NPC Natural Product Communications

2017
Vol. 12
No. 4
533 - 535

Main Constituents and Antidiabetic Properties of *Otholobium mexicanum*

Alírica I. Suárez[a], Zaw Min Thu[b], Jorge Ramírez[b,c], Diana León[c], Luis Cartuche[c], Chabaco Armijos[c,*] and Geovanni Vidari[b,*]

[a]*Faculty of Pharmacy, Central University of Venezuela, Caracas, Venezuela*
[b]*Department of Chemistry and CEMEC, University of Pavia, Via Taramelli 12, 27100, Pavia, Italy*
[c]*Department of Chemistry and Exact Sciences, Technical University of Loja, Loja, Ecuador*

**cparmijos@utpl.edu.ec, vidari@unipv.com*

Received: December 5th, 2016; Accepted: January 31st, 2017

Two phenols, bakuchiol (**1**) and 3-hydroxybakuchiol (**2**), and two isoflavone glycosides, daidzin (**3**) and genistin (**4**) were isolated from *Otholobium mexicanum* J. W. Grimes (Fabaceae). Moreover, the ability of the raw extract and isolated metabolites to inhibit the enzymes α-amylase and α-glucosidase was evaluated *in vitro*. In the α-amylase assay, the methanolic extract exhibited a moderate inhibitory activity with an IC_{50} of 470 μg/mL, while inhibition percentages of bakuchiol (**1**), 3-hydroxybakuchiol (**2**), and daidzin (**3**) were less than 25% at the maximum dose tested (1 μM). Genistin (**4**) exhibited a poor activity with an IC_{50} of 805 μM. In the α-glucosidase assay, the methanolic extract exhibited a strong inhibitory activity with an IC_{50} value of 32 μg/mL, while 3-hydroxybakuchiol (**2**) exhibited a moderate inhibitory activity with an IC_{50} of 345 μM. Daidzin (**3**) and genistin (**4**) exhibited lower inhibitory activity with IC_{50} values of 564 μM and 913 μM, respectively. Bakuchiol (**1**) exhibited a poor inhibitory activity with an inhibition percentage less than 10% at the maximum dose tested (1 mM).

Keywords: *Otholobium mexicanum*, α-Amylase, α-Glucosidase, Bakuchiol, 3-OH-Bakuchiol, Diabetes.

According to the World Health Organization (WHO), diabetes affects over 422 million people, in developed as well as in developing countries [1]. Moreover, hyperglycemia and dyslipidemia factors, increased in the diabetes, are involved in cardiovascular failures frequently affecting patients with this complex metabolic disease. In this context, the WHO has recommended that efficiency, potency and security of plants, traditionally used as anti-diabetic remedies, should scientifically be evaluated [2]. In fact, in spite of many documented beneficial properties, some plants may produce toxic effects in long-term treatments, such as those used in diabetes therapies [3].

Diabetes is responsible for 4% of all deaths in Ecuador, ranking sixth as the most common cause of death, followed by respiratory diseases [4]. Treatments with plants for the primary health care are practiced in Ecuador by nearly 80% of the people [5]. *Otholobium mexicanum* (L.f.) J.W. Grimes (Fabaceae), locally called *culen*, is considered as a medicinal plant in Ecuador and is useful for different purposes, including diarrhea, as a contraceptive, against stomach pain, indigestions, and sheep ticks [5]. Moreover, the leaves are used to prepare a refreshing drink, and the stems infusion is considered an antibacterial remedy. Indeed, this plant is not specifically used as antidiabetic; however, the related species *O. pubescens* [6] and *O. glandulosum* [7] have well-known antidiabetic properties. In addition, the antiproliferative activity of *O. mexicanum*, collected in Peru, has previously been investigated [8].

One therapeutic approach to treat diabetes is based on the inhibition of hydrolytic enzymes, such as α-amylase and α-glucosidase. α-Amylase hydrolyses α-bonds of large, α-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. On the other hand, the membrane-bound intestinal α-glucosidases degrade complex carbohydrates into glucose and other monosaccharides in the small intestine, facilitating their digestion. Thus, competitor

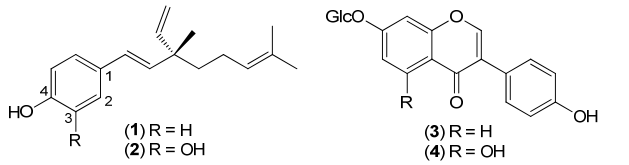

Figure 1: Structures of bakuchiol (**1**), 3-hydroxybakuchiol (**2**), daidzin (**3**) and genistin (**4**) isolated from *Otholobium mexicanum*.

inhibitors of these enzyme systems reduce the rate of digestion of carbohydrates, resulting in less glucose absorption and consequently, in the short-term, in a decreased blood glucose level. Clinically, α-amylase and α-glucosidase inhibitors are therefore useful to establish greater glycemic control over hyperglycemia.

