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Antidiabetic Activity Test by Inhibition of α-Glucosidaseand Phytochemical Screening from the Most Active Fraction of Buni (Antidesma bunius L.) Stem Barks and Leaves.

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Abstract: Fractions of 80% ethanol (EtOH) extract from buni (*Antidesma bunius* L.) stem barks and leaves were evaluated by α-glucosidase inhibitory activity.Ethyl acetate(EtOAc) fraction of buni stem barksand methanol(MeOH) fraction of buni leaves showed the highestα-glucosidase inhibitory activity with IC_{50} values 5.73 and 8.04 ppm. Phytochemical analysis ofethyl acetate fraction of buni stem barks displayed the presence of sugars, terpenes, and flavonoids, while MeOH fraction of buni leaves contains sugars, saponins, flavonoids, and tannins.Thosewere fractionated using vacuum liquid chromatography and combined based on TLC profiles. The result showed that G subfraction (EtOAc: MeOH (180:20) fraction) of buni stem barks and F subfraction(EtOAc: MeOH (60:140), (40:160), (20:180), (0:200)) of buni leaves are the most active with IC_{50} values 1.16 and 4.79 ppm. Phytochemical screening indicated the presence of sugars, and flavonoids in both G subfraction and Fsubfraction. Kinetic analyses both of simplisias confirmed that the inhibition of hydrolysis is competitive mode.
Keywords: α-glucosidase, Buni, stem bark, leaves.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate, protein, and fat that characterized by hyperglycemia[1]. Diabetes mellitus and its complications are the most important health problem in modern society. World Health Organization estimates that in 2010 Indonesia was in ninth position in the world's largest number of diabetic patients with ages 20 to 79. WHO predicts the number of people with diabetes mellitus in Indonesia rise from 7,0 million in 2010 to about 12 million people in 2030[2].The increasing prevalence of diabetes mellitus in developing countries, like Indonesia, because of prosperity in the country. Increasing in income per capita, the number of fast food restaurants, sophisticated technology that can lead to sedentary life, led to an ascending prevalence of degenerative diseases,especially diabetes mellitus[3].

Plant materials and herbal extracts have long been used in traditional treatment of diabetes. Plants contain compounds that are rich in polyphenols, which are known to interact with proteins and can inhibit enzyme activity[4]. A research has shown that 80% EtOHextract ofbuni (*Antidesmabunius*L.) stem barkand buni leaves have inhibitory activity against α-glucosidase with IC50 value of 3.90 and 7.90 ppm[5]. It shows that buni stem bark and leaves can be developed as an antidiabetic. Based on these results, this study tested the α-glucosidase inhibitory activity of

fractions inboth simplisias and phytochemical screening from the most active fraction.

Method and Material

Plant material. The stem barks and leaves of buni were collected in February 2012and identified by Centre for Plant Conservation-Bogor Botanical Garden.

Extraction and Fractionation of Plant - Materials:
Each dried powdered of stem barks (590.4 g) and leaves (593.7 g) were extracted by reflux with EtOH 80% then evaporated. The viscous extract was dissolve in water 1:1, followed by liquid-liquid chromatograpyusing n-hexane, EtOAc, and MeOH. Fractionwhich have the highestα-glucosidaseinhibitory activitywasfractionated using vacuum liquid chromatography.Fraction which have the highestα-glucosidase inhibitory activitywas added silica gel 60then homogenized.The column was packed with silica gel 60 Hand the mixture of fraction-silica gel 60 placed on top of silica gel 60 H. EtOAc fraction of buni stem barks eluted with n-hexane: EtOAc (200:0, 190:10, 180:20, 170:30, 160:40, 150:50, 140:60, 130:70, 120:80, 100:100, 80:120, 60:140, 20:180, and 0:200) and EtOAc: MeOH with the same proportion, then obtained ten fractions combined based on TLC profiles. Whereas, MeOH fraction of buni leaves eluted with EtOAc: MeOH (200:0, 195:5, 190:10, 185:15, 180:20, 175:25, 170:30, 165:35, 160:40, 150:50, 140:60, 130:70, 120:80, 110:90, 100:100, 80:120, 60:140, 40:160, 20:180, and 0:200), then obtained six fractions combined based on TLC profiles.

