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Screening of α-Glucosidase Inhibitory Activity of Vietnamese Medicinal Plants: Isolation of Active Principles from *Oroxylum indicum*

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Abstract – Among 38 Vietnamese medicinal plant extracts investigated for their α-glucosidase inhibitory activity, 35 extracts showed IC_{50} values below 250 μg/mL. The MeOH extracts of the heartwood of *Oroxylum indicum*, the seeds of *Caesalpinia sappan*, and the fruits of *Xanthium strumarium* exhibited strong α-glucosidase inhibitory activity with IC_{50} values less than 50 μg/mL. Fractionation of the MeOH extract of the heartwood of *O. indicum* led to the isolation of oroxylin A (**1**), oroxyloside (**2**), hispidulin (**3**), apigenin (**4**), ficusal (**5**), balanophonin (**6**), 2-(1-hydroxymethylethyl)-4*H*,9*H*-naphtho[2,3-*b*]furan-4,9-dione (**7**), salicylic acid (**8**), *p*-hydroxybenzoic acid (**9**), protocatechuic acid (**10**), isovanillin (**11**), and β-hydroxypropiovanillon (**12**). Compounds **1** - **3**, **5**, **6**, **8**, **10**, and **12** showed more potent activities, with IC_{50} values ranging from 2.13 to 133.51 μM, than a positive control acabose (IC_{50}, 241.85 μM). The kinetic study indicated that oroxyloside (**2**) displayed mixed-type inhibition with inhibition constant (*Ki*) was 3.56 μM.
Keywords – Vietnamese medicinal plants, α-glucosidase inhibitory activity, *Oroxylum indicum*

Introduction

Diabetes mellitus (types I and II) is a most serious and chronic disease whose incidence rates are increasing with increasing levels of obesity and also with aging of the general population over the world. Globally, type II diabetes (noninsulindependent diabetes mellitus) accounts for greater than 90% of the cases (Zimmet *et al*., 2001; Tewari *et al*., 2003). Postprandial hyperglycemia plays an important role in development of type II diabetes and complications associated with the diseases, such as microvascular (*i.e*., retinal, renal, possibly neuropathic), macrovascular (*i.e*., coronary, peripheral vascular), and neuropathic (*i.e*., autonomic, peripheral) complications (Baron, 1998). One therapeutic approach to decrease postprandial hyperglycemia is to retard absorption of glucose via inhibition of carbohydrate-hydrolysing enzymes, such as α-glucosidase, in the digestive organs (Holman *et al*., 1999). α-Glucosidase (EC 3.2.1.20, α-D-glucoside glucohydrolase) is widely distributed in microorganisms, plants, and animal tissues that catalyze the liberation of α-glucose from the non-reducing end of the substrate. In type II diabetes, delaying glucose absorption after meals by inhibition of α-glucosidase is known to be beneficial in therapy (Tewari *et al*., 2003).

To identify potential α-glucosidase inhibitory agents from natural sources, we have tested 38 extracts prepared from selected medicinal plants, which are used by indigenous people in Vietnam for their use in diabetes treatment. In addition, the active constituents of *Oroxylum indicum*, which showed the most potent α-glucosidase inhibitory activity in this screening, have been determined.

Experimental

General Experimental Procedures – NMR spectra were taken on a Bruker Avance III 500 spectrometer (Bruker Biospin) with tetramethylsilane as an internal standard, and chemical shifts are expressed in δ values. Analytical and preparative TLC were carried out on precoated Merck Kieselgel $60F_{254}$ or RP-$18F_{254}$ plates (0.25 or 0.5 mm thickness).

Plant Materials – Vietnamese plants used in this study were collected at the Seven-Mountain area, An Giang province, Vietnam in August 2009 (Table 1). The plants

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Table 1. Vietnamese plants used in this study, their families, part used and α-glucosidase inhibitory activities

