INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ISOLATION OF A-GLUCOSIDASE INHIBITORY ACTIVE COMPOUNDS FROM ETHANOL EXTRACT OF KAYU TUAH (*ANTIDESMA CELEBICUM* MIQ.) LEAVES

Novi Fajar Utami[1,2]*, Berna Elya[2], Katrin[2]
[1]Department of Pharmacy, University of Pakuan, Bogor, Indonesia
[2]Faculty of Pharmacy, University of Indonesia, Depok, Indonesia
*Corresponding Author Email: novisjaf@gmail.com

Article Received on: 30/11/14 Revised on: 03/12/14 Approved for publication: 02/01/15

DOI: 10.7897/2230-8407.0615

ABSTRACT
Antidesma celebicum Miq. belongs to Euphorbiaceae family has been used as antidiabetic traditional medicine. The aim of the research was to isolate the α-Glucosidase inhibitory compound from ethanol extract of *Antidesma celebicum* leaves. The isolation have been done using column chromatography silica gel 60 and the structure was determinated base on spectral data of IR, MS, NMR. The result showed that the isolation obtained gallic acid as α-Glucosidase inhibitor with IC_{50} value of 0.057 mM.

Keyword: *Antidesma celebicum* Miq., leaves, α-Glucosidase, antidiabetic

INTRODUCTION

Diabetes mellitus is well recognized as a major health problem associated with increased morbidity and mortality and high health care costs. It is characterized by hyperglycemia and alteration in carbohydrate, protein, and lipid metabolism caused by defects in insulin production or action.[1] Postprandial hyperglycemia is a prominent and early defect in diabetes which can in turn lead to various secondary complications including risk factor for cardiovascular diseases.[2,3] Therefore one therapeutic approach for treating diabetes is to control the postprandial hyperglycemia by retarding the absorption of glucose. α-Glucosidase is a key enzyme in carbohydrate digestion. It catalyzes the hydrolysis of 1,4-α-glucosidic bonds within carbohydrates with release of α-glucose and promotes the increase of blood glucose levels after meal. Alpha-Glucosidase inhibitors antagonize the activity of α-Glucosidase, there by delaying intestinal carbohydrate absorption and slowing the sharp rise in blood sugar levels that diabetic patients typically experience after meal.[4] For this reason, α-Glucosidase inhibitors, such as acarbose and voglibose, are clinically used as oral anti hyperglycemic agents.[5,6] However, they often cause severe gastrointestinal side effects such as flatulence and diarrhea. Therefore, search for new α-Glucosidase inhibitors from natural resources has become an attractive approach for the treatment of postprandial hyperglycemia. Plant materials and herbal extracts have been used in diabetes traditional medicine. Plants that contain polyphenolic compounds have been known interact with proteins and inhibit enzyme activities.[7] The previous research showed that plants from Euphorbiaceae family possess α-Glucosidase inhibitory activity, including kayu tuah. Based on the previous study, the IC_{50} value of 80 % ethanol extract from kayu tuah leaves is 3.92 µg/mL but it has not known the active compound that has the highest inhibitory activity of α-Glucosidase.[8] Therefore, this reseach was performed to isolate the α-Glucosidase inhibitory compound from ethanol extract of *Antidesma celebicum* leaves.

MATERIALS AND METHODS

Leaves of kayu tuah were collected in January 2013 and identified by The Center for Plant Conservation from Bogor Botanical Garden with the authentic number is 2466/IPH.3.02/KS/VI/2013. The specimen was deposited by Herbarium of Pharmacognosy Laboratorium Faculty of Pharmacy, University of Indonesia (24/A/HLF/UI).

Chemicals

n-hexane, ethyl acetate, methanol,α-Glucosidase enzyme (Sigma Chemical Co.), p-nitrofenil-α-D-glucopiranoside (Sigma Chemical Co.), acarbose.

Extraction and Isolation

The simplisia powder (3.013 kg) was refluxed for 1 hour with 70 % ethanol for 3 times and then evaporated. Extract was dispersed in water with the ratio of 1:1, and then performed with a liquid chromatography used n-hexane, ethyl acetat, and methanol. A number of 20.0 g fraction with the highest α-Glucosidase inhibitory activity was isolated by coloumn chromatography silica gel 60 with n-hexane, ethyl acetate and methanol as mobile phases. Then the highest α-Glucosidase inhibitory activity fraction obtained some subfractions.