Inhibition Assay for α-glucosidase activity:
The reaction mixture consisting 5 µL of sample at varying concentrations (0.1 to 33 µg/mL) was premixed with 245 µL phosphate buffer pH 6,8 and 125 µL of 5 mM*p*-nitrophenylα-D-glucopyranoside(Sigma Chemical Co.). After pre-incubating at 37^{o}C for 5 min, 125 µL α-glucosidase(0.15 unit/mL)(EC 3.2.1.20 from Sigma Chemical Co.)was added and incubated at 37^{o}C for 15 min. The reaction was terminated by the addition of 1000 µL Na_2CO_3 200mM.α-Glucosidase activity was determined by measuring release of p-nitrophenol at 400 nm. Acarboseand quercetinwas used as positive control of α-glucosidase inhibitor. The concentration of the extract required to inhibit 50% ofα-glucosidase activity under the assay conditions was defined as the IC_{50} value.

Kinetics of Inhibition against α-glucosidase:
Inhibition type of fraction thathad the highest α-glucosidase inhibiting activity in bunistem barks and leaveswere measured with increasing concentration of *p*-nitrophenyl α-D-glucopyranosideas a substrate in the absence or presence of extract. Enzyme kinetics data fit to Lineweaver-Burk plot to point out mode of inhibition.

Phytochemistry Test:
Phytochemistry test of fractions which have the highestα-glucosidase inhibitingactivity,has been performed. Standard protocols according to Harborne, Farnsworth, and Wagner andBladt were followed to find the presence of flavonoids, tannins, anthraquinones, terpenes, saponins, sugars, and alkaloids.

Results and Discussion

Assay for α-glucosidase inhibitory activity:
Inhibitory activity of fractions from 80% ethanol extract of buni stem barks and leaves against yeast α-glucosidasewas evaluated. In Table 1, EtOAcfraction of buni stem barks and MeOH fraction of buni leaves from liquid-liquid chromatography showed the highest α-glucosidase inhibitoryactivity with an IC_{50} of 5.73 and 8.04 µg/mL.G subfractionof buni stem barks and F subfraction of buni leaves from vacuum liquid chromatographyshowed the highest α-glucosidase inhibitory activity with an IC_{50} of 1.16 and 4.79 µg/mL.Inhibitory activity of the enzymeα-glucosidase at G and F subfractions may be due to the glycoside content in each fraction. Glycosides consist of sugars that may be structurally similar to carbohydrate which is a substrate of the enzyme α-glucosidase. IC_{50} value of fractionsfrom80% ethanol extract of stem barks and leaves of buniare lower than acarbose because acarbose had high inhibitory effects only on mammalianα-glucosidase but no inhibitory activity against yeast α-glucosidase[6]. Due to the reason above, quercetin was used as the second positive control based on its ability to inhibit yeast α-glucosidase[7].

Table 1.IC_{50} values of fractions from 80% ethanol extract of bunistem barks and bunileaves against yeast α-glucosidase

No	Sample	IC_{50} (µg/mL)
1.	Acarbose	129.75
2.	Quercetin	3.47
Fractions from liquid-liquid chromatography of buni stem barks		
3.	n-Hexane	29.78
4.	Ethyl acetate	5.73
5.	Methanol	27.68
Subfractionsfromvacuum liquid chromatographyofbuni stem barks		
6.	A (n-hexane: EtOAc (180:20))	2.38
7.	B (n-hexane: EtOAc (170:30))	2.21
8.	C(n-hexane: EtOAc (160:40), (150:50), (140:60), (130:70), (120:80), (100:100))	3.95
9.	D(n-hexane: EtOAc (80:120), (60:140))	8.61
10.	E (n-hexane: EtOAc (40:160), (0:200))	6.75
11.	F (EtOAc: MeOH (190:10))	2.17
12.	G (EtOAc: MeOH (180:20))	1.16
13.	H (EtOAc: MeOH (170:30))	11.63
14.	I (EtOAc: MeOH (160:40), (150:50), (140:60), (130:70), (120:80), (100:100))	16.28
15.	J (EtOAc: MeOH (80:120), (60:140), (40:160), (20:180), (0:200))	49.81
Fractions from liquid-liquid chromatography of buni leaves		
16.	n-Hexane	26.45
17.	Ethyl acetate	15.44
18.	Methanol	8.04
Subfractions from vacuum liquid chromatographyofbuni leaves		
19.	A (EtOAc : MeOH (200:0), (195:5))	13.24
20.	B (EtOAc : MeOH (190:10), (185:15), (180:20))	11.36
21.	C (EtOAc : MeOH (175:25), (170:30), (165:35), (160:40), (150:50), (140:60))	25.88
22.	D (EtOAc : MeOH (130:70), (120:80), (110:90))	15.56
23.	E (EtOAc : MeOH (100:100), (80:120))	7.23
24.	F (EtOAc: MeOH (60:140), (40:160), (20:180), (0:200))	4.79

