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α-Glucosidase Inhibition by *Red Yeast Rice* Extract and Fractions as *In vitro* Antidiabetes

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ABSTRACT

Red yeast rice has many biological activities such as antihyperglycemic, antihypercolesterolemic, antidiabetic and antioxidant. This study was conducted to search for the most active extracs of red yeast rice as an in vitro antidiabetes. Antidiabetic activity was measured through α-glucosidase inhibition assay. Samples were ethanol 95% extract of red yeast rice, as well as its fractionated products using n-hexane, dicloromethane, ethyl acetate and water. Extraction of red yeast rice powder with 95% ethanol had a yield of 9.44%. The yield from fractionation of ethanol 95% extract using n-hexane, dicloromethane, ethyl acetate and the water were 1.99%, 1.13%, 0.59% and 1.36% respectively. The phytochemical test showed that ethanol 95% extract of extract contained flavonoids, alkaloids and saponin. The highest antidiabetic activity was shown by ethanol 95% extracts with IC_{50} value of 20.86 µg/mL and exhibited an uncompetitive inhibition. Therefore, red yeast rice extracts is potential for antidiabetes.

Keywords: α-Glucosidase, Antidiabetes, Fractionation, IC_{50}, Red yeast rice

INTRODUCTION

Diabetes mellitus is a metabolic disorder of carbohydrate metabolism characterized by high blood glucose levels in the body (hyperglycemia), which is caused by the body's inability to produce insulin or the cells being unresponsive to the insulin produced (insulin resistance). The disease is divided into several types: type I diabetes, type II diabetes and gestasional. Type I diabetes is known as Insulin Dependent Diabetes Mellitus (IDDM). Type II diabetes is known as Non-Insulin Dependent Diabetes Mellitus (NIDDM) which is a common form of diabetes. Approximately 90-95% of people with diabetes in developing countries suffer from type II diabetes [1]. Gestasional DM namely diabetes suffered by pregnant women. This disease usually occurs in the third trimester of pregnancy and will return to normal after delivery [2].

Diabetes does not cause death directly, but can be fatal if not managed properly. Various treatment of diabetes that has been done is a therapeutic drug and non-drug therapies (diet and exercise planning). Drug therapy can be done with insulin injection or with commercial drugs. However, this treatment can cause some side effects such as flatulence, abdominal pain, diarrhea and hepatotoxicity [2].

Angkak (Red Yeast Rice), rice fermented with *Monascus purpureus*, was also reported to show blood-glucose-lowering effect by increasing insulin secretion in mice [3,4]. The mechanism involves the synthesis of acetylcholine from the central chlorinergic nerves that stimulates the *Muscarinic* M3 receptor on the β-cells of pancreas [5]. According to Su et al. [6], red yeast rice has the ability to improve insulin sensitivity and reduce insulin resistance in streptozocin-induced mice.

MATERIAL AND METHODS

MATERIAL

Red yeast rice was obtained from market in Bogor with brand 'Pazola'. The inhibitory activity of enzyme α-glucosidase was tested

using a microplate reader with p-nitrophenyl-α-D-glycopyranoside (p-NPG (Sigma Aldrich, USA)) as substrate and the enzyme α-glucosidase (Sigma Aldrich, USA) dissolved in 0.1 M phosphate buffer. The enzyme was derived from *Bacillus stearothermophillus* and had enzyme activity of 0.125 U/mL.

METHODS

Preparation of red yeast rice (Zubaidah and Sari)

Red yeast rice was oven-dried at a temperature of 50˚C for 6 hours, grounded, and sieved with a 40-mesh sieve.

Extraction of red yeast rice (Singgih et al.)

Extraction was done by maceration, in which 20 g of red yeast rice powder was put into a 400 mL erlenmeyer added with 400 mL ethanol 95%. The suspension was shaken for 3 hours at 130 rpm. The suspension was separated and the filtrate obtained was concentrated with a rotary evaporator and then re-macerated in order to obtain ethanol 95% extract of red yeast rice.

Fractionation of ethanol extract of red yeast rice (Andrianto et al.)

The ethanol extract of red yeast rice and its bran were subsequently fractionated by liquid-liquid extraction using solvents with increasing polarity, namely n-hexane, dichloromethane, ethyl acetate and water. The layers formed through fractionation were concentrated with rotary evaporator.

