms or living things from the marine. They produce bioactive compounds developed as drugs [18], [19]. Several symbiotic fungi from marine biota had been reported to be developed as antidiabetic drugs [20].

The α-glucosidase is a type of hydrolase enzyme that catalyzes the hydrolysis of glucose. As the inhibitor of α-glucosidase, acarbose could inhibit carbohydrate absorption in the digestive tract, postprandial hyperglycemia, -hyperinsulinemia, and reduce HbA1c [21]. Postprandial hyperglycemia is a characteristic and early symptom of diabetes. Blood glucose levels that are too high can raise the chance of developing diabetic complications [22]. According to Ali *et al.* study, *P. citrinum* is a symbiotic fungus that can act as an antidiabetic by inhibiting the α-glucosidase [11]. The inhibitory study of α-glucosidase was determined using *P. citrinum* in symbiosis with the marine sponge *X. testudinaria*.

The extraction process of *P. citrinum* cultured with ethyl acetate solvent obtained 4.7539 grams. About 3 g of the extract was fractionated with methanol and n-hexane as a solvent to obtain 2.1669 g of methanol fraction and 1.0821 g of n-hexane fraction. The extract and fraction's secondary metabolite compounds were identified [15], [16]. Alkaloids and phenolic compounds were detected in the *P. citrinum* extract and methanol fraction, whereas phenolic compounds were detected in the n-hexane fraction.

The assay of the inhibitory activity of the α-glucosidase enzyme in this study was carried out by *in vitro* study. The absorption obtained from the enzymatic reaction was measured using an ELISA reader. The product formed is a compound with a chromophore group, an atom in an organic compound that can absorb ultraviolet-visible light. The reaction between the enzyme and PNPG substrate is a hydrolysis reaction. The substrate will be converted into yellow p-nitrophenol and α-D-glucose, so the absorbance measurement is carried out at a visible wavelength of 405 nm. The yellow color produced indicates the ability to inhibit the reaction. After all the enzyme's active sites are filled with substrate, the reaction will stop. The substrate concentration was 5 mM because it gave the highest enzyme activity [17].

The percentage of the α-glucosidase inhibition and IC_{50} by ethyl acetate extract, methanol fraction, n-hexane fraction of *P. citrinum,* and acarbose was obtained as shown in Table 1. The IC_{50} of ethyl acetate extract, methanol fraction, n-hexane fraction, and acarbose were 37.39; 60.01; 75.45; and 124.39 μg/mL.

According to the IC_{50} value, the ethyl acetate extract, methanol, and n-hexane fraction of *P. citrinum* inhibited α-glucosidase activity better than acarbose.

Table 1: Percentage of inhibition of α-glucosidase

Sample	Concentration (μg/mL)	Percent of Inhibition (%)	IC_{50} (μg/mL)
Ethyl acetate extract	40	57.29	37.39
	60	58.31	
	80	60.79	
	100	90.85	
	120	92.54	
Methanol fraction	40	21.58	60.01
	60	47.91	
	80	88.25	
	100	93.45	
	120	100	
n-hexane fraction	40	9.49	75.45
	60	23.84	
	80	75.93	
	100	76.38	
	120	88.25	
Acarbose	40	21.19	124.39
	60	29.54	
	80	33.19	
	100	42.38	
	120	48.74	

The ethyl acetate extract of *P. citrinum* had more potential in inhibiting α-glucosidase activity than the methanol and n-hexane fractions. The alkaloids and phenolic compounds in the extract were associated with the α-glucosidase inhibitory activity of *P. citrinum* [5], [23].

Alkaloids may bind to the competitive or non-competitive sites of the enzyme involved in digestion, preventing the formation of an enzyme-substrate complex; as a result, lowering enzyme activity. Inhibiting these enzymes is one strategy for reducing blood glucose levels [24]. Likewise, phenolic compounds were known to be able to interact with enzymes or substrates. The chemical structure of phenolics affects their capacity to interact with enzymes, and dipole-dipole interactions with phenols are required to block enzymes effectively [25].

Other studies revealed the mode of inhibition by phenolic compounds. Inhibitors can interact with enzymes in a variety of ways. There were competitive, non-competitive, and mixed inhibitors from the phenolic compounds used in the α-glucosidase inhibition assay. The variation in inhibitor potency and the mode of enzyme inhibition was caused by several factors, including the inhibitor's structure and stability [26].

DeCarvalho *et al.* also stated that endophytic fungi are a rich source of bioactive phenolic compounds [27]. In Ali *et al.* study, the α-glucosidase inhibitory activity of the bioactive compound isolated from the endophytic fungus *P. citrinum* FEF6 showed that 2-hydroxyphenyl acetic acid, 11-oxoursonic acid benzyl ester, and glochidacuminosides A showed high potential for α-glucosidase inhibition [11]. It was shown that *P. citrinum* Xt6 also might be developed into an antidiabetic agent by inhibiting the α-glucosidase enzyme.

Conclusion

The study finds that by inhibiting the α-glucosidase enzyme, the marine sponge-derived fungus *P. citrinum* Xt6 has the potential as an antidiabetic agent.

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