Kinetic analysis of inhibition

Inhibition mode of EtOAc fraction from 80% ethanol extract of buni stem barks and MeOH fraction of buni leaves were investigated. Double-reciprocal plots of kinetic of inhibition on yeast α-glucosidase between absence inhibitor and both fractions as inhibitor were shown in Fig.1. These results comfirmed that the inhibition of EtOAc fraction and MeOH fraction on yeastα-glucosidase were competitive because thevalue of V_{max} wereconstant, while the K_m value were increased.

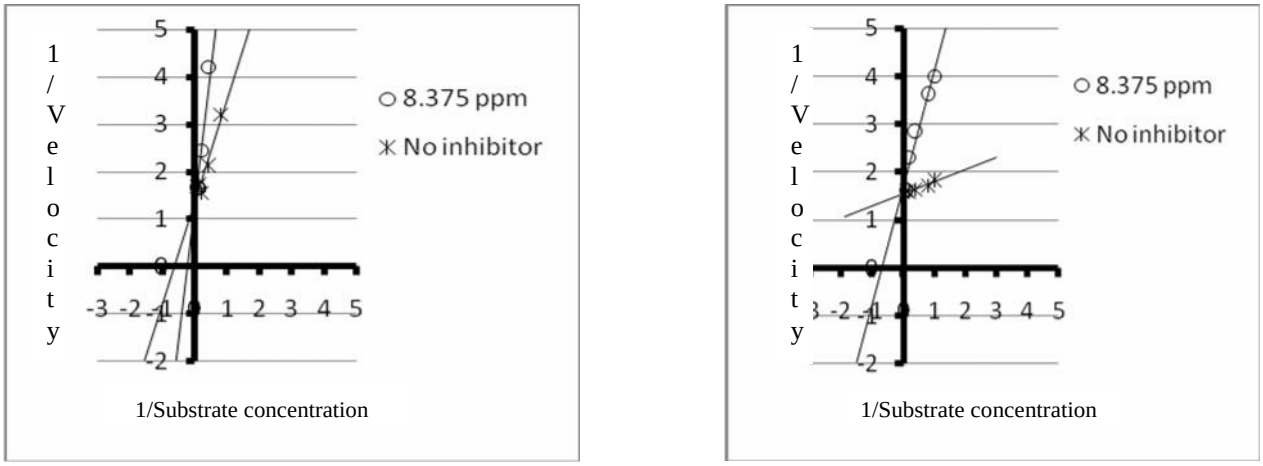

Figure 1.Lineweaver-Burke plots of the reaction α-glucosidase in the presence of EtOAc fraction from 80% ethanol extract of buni stem barks (A) and MeOH fraction 80% ethanol extract of buni leaves (B).

Phytochemistry Test

Compounds with α-glucosidase inhibitory activity were preliminary identificated by the existence of alkaloid, terpene, saponin, tannin, glycoside, flavonoid and quinone (Table 2).

Conclusion

In vitroassays of α-glucosidase inhibitory activity showed that G subfractionand F subfraction were the most active with IC_{50} values 1.16 and 4.79 µg/mL. Both subfractios indicated the presence of sugars, and flavonoids. Meanwhile, types of enzyme inhibition mechanism from EtOAc fraction of buni stem barks andMeOH fraction of bunileaveswere competitive inhibitor.

Table 2.Phytochemical screening of fractions from 80% ethanol extractsof buni (*Antidesmabunius*L.)

Compounds	Buni (*Antidesmabunius*L.)			
	Stem barks		Leaves	
	EtOAc Fraction	G Subfraction	MeOH Fraction	F Subfraction
Terpenes	+	-	-	-
Saponins	-	-	+	-
Alkaloids	-	-	-	-
Sugars	+	+	+	+
Flavonoids	+	+	+	+
Tannins	-	-	+	-
Anthraquinones	-	-	-	-

Key: + = present; - = absent

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