Inhibition Assay for α-Glucosidase activity

Inhibition of the α-Glucosidase assay was performed on all fractions and isolate. Research procedure refers to method of Kim *et al*.[9] Samples (5 to 500 µg/mL) as much as 30 ml was added with 36 ml phosphate buffer pH 6.8 and 17 ml p-nitrofenil-α-D-glucopiranoside, incubated for 5 minutes at 37°C. Into the sample solution was added 17 ml α-Glucosidase enzyme (0.15 unit/mL), then incubated again for 15 minutes at 37°C. After incubation period was completed, added 267 mM sodium carbonate to stop the reaction. Solution absorbance was measured with a microplate reader at λ 405 nm. In addition to test the extract performed as well as a positive control inhibition activity (acarbose). The IC_{50} were performed which the concentration of extract that inhibit 50 % α-Glucosidase activity.

Structure Identification

The structure of isolate was determinated base on spectral data of IR, MS, NMR.

RESULTS

Assay for α-Glucosidase inhibitory activity

Inhibitory activity of α-Glucosidase was tested in 70 % ethanol extract of kayu tuah leaves. Table 1 show that the ethyl acetate fraction of kayu tuah leaves from the liquid chromatography has IC_{50} value 57.60 µg/mL and subfraction (E) has IC_{50} value 68.30 µg/mL. Table 2 shows that the isolate have the high inhibitory activity of α-Glucosidase than acarbose with IC_{50} 0.057mM.

Isolation

Isolated from ethyl acetate fraction obtained 8 sub fractions and subfraction (E) has the highest α-Glucosidase inhibitory activity with IC_{50} value 68.30 µg/mL. Subfraction (E) then isolated again using a smaller column chromatography to obtain pure compounds. Purification results obtained isolate.

Structure Identification

Isolate is soluble in methanol and with reagent $FeCl_3$ 3 % is dark blue. Data LC-MS showed that the isolate has a molecular weight $[M^+]$ = 170 with molecular formula $C_7H_6O_5$. Data spectra FTIR of isolate on the wavelength υ = 3286.81 cm^{-1}, 3497.06 cm^{-1} indicates the presence of the O-H bond stretching vibration, the wavelength υ = 1701.27 cm^{-1} indicates the presence of the carboxylic group stretching vibration =COOH, and the wavelength υ = 1541.18 cm^{-1} indicates the presence of an aromatic ring -C=C-. Data ^{1}H-NMR spectra (solvent CD_3OD, 500 MHz) obtained δ 7:05 (1H, s). Data ^{13}C-NMR spectra (CD_3OD, 500 MHz) obtained δ 110.37; 122.71; 139.45, and 146.44 atom C in the aromatic ring, δ 170.95 indicates the presence of C=O carbonyl. Based on the literature it is known that isolate were obtained from the results of this study are similar to the structure of gallic acid (3, 4, 5 acid trihidoksibenzoat) Figure 2 Structure Gallic acid.

Table 1: Data of IC_{50} value from extract ethanol 80 % from kayu tuah leaves

No	Sample	IC_{50} (µg/mL)
1	Acarbose	38,37
	Fraction from liquid chromatography of kayu tuah leaves	
2	n-Hexane	126,18
3	Ethyl acetate	57,60
4	Methanol	61,91
	Subfraction from chromatography column of kayu tuah leaves	
5	A (n-hexane:ethyl acetate)	141,51
6	B (n-hexane:ethyl acetate)	141,64
7	C (n-hexane:ethyl acetate)	94,00
8	D (n-hexane:ethyl acetate)	92,58
9	E (n-hexane:ethyl acetate)	68,30
10	F (n-hexane:ethyl acetate)	84,67
11	G (ethyl acetate:methanol)	99,62
12	H (ethyl acetate:methanol)	166,68

Table 2: Data of IC_{50} value from gallic acid

Sample	IC_{50} (mM)
Acarbose	0,005
Gallic Acid	0,057

Figure 1: Kayu tuah (*Antidesma celebicum* Miq.)

