JOPS: *Journal of Pharmacy and Science*
p-ISSN: 2622-9919 | e-2615-1006
Homepage: http://jurnal.univrab.ac.id/index.php/jops

J Pharm & Sci Vol. 7, No. 2 (June 2024), pp. 161-166 **ORIGINAL RESEARCH**

Inhibitory Activity Test of α-amylase Methanol , n-Hexane Fraction and Ethanol Extract of Moringa Leaves (*Moringa oleifera L*)

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ABSTRACT

Diabetes mellitus is a disease characterized by increased blood sugar levels (hyperglycemia). One of the enzymes that plays an important role in breaking down oligosaccharides and disaccharides into monosaccharides so that they are ready for absorption is the α-amylase enzyme which can delay and lengthen the digestion time of carbohydrates. Moringa (*Moringa oleifera L*) is a plant that originates from India and has now spread widely to tropical and subtropical areas throughout the world. Moringa leaves are known to contain the flavonoid, β-sitosterol, which can reduce sugar levels. The aim of this research was to examine inhibitory activity of α-amylase on methanol, n-hexane fraction and ethanol extract of Moringa leaves. The method for data analysis was carried out by obtaining antidiabetic test data, namely the percent inhibition value of the α-amylase enzyme activity. From the results of the research that has been carried out, it can be concluded that there is α-amylase enzyme inhibitory activity with moderate strength in samples of methanol partition and n-hexane partition of Moringa leaves carried out in vitro, with IC_{50} values of 105.302µg/ ml and 158.29µg/ml. Meanwhile, the Moringa leaves ethanol extract sample was carried out in vitro, with an IC_{50} value of 81,74 µg/ml.

Keywords: Moringa leaves, α-amylase, IC_{50}

Introduction

Diabetes mellitus is a disease characterized by an increase in blood sugar levels (hyperglycemia), which occurs due to abnormalities in insulin secretion, sensitivity to insulin action, or both (Hermayudi and Ariani, 2017). The prevalence of Diabetes Mellitus in Central Java Province based on doctor's diagnosis, in the population aged ≈ 15 years, showed an increase from 1.6% in 2013 to 2.1% in 2018 (BPPK, 2018).

One of the efforts to overcome diabetes mellitus (DM) is by inhibiting the work of the α-amylase enzyme to hydrolyze carbohydrates so that it can reduce glucose absorption (Alegesan, Raghupathi and Sankarnarayanan, 2012). Inhibition of the α-amylase enzyme is known to delay and prolong the digestion time of carbohydrates, resulting in a decrease in the rate of glucose absorption and inhibiting the increase in postpandrial plasma glucose levels. (Sales *et al.*, 2012).

The high prevalence of diabetes in Indonesia has resulted in an increase in the use of oral antidiabetic drugs or insulin as pharmacological therapy. Meanwhile, it is known that most oral antidiabetic drugs have undesirable side effects (Hati, Setiawan and Yuliarta., 2017). To avoid side effects, people tend to turn to herbal medicine. Herbal medicines are considered safer than conventional medicines, this is because traditional medicines come from natural ingredients which have far fewer side effects than conventional medicines on the market (Muhlisah, 2001). Therefore, herbal treatment systems for diabetes continue to be developed by experts (Hati, Setiawan and Yuliarta., 2017).

Based on description above, we are interested in examining the α-amylase enzyme inhibitory activity test from the fraction, n-hexane, methanol and ethanol extract of Moringa leaves (*Moringa Oleifera L*) using the UV-Vis Spectrophotometry Method obtained from Wonorejo Village, Wonopringgo District, Pekalongan Regency.

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Materials

The tools used in this research were a UV-Vis Spectrophotometer (Shimadzu), rotary evoporator (Heidolph), analytical balance (Ohaus), oven (Memert), blender (Isolab), water bath.

The materials used in this research were Moringa leaves (Moringa oleifera L), ethanol 96%, methanol, n-hexane, α-amylase enzyme (Sigma-Aldrich), acarbose (Glucobay), Mg powder, amyl alcohol, amylum manihot 0.5% (Merck), Iodine 0.5%, H_2SO_4, phosphate buffer solution pH 7, dimethylsulfoxide (DMSO), HCl 2N, $FeCl_3$ 1%, Meyer's reagent and Dragendorf's reagent.