Voucher specimens	Plant name	Family	Part used	Percentage inhibition (%)				IC_{50} μg/mL
				250 μg/mL	100 μg/mL	50 μg/mL	25 μg/mL	
1501	*Albizia myriophylla* Benth.	Fabaceae	Bark	98.2	45.3	18.2	12.3	113.1
1502	*Alstonia scholaris* (L.) R. Br.	Apocynaceae	Bark	78.7	34.0	24.5	12.1	153.6
1503	*Ageratum conyzoides* L.	Asteraceae	Aerial part	94.4	37.1	26.7	14.8	133.7
1504	*Antidesma ghaesembilla* Gaertn.	Euphorbiaceae	Root	85.7	34.3	25.3	15.5	145.8
1505	*Artemisia vulgaris* L.	Asteraceae	Leaf	89.9	60.3	33.3	24.2	81.8
1506	*Biota orientalis* (L.) Endl.	Cupressaceae	Leaf	87.6	64.6	39.0	6.9	71.5
1507	*Blumea balsamifera* (L.) DC.	Asteraceae	Leaf	85.3	53.1	40.1	9.3	88.1
1508	*Caesalpinia sappan* L.	Caesalpiniaceae	Seed	99.5	88.1	64.2	42.8	34.3
1509	*Catharanthus roseus* (L.) G. Don.	Apocynaceae	Whole plant	36.2	18.4	14.0	9.3	> 250
1510	*Cassytha filiformis* L.	Lauraceae	Whole plant	87.7	34.2	21.1	10.0	114.3
1511	*Chrysopogon aciculatus* (Retz.) Trin.	Poaceae	Whole plant	78.5	51.1	23.8	11.6	97.5
1512	*Circus japonicus.* (DC.) Maxim	Asteraceae	Whole plant	42.7	18.2	–	–	> 250
1513	*Coix lachryma- jobi* L.	Poaceae	Seed	82.8	29.7	13.3	–	157.3
1514	*Cycas pectinata* Griff.	Cycadaceae	Seed	87.9	37.0	26.4	13.5	138.2
1515	*Dendrobium crumenatum* Sw.	Orchidaceae	Root	95.2	89.8	45.2	43.5	55.5
1516	*Drynaria quercifolia* (L.) J. Smith.	Polypodiaceae	Buld	99.4	46.4	38.2	21.8	110.2
1517	*Erythrina orientalis* (L.) Murr.	Fabaceae	Whole plant	64.5	30.6	20.3	16.6	185.9
1518	*Hydnocarpus ilicifolia* King	Kiggelariaceae	Stem	88.5	55.3	33.5	21.3	88.0
1519	*Hypobathrum racemosum* (Roxb.) Kurz	Rubiaceae	Wood	97.2	70.5	55.4	41.1	41.1
1520	*Lasia spinosa* (L.) Thw.	Araceae	Root	94.2	61.3	17.3	21.5	79.8
1521	*Millettia pulchra* Kurz.	Fabaceae	Stem	95.5	65.1	52.7	10.1	48.8
1522	*Neuracanthus tetragonostachyus* Nees	Acanthaceae	Leaf	61.2	28.0	–	–	199.3
1523	*Oroxylum indicum* (L.) Vent	Bignoniaceae	Fruit	96.7	39.1	20.5	10.9	128.2
1524	*Oroxylum indicum* (L.) Vent	Bignoniaceae	Heartwood	98.6	89,8	57,7	47,0	31.9
1525	*Pandanus tonkinensis* Mart. ex Stone	Pandanaceae	Fruit	75.6	51.8	23 .1	20.1	96.9
1526	*Passiflora foetida* L.	Passifloraceae	Stem	11.4	–	–	–	> 250
1527	*Piper lolot* C. DC.	Piperaceae	Leaf	72.4	28.2	10.5	–	174.0
1528	*Plantago asiatica* L.	Plantaginaceae	Aerial part	74.5	59.1	49 .5	40.4	55.0
1529	*Pluchea pteropoda* Hemsl.	Asteraceae	Stem	99.7	48.4	28.5	18.5	104.7
1530	*Rhinacanthus nasuta* (L.) Kurz	Acanthaceae	Whole plant	95.3	62.7	39.4	7.4	73.3
1531	*Seoparia dulcis* L.	Scrophulariaceae	Whole plant	91.3	79.1	22.3	9.8	74.2
1532	*Sesbania javanica* Miquel	Fabaceae	Whole plant	66.0	29.6	13.0	10.4	184.0
1533	*Smilax glabra* Roxb.	Smilacaceae	Buld	85.1	61.1	38.2	23.3	75.7
1534	*Stenochlaena palustris (*Burm. F.) Bedd.	Blechnaceae	Stem	98.7	42.1	22.6	13.8	120.0
1535	*Tinospora cordifolia* (Willd.) Miers.	Menispermaceae	Stem	99.1	42.5	30.0	12.1	119.8
1536	*Vitex negundo* L.	Verbenaceae	Whole plant	90.9	36.5	24.7	10.0	137.2
1537	*Xanthium strumarium* L.	Asteraceae	Fruit	99.5	98.9	95.3	19.9	35.0
1538	*Zea mays* L.	Poaceae	Beard	84.3	42.8	37.4	26.8	126.0
	Acarbose							156.1

were identified by Ms. Hoang Viet, Faculty of Biology, University of Science, Vietnam National University-HoChiMinh City. The voucher samples are preserved at Department of Analytical Chemistry, Faculty of Chemistry, University of Science, Vietnam National University-HoChiMinh City.

