Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 281078, 6 pages
doi:10.1155/2012/281078

Research Article

Screening of α-Glucosidase Inhibitory Activity from Some Plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae

Berna Elya, Katrin Basah, Abdul Mun'im, Wulan Yuliastuti, Anastasia Bangun, and Eva Kurnia Septiana

Department of Pharmacy, Faculty of Mathematics and Sciences, University of Indonesia, Depok 16424, Indonesia

Correspondence should be addressed to Berna Elya, elya64@yahoo.com

Received 30 July 2011; Revised 8 September 2011; Accepted 9 September 2011

Academic Editor: Munekazu Iinuma

Diabetes mellitus (DM) is recognized as a serious global health problem that is characterized by high blood sugar levels. Type 2 DM is more common in diabetic populations. In this type of DM, inhibition of α-glucosidase is a useful treatment to delay the absorption of glucose after meals. As a megabiodiversity country, Indonesia still has a lot of potential unexploited forests to be developed as a medicine source, including as the α-glucosidase inhibitor. In this study, we determine the α-glucosidase inhibitory activity of 80% ethanol extracts of leaves and twigs of some plants from the Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. Inhibitory activity test of the α-glucosidase was performed *in vitro* using spectrophotometric methods. Compared with the control acarbose (IC_{50} 117.20 μg/mL), thirty-seven samples of forty-five were shown to be more potent α-glucosidase inhibitors with IC_{50} values in the range 2.33–112.02 μg/mL.

1. Introduction

Diabetes mellitus (DM) is the most common endocrine disease worldwide. About 173 million people suffer from diabetes mellitus. The number of people with diabetes mellitus will more than double over the next 25 years to reach a total of 366 million by 2030 [1]. In 2000, Indonesia is ranked the fourth largest number of people with DM, after India, China, and the United States, which is about 8.4 million people. The amount is expected to rise to 21.3 million in 2030 [2].

DM consists of several types, one of which is noninsulin-dependent diabetes mellitus (type 2 DM). This type of DM is more common, reaching 90–95% of the population with DM [3]. This increasing trend in type 2 DM has become a serious medical concern worldwide that prompts every effort in exploring for new therapeutic agents to stem its progress.

In type 2 DM, inhibition of α-glucosidase therapy is beneficial to delay absorption of glucose after a meal [4]. α-glucosidase plays a role in the conversion of carbohydrates into glucose. By inhibiting α-glucosidase, glucose levels in the blood can be returned within normal limits [5].

Natural resources provide a huge and highly diversified chemical bank from which we can explore for potential therapeutic agents by bioactivity-targeted screenings [6]. As a megabiodiversity country, Indonesia still has a lot of potential unexploited forests to be developed as a source phytopharmaca or modern medicine [7]. Opportunity exploration of medicinal plants is still very wide open in line with the development of herbal industry, herbal medicine, and phytopharmaca. Therefore, researchers try to explore the potential antidiabetic agents with the mechanism of action of α-glucosidase inhibition in several plant species from four families: Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. The four families were chosen because members of some species have been scientifically proven to have antidiabetic activity. Based on the theory of kinship through a systematic approach to plant (chemotaxonomy), plants with the same family generally have similar chemical content,

Table 1: Phytochemical screening of 80% ethanol extracts from some plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae.