Methods

1. Preparation of Extract Plant

Ethanol extract from Moringa leaves is carried out using the maceration method. Maseration was carried out for 5 days with stirring several times at room temperature and protected from light. The results of the maceration are filtered and the filtrate is collected, then evaporated and concentrated using a rotary evaporator at 55°C until a thick extract of Moringa leaves is obtained (Purwatresna, 2012).

2. Fractionation of Moringa Leaves Ethanol Extract

Fractionation was carried out by adding 100 mL of concentrated ethanol extract of Moringa leaves to the solvent methanol and n-hexane each into a separating funnel. The mixture was shaken and left for 1 day. Next, the mixture was separated between the methanol fraction and the n-hexane fraction. Then it is evaporated over a water bath at 60°C to produce a thick extract. Next, a phytochemical screening test was carried out for each fraction.

3. Inhibitory Activity Test ff α-Amylase Methanol, N-Hexane Fraction And Ethanol Extract of Moringa Leaves (Mugiyanto, 2016)

In vitro testing of the inhibitory activity α-amylase enzyme in methanol, n-hexane fraction and ethanol extract of Moringa leaves was carried out using the starch-iodine method.

a. Blank Solution Testing

1000 µL pH 7 phosphate buffer solution was incubated for 10 minutes at 37°C. Next, 500 µL of 0.5% Amylum was added and incubated again for 15 minutes at 37°C. After the incubation complete, 30 µL of 0.5% iodine was added. Absorbance the solution using a UV-Vis spectrophotometer at a wavelength of 601 nm.

b. Sample Testing

500 µL sample solution with concentrations of 20, 40, 60, 80 and 100 µg/ml was added with 500 µL α-Amylase enzyme. Then mixture was incubated for 10 minutes at 37°C. Next, 500 µL of 0.5% Amylum was added to the samples (extract and fraction) and incubated again for 15 minutes at 37°C. After the incubation complete, 30 µL of 0.5% iodine was added. Absorbance the solution using a UV-Vis spectrophotometer at a wavelength of 601 nm.

c. Blank Control Testing

1000 µL pH 7 phosphate buffer solution was incubated for 10 minutes at 37°C. Next, 500 µL of 0.5% Amylum was added and incubated again for 15 minutes at 37°C. After the incubation complete, 30 µL of pH 7 phosphate buffer was added. Absorbance the solution using a UV-Vis spectrophotometer at a wavelength of 601 nm.

d. Sample Control Testing

500 µL sample solution with concentrations of 20, 40, 60, 80 and 100 µg/ml was added with 1000 µL phosphate buffer solution pH 7. Then incubated for 10 minutes at 37°C. Next, 500 µL of 0.5% Amylum was added and incubated again for 15 minutes at 37°C. After the incubation complete, 30 µL of pH 7 phosphate buffer solution was added. Absorbance the solution using a UV-Vis spectrophotometer at a wavelength of 601 nm.

e. Positive Control Testing

500 µL acarbose solution with concentrations of 20, 40, 60, 80 and 100 µg/ml was added with 500 µL of α-amylase enzyme. Then the mixture was incubated for 10 minutes at 37°C. Next, 500 µL of 0.5% amylum was added and incubated again for 15 minutes at 37°C. After the incubation period was complete, 30 µL of 0.5% iodine was added. Absorbance the solution using a UV-Vis spectrophotometer at a wavelength of 601 nm.

Data Analysis

Analysis data on the inhibitory activity of the α-amylase enzyme in Moringa leaves samples :

1. Data from research results, namely the absorbance value of the blank, positive control and test sample solution which has been measured using a UV-Vis spectrophotometer, the percent inhibition is searched for using the formula:
   Inhibition (%) = 1- $\frac{C-S}{C}$ x 100 %
   Noted that :
   C = Absorbance (Blank – blank control)
   S = Absorbance (Sample – sample control)
2. Calculate IC_{50} using a linear regression equation with the x-axis (sample concentration) and y-axis (% inhibition). From the regression equation: y = a+bx then the IC_{50} value can be calculated using the formula: $IC_{50} = \frac{50-a}{b}$

Result and Discussion

Preparation of Extract Plant

Simplicia extraction is carried out using the maceration method. This method was chosen because it is easy to work on and the tools used are simple (BPOM, 2010). The weight of simplicia macerated with 96% ethanol is 500 g. Simplicia was soaked in 3L of ethanol for 5 days, stirring occasionally. The solvent will penetrate the cell wall and enter the cell cavity which contains the active substance. The active substance will dissolve due to the difference in concentration between the active substance in cell and solvent. This event is repeated so that there is a concentration balance between the solution outside the cell and inside the cell, while stirring is carried out to speed up the extraction process. The macerated simplicia was then evaporated to evaporate the solvent to obtain a thick extract weighing 80 g and a yield percentage of 8%. The results obtained from the thick ethanol extract of Moringa leaves are greenish yellow.

