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Research Article

In Vitro Activity Test of A-Glucosidase Enzyme Inhibition of *Moringa oleifera* L. Leaves Extract

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ABSTRACT: Diabetes Mellitus (DM) is a group of disease characterized by an increase in insulin. *Moringa oleifera* leaves contain chemical properties such as tannins, steroids, triterpenes, flavonoids, saponins, anthraquinones and alkaloids. These chemical compounds can affect a variety of biological activities and one of the is antidiabetic. The research aimed to study the potential of *Moringa oleifera* extract in relation to the a-glucosidase enzyme which is one of the transitions of antidiabetic drugs based on IC_{50} values. In this study moringa leaves were extracted by maceration method using 96% ethanol solvent. The inhibitory activity testing uses an ELISA (Enzyme-linked Immunosorbent Assay) reader measured at 405 nm wavelength with a comparator of the acarbose because it is an antidiabetic drug of the glucosidase enzyme inhibitor group. The results of the a-glucosidase enzyme showed that the a-glucosidase enzyme activity was inhibited by the ethanol extract of *Moringa oleifera* L. with C_{50} value 84,238 µg/mL included in the less active category.

KEYWORDS: *Moringa oleifera* L.; Enzyme α-glucosidase; IC_{50}; In vitro

1. INTRODUCTION

Diabetes mellitus is increasing rapidly worldwide. For example, it is estimated that more than 180 million people worldwide have diabetes and the prevalence is expected to double by 2030. Diabetes is a group of diseases characterized by an increase in blood glucose due to relative or absolute deficiency of insulin (Mycek, M.J., Harvey, R.A., 2001).

Controlling postprandial glucose levels is an important strategy in the prevention of type 2 diabetes mellitus (Piya et al., 2010) so a therapeutic approach is carried out by delaying glucose absorption by inhibiting carbohydrate hydrolysis enzymes such as α-glucosidase enzymes in the digestive organs. Inhibition of enzyme activity can be done using phytochemical compounds in plants. (Tadera et al., 2006) have proven in vitro that flavonoids are compounds that have the potential to inhibit the α-glucosidase enzyme.

The leaves of Moringa (*Moringa oleifera* L.) contain chemical substances, namely tannins, steroids, triterpenes, flavonoids, saponins, anthraquinones and alkaloids (Kasolo et al., 2010). These chemical compounds can affect various kinds of biological activities and one of them is antidiabetic (Aminah, S., Ramdhan, T. dan Yanis, 2015).

In this study, an in vitro test of the α-glucosidase enzyme inhibition activity of the ethanol extract of Moringa oleifera L. leaves was carried out. This activity test is based on the reaction mechanism of the α-glucosidase enzyme which can catalyze the reaction of the substrate breakdown of p-nitrophenol - α - D- glucopyranoside into p-nitrophenol and glucose using an ELISA (Enzyme Linked Immunosorbent Assay) reader (Apriliani et al., 2017).

2. EXPERIMENTAL SECTION

2.1. Tools and materials

The tools used in this study were a blender (Philips®), ELISA reader (Biotek®), micropipette (Eppendorf®), dropper pipette (Pyrex®), a set of glassware, a set of maceration tools, analytical scales (Ohaous®).) and waterbath (Memmert®).

The test material used in this study was *Moringa oleifera* L. leaves in the form of an extract using the maceration method. The chemicals needed in the enzyme inhibition test are acarbose (SIGMA-Aldrich®), aluminum foil, phosphate buffer (pH 7), α-glucosidase enzyme (0.25 U / mL), 96% ethanol, sodium carbonate (200 mM), p-nitrophenyl-α-D-glucopyranoside (PNPG) (5 mM).

2.2. Work procedures

The test procedure for the inhibition of α-glucosidase enzyme activity is based on a modified study (Masitoh, 2011):

2.2.1. Blank testing

Phosphate buffer pH 7 as much as 36 µL and a substrate of p-nitrophenyl-α-D glucopyranoside (PNPG) 5 mM 17 µL were put into a well and incubated in a water bath for 5 minutes at 37°C. After the incubation period was complete, 17 µL of α-glucosidase was added and incubated again in a water bath for 15 minutes at 37°C. After the second incubation period was completed, 100 µL of 200 mM of Na_2CO_3 was added to stop the reaction and the absorbance was measured using an ELISA reader at a wavelength of 405 nm.