Simplicia	Chemical contents						
	Alkaloid	Flavonoid	Terpenoid	Tannin	Glycoside	Saponin	Anthraquinone
				Apocynaceae			
Beaumontia multiflora Teijsm. & Binn. Folium	+	+	−	+	+	+	+
Beaumontia multiflora Teijsm. & Binn. Cortex	−	−	−	−	+	+	−
Carissa carandas L. Folium	−	+	+	+	+	+	+
Carissa carandas L. Cortex	−	−	−	+	+	+	+
Ochrosia citrodora Lauterb. & K. Schum. Folium	+	−	+	+	+	+	+
Rauvolfia sumatrana Jack Folium	+	−	+	−	+	+	−
Strophanthus caudatus (Blume.f.) Kurz Folium	−	−	+	−	+	+	+
Strophanthus caudatus (Blume.f.) Kurz Cortex	+	−	+	+	+	+	−
Strophanthus gratus Baill. Folium	−	−	+	−	−	+	−
Strophanthus gratus Baill. Cortex	+	−	+	−	+	+	−
Tabernaemontana sphaerocarpa Blume Folium	+	−	+	−	+	+	−
Willughbeia tenuiflora Dyer ex Hook.f Folium	−	−	+	+	+	+	−
Willughbeia tenuiflora Dyer ex Hook.f Cortex	+	−	+	+	+	+	−
				Clusiaceae			
Calophyllum tomentosum Wight. Folium	+	+	+	+	+	+	−
Garcinia bancana Miq. Folium	+	−	+	+	+	+	−
Garcinia daedalanthera Pierre. Folium	−	+	+	+	+	+	+
Garcinia daedalanthera Pierre. Cortex	−	−	+	+	+	+	+
Garcinia hombroniana Pierre. Folium	+	−	+	+	+	+	+
Garcinia kydia Roxb. Folium	−	+	+	+	+	+	+
Garcinia rigida Miq. Folium	+	+	+	+	+	+	+
				Euphorbiaceae			
Antidesma bunius (L.) Spreng Folium	−	−	+	+	+	+	+
Antidesma bunius (L.) Spreng Cortex	+	−	+	+	+	+	−
Antidesma celebicum Cortex	−	−	−	+	+	+	−
Antidesma celebicum Folium	−	+	−	+	+	+	+
Antidesma montanum (Blume) Folium	+	−	+	+	+	+	−
Antidesma neurocarpum Miq. Folium	+	+	−	+	+	−	+
Blumeodendron toksbrai (Blume.) Kurz. Cortex	+	−	−	−	+	+	−
Blumeodendron toksbrai (Blume.) Kurz. Folium	+	−	+	−	+	−	−
Croton argyratus Blume. Folium	−	−	+	−	+	−	−

Table 1: Continued.

Simplicia	Chemical contents						
	Alkaloid	Flavonoid	Terpenoid	Tannin	Glycoside	Saponin	Anthraquinone
Cephalomappa malloticarpa J.J.Sm. Cortex	−	−	+	+	+	+	+
Cephalomappa malloticarpa J.J.Sm. Folium	−	−	+	+	+	−	+
Galearia filiformis Blume. Folium	+	−	+	+	+	−	+
Sumbaviopsis albicans (Blume) J.J.Sm. Cortex	−	−	+	−	+	+	−
Sumbaviopsis albicans (Blume) J.J.Sm. Folium	−	−	+	−	+	+	−
Suregada glomerulata (Blume) Baill. Folium	+	−	+	−	+	−	−
			Rubiaceae				
Adina trichotoma Zoll. & Moritzi. Folium	+	−	+	−	+	−	+
Amaracarpus pubescens Blume. Folium	+	+	+	−	+	−	+
Canthium glabrum Blume. Folium	+	+	+	+	+	−	+
Chiococca javanica Blume. Folium	+	+	+	−	+	−	+
Hydnophytum formicarum Folium	+	−	+	+	+	+	−
Hydnophytum formicarum Cortex	+	+	+	−	+	−	−
Nauclea calycina (Batrl.ex DC.) Merr. Folium	+	−	−	+	+	−	−
Nauclea calycina (Batrl.ex DC.) Merr. Cortex	−	+	+	+	+	−	+
Posoqueria latifolia (Lam.) Roem. & Schult. Folium	−	−	+	+	+	−	−

Key: +: present; −: absent.

so it may just have the same potential for the treatment of a disease [8].

2. Method and Material

2.1. Plant Material. The stem bark and leaves of plants material were collected in November 2010 and identified by Center for Plant Conservation-Bogor Botanical Garden.

2.2. Extraction. Each dried powdered of wood bark, twig and leaves (20 g) were extracted by reflux with ethanol 80% then evaporated.