Fractionation of Moringa Leaves Ethanol Extract

The methanol partition results obtained were 18 grams and the n-Hexane partition was 1.077 grams. Methanol partition results are greater than n-hexane partitions, these results occur because methanol has carbon groups (non-polar) and hydroxyl groups (polar) so that methanol can attract compounds that are both non-polar and polar. The n-hexane partition results are small because the few chemical compounds in basil leaves are non-polar.

Research on α-Amylase inhibitory activity test of the ethanol extract of Moringa leaves was carried out in vitro using the UV-Vis spectrophotometric method. The basic principle of this research is to determine the presence of α-Amylase inhibitory activity in the ethanol extract of Moringa leaves by observing a decrease in the intensity of the blue color in the starch-iodine complex, which is caused by the breakdown of the substrate by α-amylase enzyme (Vengadaramana, A. and Arasaratnam, 2014).

The α-amylase enzyme inhibitory activity test is a test to determine whether there is a decrease in the work activity of α-amylase enzyme in breaking down starch so that by inhibiting α-amylase enzyme (starch blockers) it can control the increase in postprandial blood glucose (Thilagam *et al.*, 2013). The α-amylase enzyme is known to be the main enzyme that plays a role in the hydrolysis of starch into simpler sugars (oligosaccharides, dextrin, limit dextrin and cyclodextrin derivatives). Dextrin is a

complex oligosaccharide mixture which is a product of starch and glucose or dextrose. Meanwhile, limit dextrin is a mixture of oligosaccharides with shorter chains.

In this research, amylum was used as a substrate for enzymes. pH 7 was used in this research because it is the optimum pH for α-amylase enzyme reaction is 9. Phosphate buffer is used as a buffer solution which functions to maintain pH of enzyme, so that during the reaction enzyme can still work optimally. Meanwhile, the reason for using DMSO as an extract solvent was chosen because it is an organic solvent which is commonly used as a chemical solvent. Iodine solution (iodine-iodide complex) is used as a reagent or dye for the starch remaining in the reaction, so that its absorption can be read using UV-Vis spectrophotometry at visible wavelengths. In testing the inhibition of α-amylase enzyme, a 2-stage incubation process was carried out. The first incubation (preincubation) is carried out for 10 minutes, this aims to adapt the test solution to optimal environmental conditions and to activate the enzyme. And the second stage, carried out for 15 minutes for enzymatic reaction (between the enzyme and starch). Where the incubation process is carried out at 37°C which is the optimum temperature for α-Amylase enzyme to work (Park, K. H., 1998)

Based on research, all extract sample solutions are blue with a fairly low absorbance value, this shows that α-amylase enzyme has hydrolyzed almost all the starch contained in the extract sample solution into simpler sugars (oligosaccharides). The fading intensity of the blue color indicates that less starch is absorbed so the percent inhibition is higher. During the test, the blue color that formed quickly disappeared, this was possibly due to an enzymatic reaction. Therefore, after the incubation period is complete, the extract sample solution must be quickly added with iodine reagent to stop the enzymatic reaction of α-amylase enzyme.