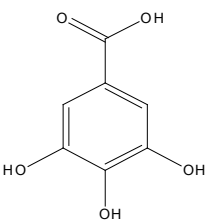

Figure 2: Structure of gallic acid

DISCUSSION

Ethyl acetate fraction of kayu tuah (*Antidesma celebicum* Miq.) leaves had the highest α-Glucosidase inhibitory activity. The ethyl acetate fraction was conducted by chromatographic coloumn using various organic solvent and obtained 8 sub fractions (A-J). The subfraction E [n-hexane:ethyl acetate (20:80)] had the highest activity as antidiabetic. Subfraction (E) then isolated again using a smaller column chromatography and the isolation obtained gallic acid as α-Glucosidase inhibitor with IC_{50} value of 0.057 mM. In this study, gallic acid illustrate as potential antidiabetic treatment. Ellagic acid (gallic acid polymer) in meniran leves (*Phyllantus niruri* L; Euphorbiaceae) have hypoglycemic activity in experimental animals as well as diabetic patient. Ellagic acid in plants can inhibit enzyme aldose reductase. This enzyme works in the polyol pathway (formation of sorbitol and fructose than

glucose). In diabetic patients, the process conversion of glucose to fructose was disturbed. Since the action of the enzyme aldose reductase is inhibited by ellagic acid so that the increase in blood glucose can be lowered and the glucose metabolism toward equilibrium.[10]

ACKNOWLEDGEMENT

The authors thank to Faculty of Pharmacy, University of Indonesia for supporting this project.

REFERENCES

1. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 1998; 21: 1414-1431. http://dx.doi.org/10.2337 /diacare.21.9.1414
2. Carroll MF, Gutierrez A, Castro M, Tsewang D, Schade DS. Targeting postprandial hyperglycemia: a comparative study of insulinotropic agents in type 2 diabetes. J Clin Endocrinol Metab 2003; 88: 5248-5254. http://dx.doi.org/10.1210/jc.2003-030649
3. Slama G, Elgrably F, Sola A, Mbemba J, Larger E. Postprandial glycaemia: a plea for the frequent use of delta postprandial glycaemia in the treatment of diabetic patients. Diabetes Metab 2006; 32: 187-192. http://dx.doi.org/10.1016/ S1262-3636(07)70268-9
4. M Koyasu, H Ishii, M Watarai, K Takemoto, Y Inden, K Takeshita, T Amano, D Yoshikawa, T Matsubara and T Murohara. Impact of acarbose on carot id intima-media thickness in patients with newly di agnosed impaired glucose tolerance or mild type 2 diabetes mellitus: A one-year, prospective, randomized, open-label, parallel-group study in Japanese adults with establ ished coronary artery disease, Clin. Ther 2010; 32: 1610-1617. http://dx.doi.org/10.1016 /j.clinthera.2010.07.015
5. S Frantz, L Calvillo, J Tillmanns, I Elbing, C Dienesch, H Bischoff, G Ertl and J Bauersa Chs. Repetitive postprandial hyperglycemia increases cardiac ischemia/reperfusion injury: prevention by the alpha-Glucosidase inhibitor acarbose, FASEB J. 2005; 19: 591-593.
6. M Shimabukuro, N Higa, I Chinen, K Yamakawa and N Takasu. Effects of a single administration of acarbose on postprandial glucose excursion and endothelial dysfunction in type 2 diabetic patients: a randomized crossover study. J. Clin. Endocrinol. Metab 2006; 91: 837-842. http://dx.doi.org/ 10.1210/jc.2005-1566
7. Oboh, Ademiluyi, Akinyemi, Henle, Saliu and Schwarzenbolz. Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorus olitorius*) on key enzyme linked to type 2 diabetes (a-amylase and a-Glucosidase) and hypertension (angiotensin I converting) *in vitro*. Journal of Functional Foods 2012; 4: 450-458. http://dx.doi.org/10.1016/j.jff.2012.02.003
8. Elya, Berna, Basah Katrin, Munim Abdul, Yuliastuti Wulan, Bangun Anastasia and Eva Septiana Kurnia. Screening of α-Glucosidase Inhibitory Activity from Some Plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. Journal of Biomedicine and Biotechnology; 2012. http://dx. doi.org/10.1155/2012/281078
9. Kim KY, Nam KA, Kurihara H and Kim SM. Potent α-Glucosidase Inhibitors Purified from the Red Alga *Grateloupia elliptica*. Phytochemistry 2008; 69: 2820-2825. http://dx. doi.org/10.1016/j.phytochem.2008.09.007
10. Hernawan UE, Setyawan AD. Review : Ellagitanin ; Biosynthesis, Isolation, and Biologic Activity. Surakarta: Sebelas Maret University(1); 2003. p. 25–38.

Cite this article as:
Novi Fajar Utami, Berna Elya, Katrin. Isolation of α-glucosidase inhibitory active compounds from ethanol extract of Kayu tuah (*Antidesma celebicum* Miq.) leaves. Int. Res. J. Pharm. 2015; 6(1): 22-24 http://dx.doi.org/10.7897/2230-8407.0615

Source of support: Nil, Conflict of interest: None Declared