2.3. Inhibition Assay for α-Glucosidase Activity. The inhibition of α-glucosidase activity was determined using the modified published method [9]. One mg of α-glucosidase (*Saccharomyces cerevisiae*, Sigma-Aldrich, USA) was dissolved in 100 mL of phosphate buffer (pH 6.8) containing 200 mg of bovine serum albumin (Merck, German). The reaction mixture consisting 10 μL of sample at varying concentrations (0.52 to 33 μg/mL) was premixed with 490 μL phosphate buffer pH 6.8 and 250 μL of 5 mM *p*-nitrophenyl α-D-glucopyranoside (Sigma-Aldrich, Switzerland). After preincubating at 37°C for 5 min, 250 μL α-glucosidase (0.15 unit/mL) was added and incubated at 37°C for 15 min. The reaction was terminated by the addition of 2000 μL Na_2CO_3 200 mM. α-glucosidase activity was determined spectrophotometrically at 400 nm on spectrophotometer UV-Vis (Shimadzu 265, Jepang) by measuring the quantity of *p*-nitrophenol released from p-NPG. Acarbose was used as positive control of α-glucosidase inhibitor. The concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

2.4. Kinetics of Inhibition against α-Glucosidase. Inhibition modes of sample that had the best α-glucosidase inhibiting activity in Clusiaceae, Euphorbiaceae, and Rubiaceae were measured with increasing concentration of *p*-nitrophenyl α-D-glucopyranoside as a substrate in the absence or presence of ethanolic extract at different concentrations. Inhibition type was determined by the Lineweaver-Burk plots analysis of the data, which were calculated from the result according to the Michaelis-Menten kinetics.

2.5. Phytochemistry Test. In this research we performed phytochemistry test which consists of alkaloid test with Mayer, Dragendorff, and Bouchardat reagents; Flavonoid test with Shinoda and Wilson Töubock reaction; tannin test with

Table 2: IC_{50} values of rude extracts against α-glucosidase.

Number	Sample	IC_{50} (μg/mL)
(1)	Acarbose	117.20
	Apocynaceae	
(2)	*Beaumontia multiflora Teijsm. & Binn.* Folium	79.80
(3)	*Beaumontia multiflora Teijsm. & Binn.* Cortex	130.20
(4)	*Carissa carandas* L.Folium	21.14
(5)	*Carissa carandas L.*Cortex	20.44
(6)	*Ochrosia citrodora Lauterb. & K. Schum.* Folium	112.02
(7)	*Rauvolfia sumatrana* Jack Folium	174.27
(8)	*Strophanthus caudatus* (Blume.f.) Kurz Folium	706.81
(9)	*Strophanthus caudatus* (Blume.f.) Kurz Cortex	13.93
(10)	*Strophanthus gratus* Baill.Folium	50.61
(11)	*Strophanthus gratus* Baill. Cortex	202.17
(12)	*Tabernaemontana sphaerocarpa* Blume Folium	554.32
(13)	*Willughbeia tenuiflora* Dyer ex Hook.f Folium	8.16
(14)	*Willughbeia tenuiflora* Dyer ex Hook.f Cortex	42.11
	Clusiaceae	
(15)	*Calophyllum tomentosum* Wight. Folium	15.83
(16)	*Garcinia bancana* Miq. Folium	22.41
(17)	*Garcinia daedalanthera* Pierre. Folium	2.33
(18)	*Garcinia daedalanthera* Pierre. Cortex	3.71
(19)	*Garcinia hombroniana* Pierre. Folium	11.30
(20)	*Garcinia kydia* Roxb. Folium	3.88
(21)	*Garcinia rigida* Miq. Folium	24.48
	Euphorbiaceae	
(22)	*Antidesma bunius* (L.) Spreng Folium	7.94
(23)	*Antidesma bunius* (L.) Spreng Cortex	3.90
(24)	*Antidesma celebicum* Cortex	3.93
(25)	*Antidesma celebicum* Folium	2.34
(26)	*Antidesma montanum* (Blume) Folium	2.83
(27)	*Antidesma neurocarpum* Miq. Folium	4.22
(28)	*Blumeodendron toksbrai* (Blume.) Kurz. Cortex	22.82
(29)	*Blumeodendron toksbrai* (Blume.) Kurz. Folium	64.78
(30)	*Croton argyratus* Blume. Folium	366.07
(31)	*Cephalomappa malloticarpa* J.J.Sm. Cortex	12.22
(32)	*Cephalomappa malloticarpa* J.J.Sm. Folium	2.66
(33)	*Galearia filiformis* Blume. Folium	21.54
(34)	*Sumbaviopsis albicans* (Blume) J.J.Sm. Cortex	42.66
(35)	*Sumbaviopsis albicans* (Blume) J.J.Sm. Folium	43.40
(36)	*Suregada glomerulata* (Blume) Baill. Folium	57.46
	Rubiaceae	
(37)	*Adina trichotoma Zoll. & Moritzi. Folium*	28.22
(38)	*Amaracarpus pubescens* Blume. Folium	3.64
(39)	*Canthium glabrum Blume.* Folium	117.85
(40)	*Chiococca javanica Blume.* Folium	23.86
(41)	*Hydnophytum formicarum* Folium	181.90
(42)	*Hydnophytum formicarum* Cortex	11.04
(43)	*Nauclea calycina (Batrl.ex DC.) Merr.* Folium	18.81
(44)	*Nauclea calycina (Batrl.ex DC.) Merr.* Cortex	25.99
(45)	*Posoqueria latifolia (Lam.) Roem. & Schult.* Folium	80.27