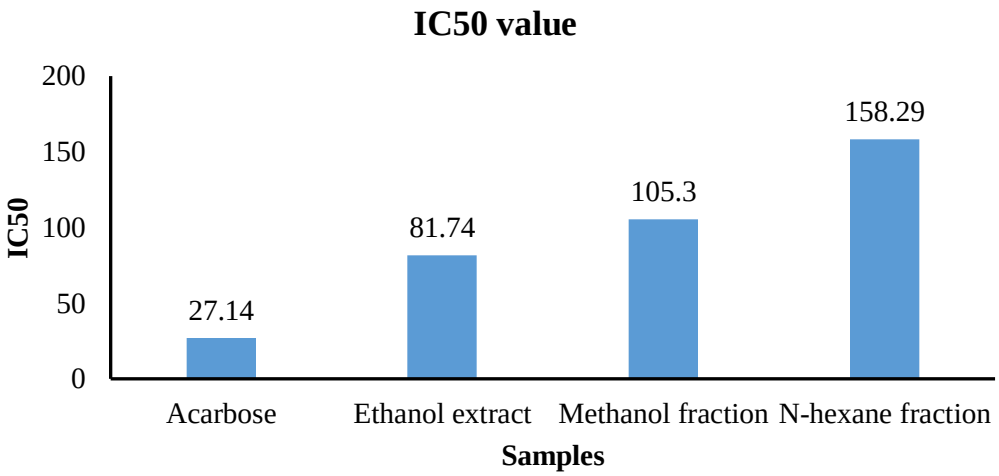

Figure 1. IC_{50} values for samples with acarbose

Based on figure 1 can be seen that samples of ethanol extract, methanol partition and n-hexane partition of Moringa leaves have a smaller percent inhibition value compared to acarbose as the positive control. This shows that with increasing test concentration, the greater the percent inhibition value obtained. Where the greater the percent inhibition value, the more potential it has as an antidiabetic. The inhibitory activity parameter of an extract is expressed in the IC_{50} value (a value that states the concentration of an extract that has an α-Amylase enzyme inhibitory activity of 50%). This value can be obtained from the measurement results of linear regression which states the relationship between the concentration of the extract sample as the x-axis and the percent Inhibition value as the y-axis. Based on tests that have been carried out, it shows that acarbose has an IC_{50} value = 27.14 µg/ml. Meanwhile, in the Moringa leaves ethanol extract sample, IC_{50} = 81.74 µg/ml and in the methanol partition and n-hexane partition samples, the Moringa leaves has an IC_{50} value of 105.30 µg/ml and 158.29 µg/ml respectively. A comparison graph of the IC_{50} value of samples with acarbose can be seen in Figure 4.

The level of strength of the IC_{50} value, namely that a compound is grouped as very active (IC_{50}: < 50 µg/ml), active (IC_{50}: 50-100 µg/ml). ml), moderate (IC_{50}: 101-250 µg/ml), weak (IC_{50}: 250-500 µg/ml) and inactive (IC_{50}: > 500 µg/ml)(Jun *et al.*, 2006). This shows that the ethanol extract sample of Moringa leaves has active activity in inhibiting α-amylase enzyme, while the methanol partition and n-hexane partition samples of Moringa leaves have moderate activity in inhibiting α-amylase enzyme.

From the research that has been carried out, it is known that acarbose has greater α-amylase enzyme inhibitory activity compared to the sample. It is known that the smaller the IC_{50} value obtained, the greater the inhibitory activity. This quite high difference in value can be caused because acarbose is a drug that works as a competitive inhibitor of the α-amylase enzyme which has been widely used in diabetes mellitus sufferers (Park, K. H., 1998).

From the results of the phytochemical screening test, it can be seen that there is a relationship between the compounds contained in the Moringa leaves samples which have the ability to inhibit α-amylase enzyme. Compounds contained in the Moringa plant which are thought to play a role in inhibiting the α-amylase enzyme are flavonoids, alkaloids, polyphenols and tannins (Al, 2017 ;Himmah and Handayani, 2012). In vitro (Samudra, Nugroho and Husni, 2015), has proven that flavonoids have the potential to inhibit α-Amylase enzyme and the α -glucosidase enzyme. Where flavonoids are known to play a role in antidiabetic activity by significantly reducing blood glucose levels. Apart from that, polyphenolic compounds and tannins can also play a role in inhibiting α-amylase enzyme. Polyphenols will selectively reduce starch hydrolysis in vitro by the enzyme α-amylase (Himmah and Handayani, 2012).

Conclusion

From the results of the research that has been carried out, it can be concluded that there is α-amylase enzyme inhibitory activity with moderate strength in samples of methanol partition and n-hexane partition of Moringa leaves carried out in vitro, with IC_{50} values of 105.30 µg/ml and 158.29 µg/ml. Meanwhile, the Moringa leaves ethanol extract sample was carried out in vitro, with an IC_{50} value of 81.74 µg/ml

Acknowledgement

Thanks to the University of Muhammadiyah Pekajangan Pekalongan.

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