2.2.2. Testing control blanks

A pH 7 phosphate buffer of 36 µL and a substrate of 17 µL of p-nitrophenyl-α-D-glucopyranoside (PNPG) 5 mM were put into the well, then incubated in a water bath for 20 minutes at 37°C. After the incubation period was completed, 100 µL of 200 mM of Na_2CO_3 was added to stop the reaction. The absorbance was measured by means of an ELISA reader at a wavelength of 405 nm.

2.2.3. Comparative testing (acarbose)

Phosphate buffer pH 7 as much as 36 µL was put into the well, then a comparison of the concentration of 0.2 ppm was added to 30 µL and put into the well, as well as for the concentration of 0.4 ppm; 0.6 ppm; 0.8 ppm and 1 ppm. The substrate of p-nitrophenyl-α-D-glucopyranoside (PNPG) 5 mM 17 µL was added and incubated in the water bath for 5 minutes at 37°C, after the incubation period was completed, 17 µL of α-glucosidase enzyme was added to each well and incubated again on a water bath for 15 minutes at 37°C. After the incubation period was completed, 100 µL of 200 mM Na_2CO_3 was added to stop the reaction. The absorbance of the sample was then measured using an ELISA reader at a wavelength of 405 nm.

2.2.4. Comparative control testing

Phosphate buffer pH 7 as much as 36 µL was put into the well, then added with a comparison of the concentration of 0.2 ppm as much as 30 µL and put into the well, as well as for the concentration of 0.4 ppm; 0.6 ppm; 0.8 ppm and 1 ppm. P-nitrophenyl-α-D-glucopyranoside (PNPG) 5 mM 17 µL substrate was added and incubated in the water bath for 20 minutes at 37°C. After the incubation period was completed, 200 mM 100 µL of Na_2CO_3 was added to stop the reaction. The absorbance of the sample was then measured using an ELISA reader at a wavelength of 405 nm.

2.2.5. Sample testing (moringa leaf ethanol extract)

Phosphate buffer pH 7 of 36 µL was put into the well, then 30 µL of 100 ppm of Moringa leaf ethanol extract was added and put into the well, as well as for the ethanol extract of Moringa leaves with a concentration of 150 ppm, 200 ppm, 250 ppm, and 300 ppm. The substrate of p-nitrophenyl-α-D-glucopyranoside (PNPG) 5 mM 17 µL was added and incubated in the water bath for 5 minutes at 37°C, after the incubation period was completed, 17 µL of α-glucosidase enzyme was added to each well and incubated again on a water bath for 15 minutes at 37^0C. After the incubation period was completed, 100 µL of 200 mM Na_2CO_3 was added to stop the reaction. The absorbance of the sample was then measured using an ELISA reader at a wavelength of 405 nm.

2.2.6. Sample control testing

Phosphate buffer pH 7 of 36 µL was put into the well, then 30 µL of 100 ppm of Moringa leaf ethanol extract was added and put into the well, as well as for the ethanol extract of Moringa leaves with a concentration of 150 ppm, 200 ppm, 250 ppm, and 300 ppm. The substrate of p-nitrophenyl-α-D-glucopyranoside (PNPG) 5 mM 17 µL was added and incubated in the water bath for 20 minutes at 37°C, after the incubation period was complete, 100 µL of Na_2CO_3 200 mM was added to stop the reaction. The absorbance of the sample was then measured using an ELISA reader at a wavelength of 405 nm.

3. RESULTS

Table 1. Extraction results and% yield of ethanol extract of Moringa leaves (*Moringa oleifera* L.)

Sample (gram)	Solvent (mL)	Extract (gram)	Extract (%)
300	2,700	43.06	14.35

Table 2. The results of the test for the inhibition of α-glucosidase enzyme activity by comparison (acarbose) and ethanol extract of Moringa leaves (*Moringa oleifera* L.)

