Inhibition of α-glucosidase activity by ethanolic extract of *Melia azedarach* L. leaves

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Abstract. Development of α-glucosidase inhibitor derived from natural products is an opportunity for a more economic management of diabetes prevention. The objective of this study was to test the activity of α-glucosidase with or without potential inhibitor compounds. By *in vitro* method, α-glucosidase hydrolizes *p*-nitrophenyl-α-D-glucopiranoside to glucose and the yellow of *p*-nitrophenol which can be determined with spectrophotometry at 400 nm. The ability of ethanolic leaf extract of *Melia azedarach* L. as α-glucosidase inhibitor was compared with that of commercial acarbose (Glucobay®). Acarbose showed strong inhibitory activity against α-glucosidase with IC_{50} values of 2.154 µg/mL. The crude ethanolic leaf extract of *M. azedarach,* however, showed less inhibitory activity with IC_{50} value of 3,444.114 µg/mL. Total phenolics of *M. azedarach* leaves EtOH extract showed 17.94 µg GAE/mg extract and flavonoids total compound of 9.55 µg QE/mg extract. Based on the published wide range of IC_{50} values of extracts reported as α-glucosidase inhibitor which were between 10,000 ppm-0.66 ppm, our result suggests that extract of *M.azedarach* leaves is potential candidate for development of anti-hyperglycemic formulation.

1. Introduction

α-Glucosidase is an intestinal enzyme that catalyzes the break of α-1.4-glycosidic bond in oligosaccharides into α-glucose molecules which can be absorbed by the intestine[1]. Development of α-glucosidase inhibitor derived from natural products is an opportunity for a more economic management of diabetes mellitus prevention. Diabetes mellitus is a disease characterized by hyperglycemia which increased levels of sugar in the blood exceeds normal levels with fasting glucose levels ≥ 126 mg / dL and 2 hours after eating ≥ 200 mg / dL. The disease is caused by lack of pancreas β cells to produce insulin or the cell resistance of insulin. One therapeutic approach to decreasing postprandial hyperglycemia is to retard the absorption of glucose via inhibition of carbohydrate-hydrolyzing enzymes, such as glucosidase, in the intestine[2]. The glucosidase enzymes are located in the brush border of the small intestine and are required for the breakdown of carbohydrates before monosaccharide absorption. The α-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glycemia and insulin peaks[3].

The objective of this research was to test the activity of α-glucosidase with or without potential inhibitor compounds. By in vitro method, α-glucosidase hydrolizes p-nitrophenyl-α-D-glucopiranoside to glucose and the yellow of p-nitrophenol which can be determined with spectrophotometry at 400

nm. The ability of ethanolic leaf extract of *Melia azedarach* L. as α-glucosidase inhibitor was compared with that of commercial acarbose (Glucobay®). *M. azedarach* L., known as mindi, is a forest plant that has been used traditionally as medicinal plant for diabetes mellitus. Jo et al.[4] reported that flavonoid which is one of its phytochemical compounds can inhibit α-glucosidase by hydroxylating C-3 of the flavonol ring carbon. Acarbose is structurally similar to oligosaccharides, but it has a higher affinity around 10^4-10^5 to bind α-glucosidase, thus acarbose is a competitive inhibitor, which resulted in the decreased formation of monosaccharides from oligosaccharides[5]. Therefore, the results will provide scientific informations on the potency of *M.azedarach* L. leaf extract in the development of antidiabetic natural products formulation with antihyperglicemic mechanism of action.

2. Materials and Methods

2.1 Total phenolic compound determination[6]

Plant extract stock solution with concentration of 1000 µg/mL was made with methanol. Five mL of extract solution was added with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.5 mL of 7.5% Na_2CO3. The mixture in triplicate were then incubated in the waterbath at 45°C for 45 minutes. Absorbancy was determined with spectrophotometer at 765 nm wavelength. Similar protocol was done to prepare the galic acid standard curve with the following concentrations of 10, 20, 30, 40, and 50 µg/mL. The extract total phenolic content was expressed as mg galic acid equivalent (GAE)/g of extract.