gelatin test, gelatin-salt test, and test with ferrous (III) chloride; glycoside test with Molisch reaction; saponin test with honeycomb froth test; anthraquinone test with Bornträger reaction; terpenoid test with Liebermann-Burchard reagent.

3. Results and Discussion

3.1. Phytochemistry Test. Compounds with α-glucosidase inhibitory activity were preliminary identificated by the existence of alkaloid, terpene, saponin, tannin, glycoside, flavonoid, and quinone (Table 1).

3.2. Assay for α-Glucosidase Inhibitory Activity. The α-glucosidase of *S. cerevisiae* is used to investigate the inhibitory activity of the rude extracts. α-glucosidase inhibitory activity of rude extracts compounds against α-glucosidases were determined using *p*-nitrophenyl-α-D-glucopyranoside (p-NPG) as a substrate and these were compared with acarbose (Table 2). The IC_{50} values of compounds range from 2.33 μg/mL to 706.81 μg/mL. There are thirty-seven of samples which have IC_{50} lower than acarbose. Extracts derived from leaves of *Garcinia daedalanthera* showed inhibitory activity against α-glucosidase enzyme significantly, with IC_{50} value of 2.33 μg/mL. Inhibitory activity of the enzyme α-glucosidase at forty-five extracts may be due to the glycoside content in each extract. Glycosides consist of sugars that may be structurally similar to carbohydrate which is a substrate of the enzyme α-glucosidase [10]. IC_{50} value of samples of plant extracts are lower than acarbose because their active chemical compounds have no further fractionation and may have a synergistic effect in inhibiting α-glucosidase [11].

Inhibition mode of leaves extract of *Antidesma celebicum* from Euphorbiaceae was investigated. Inhibition mode of 80% ethanol extract showed competitive inhibitory mode. This mode may have been due because the structure is similar with glucose. This result is similar with inhibition mode of Nojirimycin which has a competitive inhibition against α-glucosidase [9] (Figure 1).

Inhibition mode of leaves extract of *Garcinia kydia* from Clusiaceae was investigated. Inhibition mode of 80% ethanol extract showed noncompetitive inhibitory mode [12] (Figure 2).

Inhibition mode of 80% ethanol extract from *Amaracarpus pubescens* Blume. leaves had a combination of competitive and uncompetitive inhibition. Combination of competitive and noncompetitive may have been due to the extract having more than one compound that has α-glucosidase inhibitory activity [13] (Figure 3).

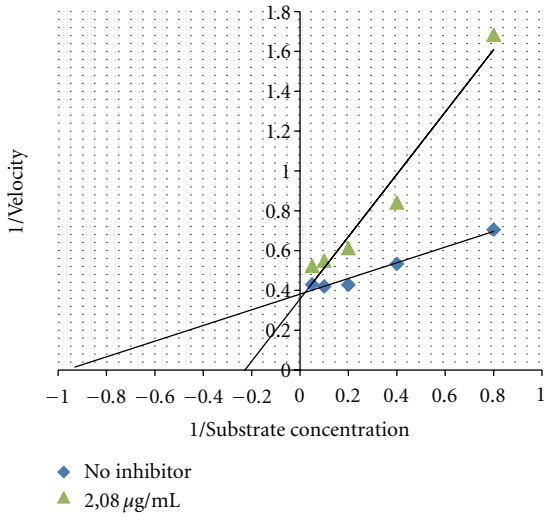

Figure 1: Lineweaver-Burk plot of 80% ethanol extract of leaves of *Antidesma celebicum* with concentration of 2.08 μg/mL with pNPG substrate concentration of 1.25, 2.5, 5, 10, and 20 mM.