Chemicals – α-Glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisae* (750UN) and *p*-nitrophenyl-α-D-

glucopyranoside were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acarbose and DMSO were purchased from Merck (Darmstadt, Germany). Other chemicals were of the highest grade available.

Preparation of Samples – Each plant (100 - 200 g) was cut into small pieces and extracted with MeOH (300 mL, reflux, 3 h, × 3). The MeOH solution was evaporated under reduced pressure to give a MeOH extract.

***α*-Glucosidase Inhibitory Assay** – The inhibitory activity of *α*-glucosidase was determined according to the modified method of Kim *et al*. (Kim *et al*., 2008). 3 mM *p*-nitrophenyl-*α*-D-glucopyranoside (0.01 mL) and 20 U/mL *α*-glucosidase (0.01 mL) in 0.01 M phosphate buffer (pH 7) were added to the sample solution (2.2 mL) to start the reaction. Each reaction was carried out at 37 °C for 30 min and stopped by adding 0.1 M Na_2CO_3 (2 mL). Enzymatic activity was quantified by measuring absorbance at 405 nm. One unit of *α*-glucosidase activity was defined as amount of enzyme liberating *p*-nitrophenol (1.0 μM) per min. The IC_{50} value was defined as the concentration of *α*-glucosidase inhibitor that inhibited 50% of *α*-glucosidase activity. Acarbose, a known *α*-glucosidase inhibitor, was used as positive control.

Extraction and Isolation of the Active Compounds from the Heartwood of *Oroxylum indicum* – The dried powder of heartwood of *O. indicum* (2.2 kg) was extracted with MeOH (15 L, reflux, 3 h, × 3) to yield a MeOH extract (338 g; IC_{50}, 31.9 μg/mL). The extract was partitioned between EtOAc and water to give an EtOAc-soluble fraction (69 g; IC_{50}, 17.5 μg/mL). The EtOAc-soluble fraction was subjected to silica gel column chromatography with acetone/hexane to give four fractions: fr. 1 (38.2 g; IC_{50}, 17.5 μg/mL), fr. 2 (0.8 g; IC_{50}, 84.6 μg/mL), fr. 3 (15 g; IC_{50}, 9.1 μg/mL), and fr. 4 (12 g; IC_{50}, 8.0 μg/mL).