2.2 Total flavonoid determinatiorn[7]

Plant extract solution with concentration of 1000 µg/mL was prepared in methanol. Five mL of extract solution in triplicate were added with 0.3 mL of 5% $NaNO_2$ and 0.3 mLof 10% AlCl3 and was kept at room temperature for 5 minutes. These mixtures were then added with 2 mL of 1M NaOH and the volume was made up to 10 ml with distilled water. Absorbancy was determined with spectrophotometer at 510 nm wavelength. Similar protocol was used to prepare quercetin standard curve in varying concentrations. The extract total flavonoid content was expressed as mg quercetin equivalent (QE)/g of extract.

2.3 *In vitro assay of α-glucosidase activity (modified from Lelono & Tachibana* [8]*)*

Enzyme solution was prepared by dissolving 1 mg of α-glucosidase in 100 mL of phosphate buffer (pH 7) which contained 200 mg of bovine serum albumin. Prior to use, 1 mL of enzyme solution was diluted 25 times with phosphate buffer (pH 7). The reaction mixture was prepared in the microplate wells which consisted of 25 µl of 10 mM *p*-nitrophenyl-D-glucopyranose as substrate and 50 µl of 100mM phosphate buffer (pH 7). Briefly, plant extract was dissolved in DMSO and aliquots of extract samples (10 µL) was added to the reaction mixture to final concentrations of: 50, 100, 200, 500, 1000, 5000, 7500, and 10,000 µg/mL. Solution of 1% acarbose (Glucobay®) was prepared with phosphate buffer pH 7. Then it was mixed with 2N HCl of equal volume (1:1) and was centrifuged. Aliquots of supernatant (10 µL) was taken and added into the reaction mixture at final concentration of 0.1, 0.5, 1, 5, and 10 µg/mL. Blanks, controls and each concentration of samples were done in triplicate. Following incubation at 37°C for 5 minutes, 25 µl of enzyme solution was added into the reaction mixture and incubated further for 15 minutes. Enzyme reaction was stopped by adding 100 µl of 200mM Na_2CO_3. Blanks, controls, and samples absorbance of the *p*-nitrophenol product was measured by microplate reader spectrophotometer at 400 nm wavelength.

Percent of inhibition of the enzyme activity was calculated using the following formula:

$$\% \text{ of inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

All data analyses were done using Microsoft Excel and expressed as the average of triplicate.

3. Results and Discussion

Acarbose, a commercially known α-glucosidase inhibitor[9], showed strong inhibitory activity against α-glucosidase with IC_{50} values of 2.154 μg/mL (Figure 1). This data is consistent with study by Permasku [10] who reported smaller acarbose's IC_{50} value of 1.46 μg/mL. Using similar in vitro system, Septiawati[11] reported that acarbose at concentration of 10,000 μg/mL inhibited αglucosidase activity by 99.34%. Acarbose is an oligosaccharide derived from the *Actinoplanes* strain of fungi[9]. Due to its similarity to the structure of oligosaccharide, acarbose acted as competitive inhibitor of the enzyme in this study. According to Arungarinthan *et al*.[9] the mechanism of action is predominantly through competitive, reversible inhibition of intestinal brush border α-glucosidase, with a weaker effect on pancreatic α-amylase. Acarbose, the first α-glucosidase inhibitor to be identified, is currently used for the treatment of type 2 diabetes [9].

On the other hand, crude ethanolic leaf extract of *M. azedarach* showed less inhibitory activity with IC_{50} value of 3444.114 μg/mL (Figure 2). The inhibitory activity of the ethanolic extract of *M.azedarach,* however, was higher than that of ethanolic extract of *Graptophyllum pictum Griff* (Daun Wungu)[12] and *Orthosiphon stamineus* Benth (Kumis Kucing)[13] which inhibited 50% of the α-glucosidase at concentration of 10,000 ppm. The IC_{50} of ethanolic bark extract of *Toona sinensis* Merr. (suren) was 0.66 ppm [14] and that of ethanolic seed extract of *Swietenia mahagony* Jacq was 100 ppm [15]. Based on these IC_{50} values, *M.azedarach* ethanolic extract was better and more potential enzyme inhibitor than *Graptophyllum pictum Griff* and *Orthosiphon stamineus* Benth. The inhibition of this enzyme can be achieved by various natural compounds such as the phenolic group, the flavonoids, luteolin, miricetin, and quercetin[16]. Therefore, inhibitory activity of mindi leaf extracts was likely due to phytochemical compounds contained in the extract.