Phytochemical screening (Harbone)

Phytochemical screening was carried out on the ethanol extract of red yeast rice, including qualitative analysis of tannins, alkaloids, flavonoids, saponins, steroids and triterpenoids.

α-Glucosidase inhibition assay (Cengiz et al.)

The reaction mixture consisted of the blank, blank control, samples and control sample of 11.5 µL. They were incubated for 10 min at 37˚C. As much as 11.5 µL of 0.025 M p-nitrophenyl-α-D-glucopyranoside (p-NPG) was added followed by re-incubation at 37˚C for 20 min. The reaction was stopped by the addition of 41 µL of 2 M Na_2CO_3. The mixtures were diluted with 116 µL of distilled water and measured at a wavelength of 410 nm. Acarbose solution was used as a positive control, treated the same as the samples. The experiments were performed in 3 replicates. The percentage of inhibition was then calculated to determine the IC_{50} value.

The percentage of inhibition against α-glucosidase was calculated as follow:

% Inhibition=[1-(absorbance of sample/absorbance of control)] × 100%

Kinetic assay of α-glucosidase inhibition (Alfarabi)

The kinetic of α-glucosidase inhibition was measured by increasing the concentrations of p-nitrophenyl-α-D-glucopyranoside as substrate derived from the *Michaelis-Menten* curves. Extract from red yeast rice which was used as the enzyme inhibitors was the most active fraction with the highest inhibitory activity on α-glucosidase inhibition test. The kinetics of α-glucosidase inhibition was studied with two reaction systems, with and without inhibitor. The mixture reaction systems used were same as in the α-glucosidase inhibition test. The absorbance was measured with a microplate absorbance reader at 410 nm. The test was done 3 replicates.

RESULTS AND DISCUSSION

Extraction and fractionation of samples

The yield obtained from the extraction was 9.44% for ethanol 95% extract of red yeast rice. The extract was then fractionated using solvents with increasing polarity level, namely n-hexane, dichloromethane, ethyl acetate and water. The use of solvents with different polarities were to allow the chemical compounds being extracted based on their polarities. The yields of the fractionation process using the aforementioned solvents were 1.99%, 1.13%, 0.59% and 1.36% respectively.

Phytochemical compounds

Phytochemical test is a preliminary analysis to determine the content of the existing secondary metabolites in a sample. The results of phytochemical tests of ethanol extract of red yeast rice can be seen in Table 1.

Table 1: Phytochemical compounds of ethanol extract of red yeast rice

Chemical compound	Result	
	Red yeast rice	Positive control
Tannin	-	*Camellia sinensis*
Saponin	+	*Sapindus rarak*
Flavonoid	+	*Piper ornatum*
Alkaloid	+	*Catharanthus roseus*
Steroid	-	*Talinum paniculatum*
Triterpenoid	-	*Talinum paniculatum*

Inhibitory activity of samples toward α-glucosidase

The inhibitory activity of the samples was determined using α-glucosidase derived from *Bacillus stearothermophillus* recombinant with p-nitrophenyl-α-D-glucopyroside (p-NPG) as substrate. Enzyme inhibitory activity of various samples is known based on IC_{50} values (Table 2).

Table 2: α-Glucosidase inhibitory activity of the extract and some fractions of red yeast rice

Sample	IC_{50} (µg/mL)
Ethanol extract	**20.86**
n-Hexane fraction	40.42
Dicloromethane fraction	42.50
Ethyl acetate fraction	36.63
Water fraction	59.60
Acarbose	0.19

This indicated that it was the more polar compounds which had the ability to inhibit α-glucosidase. This data in the present study was better than the results reported by Gomathi *et al.* in which the ethanol extract of *Evolvulus alsinoides* recorded the best IC_{50} value of 86 mg/mL. The ethanol extract of red yeast rice contained saponins, flavonoids and alkaloids. Secondary metabolites such as flavonoids and saponins were found in the form of glycosides, hence might have a great activity to inhibit the enzyme α-glucosidase [7-13].

Srianta et al. [10], reported that durian seeds fermented by *Monascus* sp. had IC_{50} of 70.7 µg/mL. Other studies conducted by Kim et al. [12], reported that pigment Monascus with Penicilamin (H-Pen) demonstrated an ability of inhibiting α-glucosidase with IC_{50} of 50.9 µM.