Therefore, as a part of our research project on Ecuadorian plants potentially active against diabetes mellitus, a methanolic and a hydromethanolic extracts of *O. mexicanum* leaves were investigated phytochemically. Moreover, the hypoglycemic properties of the crude extracts and the isolated secondary metabolites were evaluated by measuring their *in vitro* α-amylase and α-glucosidase enzyme inhibitory effects [9]. Noteworthy, the phytochemistry as well as the potential anti-diabetic properties of *O. mexicanum* have not yet been investigated.

Two lots of ground air-dried leaves of *O. mexicanum* were separately extracted with MeOH and MeOH-H_2O, respectively. Repetitive separations of the crude extracts on silica gel and reversed RP-18 chromatographic columns, under normal as well as medium pressure conditions, led to the isolation of bakuchiol (**1**) [10] and 3-hydroxybakuchiol (**2**) [10] from the MeOH extract, while daidzin (daidzein 7-*O*-β-D-glucopyranoside) (**3**) [11] and genistin (genistein7-*O*-β-D-glucopyranoside) (**4**) [12] were isolated from the hydromethanolic extract. The structures of these compounds were established by ESI-MS, IR, extensive 1D (^{1}H and

^{13}C) and 2D NMR spectroscopic measurements, which nicely matched with published spectral data.

α-Amylase and α-glucosidase inhibition activity: The α-amylase and α-glucosidase inhibitory effects of the methanolic extract and isolated compounds **1**-**4** were evaluated using α-amylase from porcine pancreas (Type IV-B) and α-glucosidase from *Saccharomyces cerevisiae* (Type I).

In the α-amylase assay, the methanolic extract exhibited a moderate inhibitory activity with an IC_{50} value of 470 μg/mL while bakuchiol (**1**), 3-hydroxybakuchiol (**2**) and daidzin (**3**) showed a weak inhibitory activity with inhibition percentages < 25% at the maximum dose tested (1 μM). Genistin (**4**) exhibited an IC_{50} value of 805 μM. In the α-glucosidase assay, the methanolic extract exhibited a strong inhibitory activity with an IC_{50} value of 32 μg/mL, while 3-hydroxybakuchiol (**2**) exhibited an IC_{50} of 345 μM and inhibition percentage of bakuchiol (**1**) was < 10% at the maximum dose tested (1 mM). On the other hand, IC_{50} values of the inhibitory activities of daidzin (**3**) and genistin (**4**) were 564 μM and 913 μM, respectively. Therefore, regarding their activity, 3-hydroxybakuchiol (**2**) and daidzin (**3**) did not differ significantly from the commercially available α-glucosidase inhibitor acarbose, which showed an IC_{50} = 377 μM in the same test.

The Peruvian medicinal plant *Otholobium pubescens* exhibited anti-diabetic properties that have been attributed to the phenolic compound bakuchiol (**1**) [6]. Indeed, bakuchiol was active in the db/db mice model in a dose-dependent manner; however, it displayed no hypoglycemic effect when tested in lean mice at 250 mg/Kg q.d. [6]. Bakuchiol (**1**) was also isolated from *Psoralea corylifolia* and exhibited a moderate glycosidase inhibitory activity with an IC_{50} value of 115 μM [13]. To our knowledge, no *in vitro* inhibitory activity was previously reported for 3-hydroxybakuchiol (**2**) and isoflavonoids **3** and **4**. Interestingly, daidzein, which is the aglycone of daidzin (**3**) inhibited α-glucosidase and α-amylase enzymes with IC_{50} values of 48 μM and 301 μM, respectively, thus indicating that daidzein was more effective than acarbose [14]. Similarly, the isoflavone genistein, which is the aglycone of genistin (**4**),has been reported to reduce the activity of α-glucosidase in a dose-responsive manner, showing an IC_{50} value of 50 nM [15]. Thus, the remarkable α-glucosidase inhibitory activities of daidzein and genistein were about 20 times higher than those reported here for their glucosides **3** and **4**, respectively. These differences may be related to the mode and mechanism of enzyme inhibition and deserve further investigation.

In conclusion, the α-amylase and α-glucosidase inhibitory activity of *O. mexicanum* offers the possibility to develop this plant as a promising anti-diabetic natural remedy. However, these effects can be attributed, only in part, to the isolated compounds **1**-**4**. Thus, the activity might mainly be due to synergistic effects or to the presence of minor constituents with higher bioactivity, still to be isolated. Moreover, the yeast α-glucosidase is known to be very different from mammalian digestive enzymes, suggesting that further experiments should be extended to the inhibition of mammalian intestinal α-glycosidases.