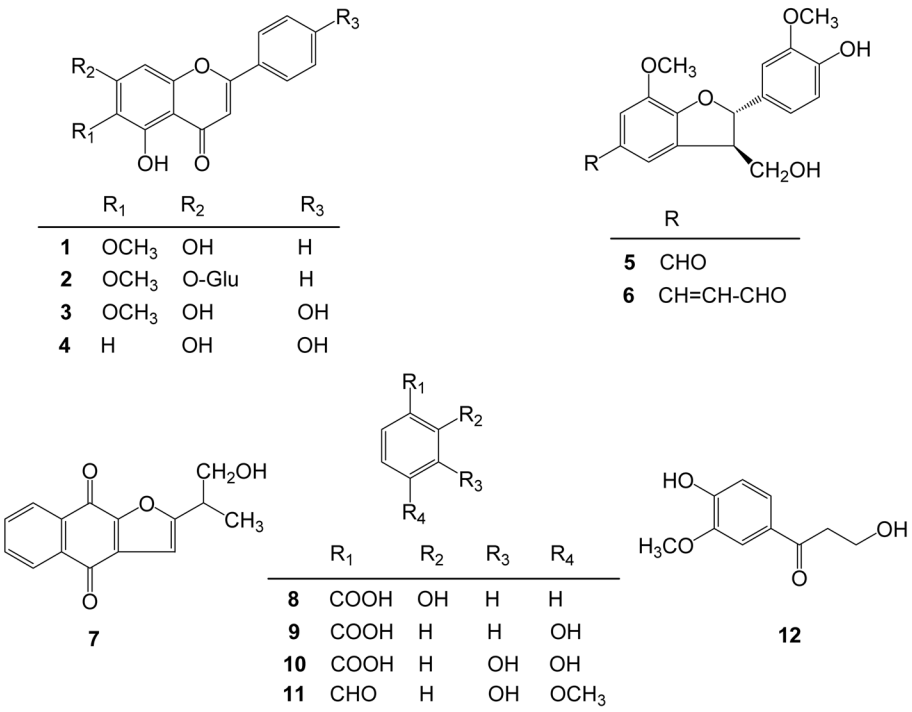
Fraction 1 was subjected to silica gel column chromatography, eluted with EtOAc/hexane (0 - 30%) to afford four subfractions (1 - 1, 340 mg; 1 - 2, 5.0 g; 1 - 3, 3.5 g; 1 - 4, 2.4 g). Subfraction 1 - 1 was separated by normal-phase preparative TLC with MeOH/$CHCl_3$ (1 : 99) to give **11** (7.0 mg). Subfraction 1 - 2 was rechromatographed with acetone/hexane (0 - 50%), and then followed by normal-phase preparative TLC with acetone/hexane (3 : 7) to give **1** (37.6 mg) and **7** (10.4 mg). Subfraction 1 - 3 was separated by normal-phase preparative TLC with acetone/hexane (4 : 6), and then followed by reversed-phase preparative TLC with acetone/H_2O (8:2) to give **12** (14.9 mg), **5** (13.5 mg), and **6** (12.1 mg). Fraction 3 was subjected to silica gel column chromatography, eluted with MeOH/$CHCl_3$ (0 - 50%) to afford three subfractions (3 - 1, 1.5 g; 3 - 2, 1.8 g; 3 - 3, 500 mg). Subfraction 3 - 1 was rechromatographed with MeOH/$CHCl_3$ gradient system, and then followed by reversed-phase preparative TLC with acetone/H_2O (7 : 3) to give **4** (4.6 mg), **9** (7.4 mg), and **10** (5.4 mg). Subfraction 3 - 2 was separated by reversed-phase preparative TLC with acetone/H_2O (8 : 2) to give **2** (18.3 mg), **3** (21.3 mg), and **8** (16.7 mg).

Results and Discussion

Glycosidases are hydrolytic enzymes that play a vital role in digestion of carbohydrates and biosynthesis of glycoproteins. Inhibitors of *α*-glucosidase may potentially reduce the progression of diabetes by decreasing the digestion and absorption of carbohydrates. In the present study, 38 plants from Vietnam were selected based on their ethnomedical use for the treatment of diabetes and were extracted with MeOH to give 38 crude extracts. All of these extracts were tested for their *α*-glucosidase inhibitory activity to identify candidates for the development of antitype II diabetics. The assay was carried out at four different concentrations of extract ranging from 25 - 250 μg/mL (Table 1).

The results of screening showed that all of extracts (100%) demonstrated *α*-glucosidase inhibitory activity at 250 μg/mL, among which 35 (92.1%) displayed an inhibition rate greater than 50%. Altogether, 37 extracts (97.4%) were found to be active at a concentration of 100 μg/mL, among which 17 (44.7%) showed inhibition of more than 50%. At 50 μg/mL, 35 extracts (92.1%) were active, and four (10.5%) of over 50% inhibition. Of the extracts assayed, 33 (86.8%) displayed activity at 25 μg/mL, but no samples showed an inhibition of over 50%. In total, 35 extracts showed IC_{50} values below 250 μg/mL. The crude extracts possessing potent *α*-glucosidase inhibitory activity with IC_{50} values less than 50 μg/mL were MeOH extracts of the heartwood of *O. indicum* (IC_{50}, 31.9 μg/mL), the seeds of *C. sappan* (IC_{50}, 34.3 μg/mL), and the fruits of *X. strumarium* (IC_{50}, 35.0 μg/mL). This is the first report on the *α*-glucosidase inhibitory activity of these plants.

