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Research Article

ALFA GLUCOSIDASE INHIBITORY ACTIVITY OF KAYU TUAH (*ANTIDESMA CELEBICUM* MIQ.)

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ABSTRACT
Ethanolic extracts of kayutuah (*Antidesma celebicum* Miq.) leaves and stem bark were evaluated for their antidiabetic activity by inhibition of α-Glucosidase. Each ethanolic extract was fractionated using n-hexane, ethyl acetate, and methanol. The result showed that the ethyl acetate fraction of kayutuahstem bark had the highest α- Glucosidase inhibitory activity with IC50 value 8.06 µg/mL. The ethyl acetate fractionwas conducted by chromatographic column using various organic solvent and obtained 10 sub fractions (A-J). The subfraction2.E [n-hexane:ethyl acetate (20:80)] had the highest activity as anti diabetic with IC_{50} value 5.60 µg/mL and itscontain of tannins, saponins, terpens, and glycosides.
Keyword: α-Glucosidase, *Antidesma celebicum*, leaves, stem bark

INTRODUCTION

Diabetes mellitus is a metabolic disorder of fats, carbohydrates, and proteins caused by defect of insulin secretion, insulin sensitivity or both.[1] If insulin is not available in the body, the glucose will be accumulated in the blood and excreted into the urine immediately. Worldwide survey reported that diabetes affects nearly 10 % of the population and a common diabetes cases are type 2 diabetes mellitus. Cases of diabetes mellitus reach 90 % of all cases of diabetes mellitus accidents.[2] Treatment of diabetes can be performed with insulin injection or by using of modern drugs such as oral antidiabetic. For type 1 diabetes mellitus, insulin injections are used for treatment. While for type 2 diabetes mellitus, oral antidiabetic treatments are commonly used. α-Glucosidase inhibitors are therapeutic agent for the treatment of carbohydrate metabolism disorders especially diabetes mellitus.[3] Acarbose is one of the α-Glucosidase inhibitors, but the synthetic α-Glucosidase inhibitors have side effects such as gastrointestinal disorders.[4,5] Therefore, the drug needs to be developed from natural materials which have relatively small side effects compared with conventional drugs. Plant materials and herbal extracts have beenused in diabetes traditional medicine. Plants that contain polyphenolic compounds have been known interact with proteins and inhibit enzyme activities.[6] The previous research showed that plants from Euphorbiaceae family possessα-Glucosidase inhibitory activity, including kayutuah. Based on the previous study, the IC_{50} value of 80 % ethanol extract fromkayutuah leaves is 2.34 and the stem bark is 3.92 µg/mL but it has not known which fraction that has the highest inhibitory activity of α-Glucosidase.[7] Therefore, this research was performed to know the highest active fraction and chemical compounds of kayutuah leaves and stem bark.

MATERIALS AND METHODS

Material Test

Leaves and stem bark of kayutuah 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 Fractionation

The simplisia powder (3.0 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 acetate, and methanol. A number of 20.0 g fraction with the highest α-Glucosidase inhibitory activity was fractionated by column chromatography with n-hexane, ethyl acetate, and methanol as mobile phases. Then the highest α-Glucosidase inhibitory activity fraction obtained some sub fractions.

Inhibition Assay for α-Glucosidase activity

Inhibition of the α-Glucosidase assay was performed on all fractions. Research procedure refers to method of Kim *et al*.[8] 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 totest 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.

Phytochemistry Test

Identification of phytochemicals was performed on the highest α-Glucosidase inhibitory activity fraction.

RESULTS

Assay for α-Glucosidase inhibitory activity

Inhibitory activity of α-Glucosidase was tested in 70 % ethanol extract of kayutuah leaves and stem bark. Table 1 show that the ethyl acetate fraction of kayu tuah leaves (2) from the liquid chromatography has IC_{50} value 57.60 and sub

fraction (2.E) has IC_{50} value 68.30 µg/mL. Table 2 show that the ethyl acetate fraction of kayu tuah stem bark (2) from the liquid chromatography has IC_{50} value 8.06 and sub fraction (2.E) has IC_{50}value 5.60 µg/mL.

Phytochemistry Test

Chemical compounds which active to inhibit α-Glucosidase activity are alkaloids, flavonoids, tannins, glycosides, saponins, terpenes, and quinones. Chemical compounds are contained in the ethyl acetate fraction are shown in Table 3.

Table 1: Data of IC_{50} value from 80 % ethanol extract of kayutuahleaves

No	Sample	IC_{50} (µg/mL)
1	Acarbose	38.37
	Fraction from liquid chromatography	
2	n-Hexane	126.18
3	Ethyl acetate	57.60
4	Methanol	61.91
	Sub fraction from chromatography column of kayutuah 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 extract ethanol 80 % from kayutuah stem bark

No	Sample	IC_{50} (µg/mL)
1	Acarbose	38.37
	Fraction from liquid chromatography of kayutuah stem bark	
2	n-Hexane	34.38
3	Ethyl acetate	8.06
4	Methanol	27.49
	Sub fraction from chromatography column of kayutuah stem bark	
5	A (n-hexane:ethyl acetate)	24.44
6	B (n-hexane:ethyl acetate)	24.33
7	C (n-hexane:ethyl acetate)	24.69
8	D (n-hexane:ethyl acetate)	26.63
9	E (n-hexane:ethyl acetate)	5.60
10	F (n-hexane:ethyl acetate)	27.23
11	G (ethyl acetate:methanol)	18.70
12	H (ethyl acetate:methanol)	30.67
13	I (ethyl acetate:methanol)	46.54
14	J (ethyl acetate:methanol)	25.75

Table 3: Phytochemical test of fractions EtOAC of kayutuah (*Antidesma celebicum* Miq.) leaves and stem bark

Chemical Compounds	EtOAC fractions of kayutuah (*Antidesma celebicum* Miq.) leaves and stem bark	
	Leaves	Stem Bark
Alkaloid	-	-
Flavonoid	+	-
Tanin	+	+
Saponin	+	+
Antrakuinon	-	-
Terpen	-	+
Glikosida	+	+

Key: +: present; -: absent

Figure 1: kayutuah (*Antidesma celebicum* Miq.)

DISCUSSION

Ethyl acetate fraction of kayutuah (Antidesma celebicum Miq.) stem bark had the highest α-Glucosidase inhibitory activity. The ethyl acetate fraction was conducted by chromatographic column using various organic solvent and obtained 10 sub fractions (A-J). The sub fraction 2.E [n-hexane:ethyl acetate (20:80)] had the highest activity as anti diabetic and it contains of tannins, saponins, terpens and glycosides. Therefore, the sub fraction 2.E can be used as novel anti diabetic treatment.

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