Kinetics of α-glucosidase inhibition by the most active sample

Determination of the kinetics of α-glucosidase inhibition aimed to investigate the type of sample's inhibition toward the enzyme. The mechanism of inhibition was determined from Lineweaver-Burk curve with two reaction systems, namely the enzyme-substrate reaction with and without inhibitor. The most active sample in α-glucosidase inhibition test, indicated with the smallest IC_{50}, was used as the inhibitor, which was ethanol extract of red yeast rice. Analysis of the kinetics was investigated through the increasing concentrations of substrate p-NPG derived from Michaelis-Menten curves, started from 1.25-5 mM (Figure 1) [14].

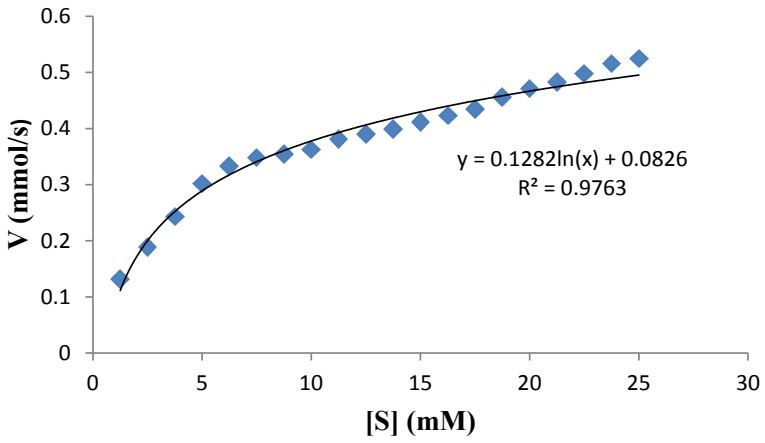

Figure 1: Michaelis-Menten curve

Lineweaver-Burk plot (Figure 2) resulted in equation y=1.5597 × +3.2394 with V_{max} values of 0.3 mmol/s and Km of 0.5 mM for the system without inhibitor and y=1.8556 × +5.9401 with V_{max} values of 0.2 mmol/s and Km of 0.3 mM for the one with inhibitor. The result showed a declining V_{max} and Km values, indicating that the inhibitor demonstrated uncompetitive inhibition.

CONCLUSION

The best inhibitory activity toward α-glucosidase was demonstrated by ethanol extract of red yeast rice, resulted in IC_{50} value of 20.86 µg/mL. The kinetics of inhibition of red yeast rice toward α-glucosidase showed an uncompetitive inhibition. Red yeast rice has a great potential as an alternative for antidiabetic.

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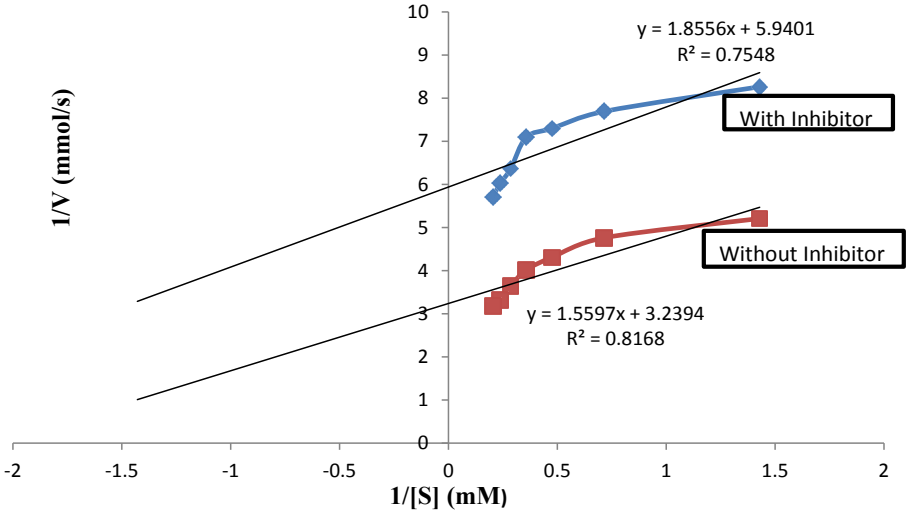

Figure 2: Kinetics of inhibition of the enzyme α-glucosidase

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