The seeds of *C. sappan* have been used as antidiabetes and anti-inflammatory in Vietnam. The phytochemical studies on this plant reported that it contains a number of cassane-type diterpenes, together with amino acids (*e.g.* alanine, cystine, glycine, isoleucine, lysine, threonone, trypophan), fatty acids (*e.g.* capric, lauric, myristic, myristoplalmatic, palmitic, palmitoleic, oleic, linoleic, and arachidic acids) (Yodsaoue *et al*., 2008). Therefore, the putative therapeutic effects of *C. sappan* and its *α*-



	R_1	R_2	R_3
1	OCH_3	OH	H
2	OCH_3	O-Glu	H
3	OCH_3	OH	OH
4	H	OH	OH

	R
5	CHO
6	CH=CH-CHO

7

	R_1	R_2	R_3	R_4
8	COOH	OH	H	H
9	COOH	H	H	OH
10	COOH	H	OH	OH
11	CHO	H	OH	OCH_3

12

Fig. 1. Structures of the isolated compounds from the heartwood of *O. indicum*.

glucosidase inhibitory activity are ascribed to its diterpene, amino acid, and fatty acid constituents.

It is interesting to note that the fruits of *X. strumarium* possess various biological activities such as antibacterial, antitumour, anticancer, anti-inflammatory, and hypoglycemic activity. Athought this plant is well-known source of xanthanolide sesquiterpenes which have been known to possess cytotoxic activities against cancer cell lines (Yoon *et al*., 2008), its antihyperglycemic effect in the rat was attributed to the presence of caffeic acid and phenolic compounds (Hsu *et al*., 2000). Thus, the α-glucosidase inhibitory activity of these plants also may be due to the presence of these constituents.

The most active extract found in the present screening was the MeOH extract of the heartwood of *O. indicum* (IC_{50}, 31.9 μg/mL), which is used for the treatment of diabetes and inflammatory diseases (Vo, 1991). There were several reports on the constituents from the seed, root, and stem bark of this plant, but no chemical constituents have been reported from its heartwood. Therefore, we carried out further investigation to isolate and identify the active constituents. The MeOH extract of the heartwood of *O. indicum* was subjected to silica gel column chromatography to give four fractions. Further separation and purification of the active fractions with preparative TLC led to the isolation of oroxylin A (**1**) (Marques, 2010), oroxyloside (**2**) (Marques, 2010), hispidulin (**3**) (Marques, 2010), apigenin (**4**) (Harborne, 1982), ficusal (**5**) (Li, 2000), balanophonin (**6**) (Haruna *et al*., 1982), 2-(1-hydroxymethylethyl)-4*H*,9*H*-naphtho[2,3-*b*]furan-4,9-dione (**7**) (Kizu *et al*., 1994), salicylic acid (**8**) (Jadrijevic *et al*., 2004), *p*-hydroxybenzoic acid (**9**) (Lee *et al*., 1995), protocatechuic acid (**10**) (Zijia *et al*., 2009), isovanillin (**11**) (Ryu *et al*., 2004), and β-hydroxypropiovanillon (**12**) (Luo *et al*., 2008) (Fig. 1). All of the isolated compounds, except for **4** and **11**, were examined for their α-glucosidase inhibitory activity (Table 2). Compounds **1** - **3**, **5**, **6**, **8**, **10**, and **12** showed more potent activities, with IC_{50} values ranging from 2.13 to 133.51 μM, than a positive control acarbose (IC_{50}, 241.85 μM). Further kinetic study indicated that the most active compound, oroxyloside (**2**) (IC_{50}, 2.13 μM), displayed mixed-type inhibition with inhibition constant (*Ki*) was 3.56 μM. These results indicated that the active compounds such as oroxyloside (**2**) can potentially be developed as a novel natural nutraceutical to decrease the blood glucose level because of their high α-glucosidase inhibitory activity. Therefore, the traditional use of *O. indicum* for the treatment of diabetes in Vietnam might be attributable to the α-glucosidase inhibitory activity of flavonoid, lignan, and phenolic constituents.

Table 2. α-Glucosidase inhibitory activity of the isolated compounds

Compounds	IC_{50} (μM)[a]	Compounds	IC_{50} (μM)[a]
1	27.91 ± 0.11	**7**	> 250
2	2.13 ± 0.12	**8**	99.98 ± 0.12
3	7.03 ± 0.14	**9**	> 250
4	NT	**10**	45.32 ± 0.18
5	78.61 ± 0.11	**11**	NT
6	133.51 ± 0.21	**12**	123.92 ± 0.17
Acarbose	241.85 ± 0.15		

NT: No tested
[a] Each value represents the mean ± SD of three determinations.

Conclusions

In conclusion, we have carried out a systematic investigation of Vietnamese plants for α-glucosidase inhibitory activity. The results indicate a number of plants that may be useful in diabetes treatment and provide the basis for further investigation on these plant species to isolate active constituents and drug development.

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