Testing	Concentration (ppm)	Absorbance (405 nm)	% inhibition	IC_{50} (µg/mL)
Comparator (Acarbose)	0.2	0.41	49.00	0.25
	0.4	0.38	53.63	
	0.6	0.35	58.11	
	0.8	0.32	63.06	
	1	0.30	68.93	
Sample	100	0.43	52.81	84.24
	150	0.40	59.74	
	200	0.38	67.10	
	250	0.34	75.90	
	300	0.31	82.68	

4. DISCUSSION

In this study, an inhibitory activity test of the α-glucosidase enzyme from the ethanol extract of *Moringa oleifera* L. leaves was carried out which aims to determine the potential of the extract in inhibiting the α-glucosidase enzyme, which is one of the mechanisms of action of antidiabetic drugs based on the IC_{50} value.

The sample used in this study was *Moringa oleifera* L. leaf because in several previous studies it was stated that *Moringa oleifera* L. leaves had biological activity as an antidiabetic. The extraction method used on *Moringa oleifera* L. leaves is the maceration extraction method.

After extraction, the α-glucosidase enzyme inhibition activity test was carried out by measuring absorbance blanks, control blanks, comparators (acarbose), comparison controls (acarbose), ethanol extract samples from *Moringa oleifera* L. leaf, ethanol extract samples from moringa leaves. (*Moringa oleifera* L.) using an ELISA (Enzyme linked Immunosorbent Assay) reader at a wavelength of 405 nm. In the same blank and enzyme work, using blank control and sample control as correction factors for the absorbance value of the sample and blank because the color of the extract can also provide the absorbance value at these wavelengths.

Acarbose is used as a comparison because acarbose is an antidiabetic drug that works to inhibit α-glucosidase circulating in Indonesia, and root Bose has become an internationally recognized benchmark.

The substrate of p-nitrophenyl-α-D-glucopyranose is used as a substitute for carbohydrates which will be broken down by the α-glucosidase enzyme. Inhibition of the α-glucosidase enzyme occurs because the α-glucosidase enzyme will hydrolyze p-nitrophenyl-α-D-glucopyranose to become p-nitrophenol and glucose. The phosphate buffer used is pH 7 because the α-glucosidase enzyme works optimally at that pH. Whereas during the incubation period at 37 ° C because at that temperature the α-glucosidase enzyme can work optimally. As it is known that enzymes are thermolabile proteins so that in their processing, temperature and pH must be maintained in optimum conditions. Na_2CO_3 is used to stop the hydrolysis reaction.

The tool used in this study is the ELISA reader at a wavelength of 405 nm. The use of this method has been widely used by enzyme inhibitor researchers before because it has several advantages such as the processing technique which is relatively simple, economical, and has a high sensitivity (Syarif et al., 2020).

In this α-glucosidase inhibition test, comparators (acarbose) were also made in 5 concentration series, namely 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm, and 1 ppm. The low concentration of acarbose used was because the acarbose used was pure acarbose. Samples were made in 5 concentration series, namely 100 ppm, 200 ppm 150 ppm, 250 ppm, and 300 ppm. The concentration series selection was used after several tests were carried out. The results of the inhibitory activity test can be seen in Table 2.

After obtaining the absorbance value, the next step is to calculate the percent inhibition to be used in determining the IC_{50} value (the concentration needed to inhibit 50% enzyme activity) from the sample and comparison. The smaller the IC_{50} value, the stronger the inhibitory effect. The inhibition level of α-glucosidase enzyme is very active if $IC_{50} \leq 25$ µg / mL, active if 25 µg / mL $\leq IC_{50} \leq 50$ µg / mL, less active if 50 µg / mL $\leq IC_{50} \leq 100$ µg / mL, and inactive if $IC_{50}>$ 100 µg / mL (Dedi, 2018).

In testing the α-glucosidase enzyme activity by the test sample showed an inhibition of the α-glucosidase enzyme activity by a comparator (acarbose) had an IC_{50} value of 0.25 µg / mL. Meanwhile, the ethanol extract of *Moringa oleifera* L. leaves with an IC_{50} value of 84.24 µg / mL. Based on the inhibitory power level of the α-glucosidase enzyme above, acarbose is in the very active category, while the *Moringa oleifera* L. leaf extract sample has activity in inhibiting the α-glucosidase enzyme even though it is in the less active category.

5. CONCLUSION

Based on the research, it can be concluded that the ethanol extract of *Moringa oleifera* L. leaf has α-glucosidase enzyme inhibitory activity with an IC_{50} value of 84.24 µg / mL and is in the less active category.

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