Quantitative analysis of total flavonoids content showed that there was 9.55 μg QE/mg extract. Previous *s*tudy by Purnama[17] showed that the total flavonoid of ethanolic extract was 5.99 mg *QE/g* extract. Marek et al.[18] reported higher flavonoids content in 70% ethanolic extract (15.91 mg QE/g extract). This is consistent with the fact that our extraction was using 96% ethanol which is less polar compare to the 70% ethanol, thus resulted in less amount of flavonoids that can be extracted. This amount of flavonoids is relatively small compared to other medicinal plant such as the rhizome of bawang dayak that had been reported by Febrinda *et al*.[19]. which contained as much as 65.35 mg QE/g extract. Flavonoids are naturally occurring phenolic compounds that are widely distributed in plants and some of them have been described as glucosidase inhibitors[20,21].

Total phenolics of *M. azedarach* leaves ethanolic extract was as much as 17.94 μg GAE/mg extract. This data is consistent with that of Purnama [17] which reported total phenolic compound of 17.77 mg GAE/g extract. Nahak & Rajani[22] reported higher content of total phenolics in ethanolic extract of *M.azedarach* as much as 360 μg CE/mg extract, and also that of Ahmed *et al.*[23] was as much as 492 μg CE/mg ekstrak. As comparison, the rhizome of bawang dayak contained as much as 217.71 mg GAE/g extract[19].

Our result suggests that extract of M.azedarach leaves is potential candidate for development of anti-hyperglycemic formulation.

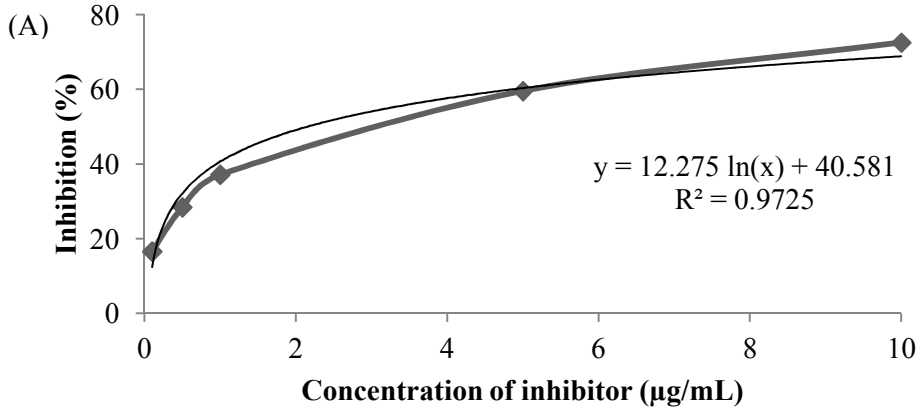

Figure 1. Inhibition of α-glucosidase by acarbose

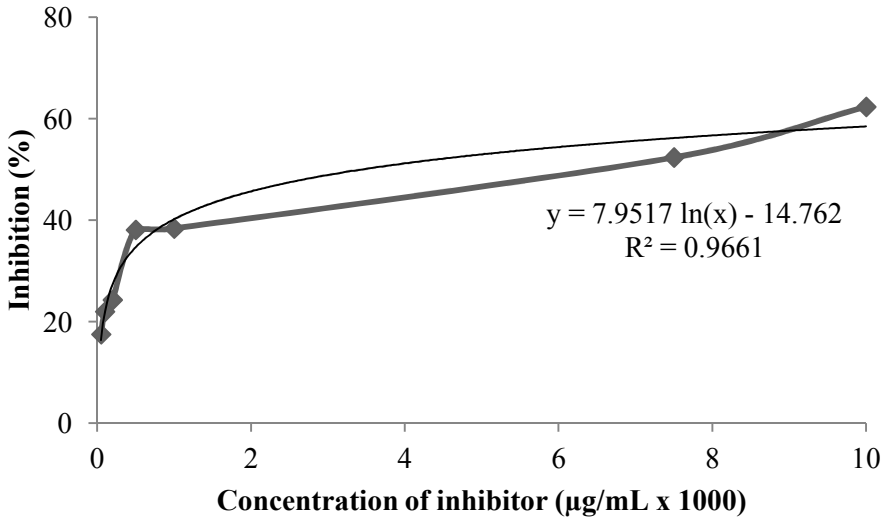

Figure 2. Inhibition of α-glucosidase by *M. azedarach* leaves EtOH extract

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