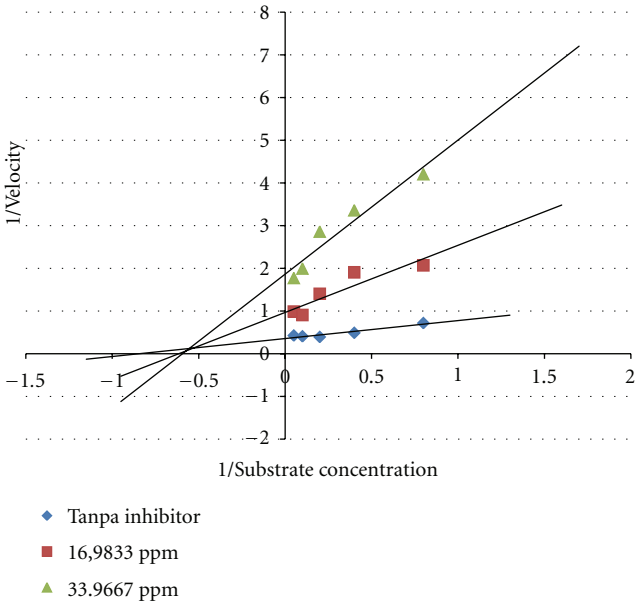

Figure 2: Lineweaver-Burke plot of the reaction α-glucosidase in the presence of 80% ethanol extract from *Garcinia kydia* Roxb. Leaves.

4. Conclusion

In vitro assays of α-glucosidase activity showed thirty-seven of forty-five samples had IC_{50} values of between 2.33 μg/mL and 112.02 μg/mL, which were lower than that of acarbose (117.20 μg/mL). Based on family, 80% ethanol extract from *Garcinia daedalanthera* Pierre. leaves (Clusiaceae), *Antidesma celebicum* leaves (Euphorbiaceae), *Amaracarpus pubescens* Blume. leaves (Rubiaceae), and *Willughbeia tenuiflora* Dyer ex Hook.f leaves (Apocynaceae) had the highest

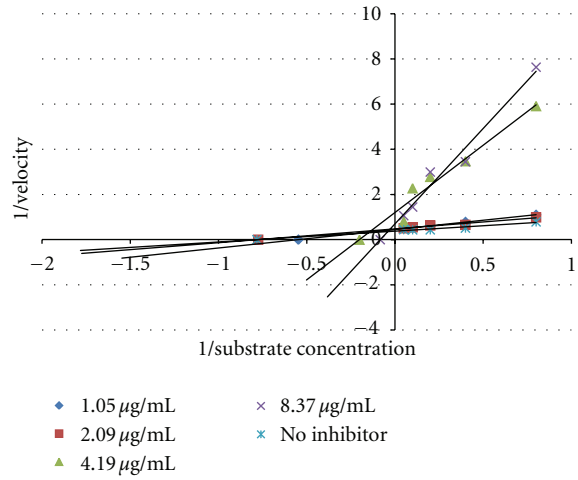

Figure 3: Lineweaver-Burke plot of the reaction α-glucosidase in the presence of 80% ethanol extract from *Amaracarpus pubescens* Blume.

α-glucosidase inhibiting activity with IC_{50} of 2.33 μg/mL, 2.34 μg/mL, 3.64 μg/mL, and 8,16 μg/mL. Meanwhile, types of enzyme inhibition mechanism from *Garcinia kydia* Roxb. leaves (Clusiaceae), *Antidesma celebicum* leaves (Euphorbiaceae), and *Amaracarpus pubescens* Blume. leaves (Rubiaceae) were noncompetitive inhibitor, competitive inhibitor, and mixed inhibitor. Currently attempts to purify the active compound from leaves extract of *Garcinia kydia* Roxb. (Clusiaceae), *Antidesma celebicum* (Euphorbiaceae), and *Amaracarpus pubescens* Blume. (Rubiaceae) are conducted to understand the inhibitory mechanisms more clearly. Moreover, further *in vivo* study is also required.

Acknowledgment

The authors would like thank to Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Indonesia for supporting this project.

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