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

Sulistiyani¹,², Mega Safithri¹, Yoana Puspita Sari¹

¹Departement of Biochemistry, Bogor Agricultural University, Jl. Agathis Dramaga, Bogor, Indonesia
²Biopharmaca Research Center, Bogor Agricultural University, Jl. Taman Kencana No.3, Bogor Indonesia

E-mail: sulistyanisoemardi@gmail.com

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 hydrolyzes p-nitrophenyl-α-D-glucopyranoside 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 IC₅₀ values of 2.154 µg/mL. The crude ethanolic leaf extract of M. azedarach, however, showed less inhibitory activity with IC₅₀ 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 IC₅₀ 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 intestine¹. 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 intestine². 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 peaks³.

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 hydrolyzes p-nitrophenyl-α-D-glucopyranoside 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 hydroxylation 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 oligosaccharides⁵. 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 antihyperglycemic 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% Na₂CO₃. 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 gallic 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 gallic acid equivalent (GAE)/g of extract.

2.2 **Total flavonoid determination**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% NaNO₂ and 0.3 mL of 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 Na₂CO₃. 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 Permasku10 who reported smaller acarbose’s IC$_{50}$ value of 1.46 μg/mL. Using similar in vitro system, Septiawati11 reported that acarbose at concentration of 10,000 μg/mL inhibited α-glucosidase 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 study by Purnama17 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 inhibitors20,21. 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 Purnama17 which reported total phenolic compound of 17.77 mg GAE/g extract. Nahak & Rajani22 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 extract. 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

\[ y = 12.275 \ln(x) + 40.581 \]
\[ R^2 = 0.9725 \]

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

\[ y = 7.9517 \ln(x) - 14.762 \]
\[ R^2 = 0.9661 \]

References

[1] Gao H, Huang Y, Gao B, Kawabata J. 2008. Chebulagic acid is a potent α-glucosidase inhibitor. *Biosci Biotechnol Biochem* 72: 601-603.

[2] Holman RR, Cull CA, Turner RC. A randomized double-blind trial of acarbose in type 2 diabetes shows improved glycemic control over 3 years. *Diabetes Care* 1999;22:960–4.

[3] Stuart AR, Gulve EA, Wang M. Chemistry and biochemistry of type 2 diabetes. *Chem Rev* 2004;104:1255–82.

[4] Jo SH, Ka EH, Lee HS, Apostolidis E, Jang HD, Kwon YI. 2009. Comparison of antioxidant potential and rat intestinal alpha glucosidases inhibitory activities of quercetin, rutin, and isoquercetin. *International Journal of Applied Research in Natural Products* 4: 52-60.

[5] Rosak C, Gabrielle M. 2012. Critical evaluation of the role of acarbose in the treatment of diabetes: patient consideration. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* 5: 357-367.
Pourmorad F, Hosseinimehr SJ, Shahabimajd N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African J. Biotech.* 5 1142-1146.

Sahu R, Jyoti S. 2013. Screening of total phenolic and flavonoid content in conventional and non-conventional species of curcuma. *J. Pharmacog and Phytochem.* 2 176-179.

Lelono RAA, Tachibana S. 2013. Preliminary studies of Indonesion *Eugenia polyantha* leaf extracts as inhibitory of key enzymes for type 2 diabetes. *Journal of Medical Sciences* 13 103-110.

Arungarinathan G, McKay GA, Fisher M. 2011. Drugs for diabetes: part 4 acarbose. *Br. J Cardiol.* 18 78-81.

Permasku G. 2014. Aktivitas inhibisi enzim α-glukosidase dan sitotoksisitas ekstrak kurkuminoid rimpang temulawak dari berbagai aksesi (*in vitro*) [skripsi]. Bogor (ID): Institut Pertanian Bogor.

Septiawati T. 2008. Daya hambat ekstrak etanol buah mahkota dewa terhadap α-glukosidase secara *in vitro* [skripsi]. Bogor (ID): Institut Pertanian Bogor.

Irwan F. 2011. Aktivitas anti diabetes dan analisis fitokimia ekstrak air dan etanol daun wungu (*Graptophyllum pictum* Griff) [skripsi]. Bogor (ID): Institut Pertanian Bogor.

Kurniawati A, Sulistiyani, Mega S. 2013. Identification of morphological characteristic, bioactive content, and alfa-glucosidase inhibitory activity of *Orthosiphon stamineus* Benth accession. *The proceeding of PPM IPB Research Seminar* 2 493-509.

Ichsan SA. 2011. In vitro activity of suren (*Toona sinensis* Merr.) bark extract as antioxidant and anti diabetic. [skripsi]. Bogor (ID): Bogor Agricultural University.

Febriyany V. 2014. Assay of alfa-glucosidase inhibitor and hypoglycemic potency of ekstrak mahoni seed (*Swietenia mahagoni* Jacq.) extract as antidiabetic agent [skripsi]. Bogor (ID): Bogor Agricultural University.

Tadera K, Yuji M, Kouta T, Tomoko M. 2006. Inhibition of α-glucosidase and α-amylase by flavonoids. *J. Nutr Sci Vitaminol.* 52 149-153.

Purnama RL. 2015. Aktivitas antioksidan, kandungan total fenol, dan flavonoid lima tanaman hutan yang berpotensi sebagai obat alami [skripsi]. Bogor (ID): Institut Pertanian Bogor.

Marek CB, Ana MI, Tereza CM, Rubiani AP, Natalie RT, Alana MR. 2012. Influence of leaf extracts from *Melia azedarach* L. on butyrylcholinesterase activity in rat liver. *J. Med Plants Res.* 6 3931-3938.

Febrinda AE, Made A, Tutik W, Nancy DY. 2013. Kapasitas antioksidan dan inhibitor alfa glukosidase ekstrak umbi bawang dayak. *J. Teknol dan Industri Pangan* 24 161-167.

Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MSRB, Folador P, Damazio RG, et al. 2008. Flavonoids: prospective drug candidates. *Mini Rev Med Chem* 8 1429–40.

Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MSRB, Folador P, Damazio RG, et al. 2008. Flavonoids: cellular and molecular mechanism of action in glucose homeostasis. *Mini Rev Med Chem* 8 1032–8.

Nahak G, Rajani KS. 2010. *In vitro* antioxidantive activity of *Azadirachta indica* and *Melia azedarach* leaves by DPPH scavenging assay. *Nat and Sci.* 8 22-28.

Ahmed MF, Rao AS, Shaik RA, Mohammed I. 2012. Phytochemical studies and antioxidant activity of *Melia azedarach* Linn leaves by DPPH scavenging assay. *International Journal of Pharmaceutical Applications* 3 271-276.