Experimental

General Experimental Procedures: Chromatographic and spectrometric instruments and procedures were reported previously [16]. In addition, a *Varian Premium Shielded* spectrometer, operating at 400 MHz (^{1}H) and 100 MHz (^{13}C) was used. Preparative MPLC separations were performed on a *Biotage Isolera One* instrument equipped with home-made silica gel and RP-18 filled cartridges and a diode UV detector set at 254-366 nm. α-Amylase and α-glucosidase were isolated from porcine pancreas (Type IV-B, Sigma A3176, Saint Louis,MO, USA) and from *Saccharomyces cerevisiae* (Type I, Sigma G5003), respectively. Starch and *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG-Sigma, N1377) were used as the substrates for α-amylase and α-glucosidase, respectively. A microplate reader (EPOCH 2, Biotek Instruments Inc. Winoosky, VT, USA) was used for both assays.

Plant Material: *Otholobium mexicanum* were collected in the San-Lucas region of the Loja Province, Ecuador, in December 2010. The plant was identified by Bolivar Merino, of the Herbarium of the Nacional University of Loja. A voucher specimen (PPN-fa-005) has been deposited in the Herbarium of the Technical University of Loja. The plant collection was authorized by the Ministry of Environment of Ecuador-MAE (nº 047-IC-FLO-DPL-MA).

Extraction and Isolation: Two lots, 300 g and 200 g, respectively, of air-dried leaves of *O. mexicanum* were separately extracted. The first sample was directly macerated in MeOH at rt, three times overnight. After solvent evaporation, the residue (32 g) was successively exposed to *n*-hexane, $CHCl_3$, EtOAc, MeOH, yielding after evaporation *in vacuo*, residues A (10.3 g), B (5.56 g), C (8.6 g) and D (7.4 g), respectively. A sample (4.0 g) of C was directly chromatographed on a silica gel column, eluted with a solvent gradient from *n*-hexane-EtOAc (1:2) to EtOAc-MeOH (9:1). Elution with *n*-hexane-EtOAc (1:9) afforded bakuchiol (**1**) (342.2 mg), whereas 100% EtOAc gave a fraction which was further purified on a silica gel column. Elution with EtOAc-$CHCl_3$ (4:1), gave 3-hydroxybakuchiol (**2**) (43.2 mg). The second leaf lot was extracted successively with EtOAc, MeOH and MeOH-H_2O (7:3, v/v) at rt, three times for 1 h each, yielding, after solvent evaporation *in vacuo*, residues A´ (8.6 g), B´ (8.81) and C´ (16.9 g), respectively. MPLC separation of C´ (1.3 g) on a RP-18 column (100 g), eluted with a gradient of MeOH in H_2O (from 20 to 100%, v/v) at 30 ml/min, afforded 220 fractions of 8 ml each. Their combination on the basis of similar composition gave eight main fractions (C´1-C´8). Chromatography of C´4 (101 mg) on a silica gel column, eluted with DCM-MeOH (85:15), gave daidzin (**3**) [11] (15.3 mg), R_f = 0.25 (DCM-MeOH, 85:15). Chromatography of C´6 (62.6 mg) on a silica gel column eluted with a gradient of MeOH in DCM, from 10 to 20%, yielded three subfractions (C´6I-C´6III). Chromatography of C´6II (10.43 mg) on a silica gel column, eluted with DCM-MeOH (85:15), gave genistin (**4**) [12] (5.8 mg), R_f = 0.63 (DCM-MeOH, 85:15).

α-Amylase inhibition assay: The method reported by Xiao *et al*. [17] was followed with minor modifications. Acarbose was used as the positive control. A starch solution was prepared by dissolving 1 g in 50 mL of 0.4 M NaOH and heating at 100 °C for 5 min. After cooling in ice-water, the pH was adjusted to 7 with 2 M HCl and then H_2O was added to a final volume of 100 mL. Tested sample solutions were prepared by dissolving 10 mg in 1 mL of MeOH-H_2O (1:1); several dilutions with PBS (SIGMA-P4417) were made in case of complete enzyme inhibition. The PBS (35 μL, pH 7.4), the starch (20 μL) and the sample (5μL) solutions were mixed in a 96-microtiter plate, and the mixture was preincubated at 37 °C for 1 min. Then 20 μL of a 50 μg/mL α-amylase solution (SIGMA-A3176) was added to each well. The plate was incubated for 15 min. The reaction was terminated by adding 0.1 M HCl (50 μL); subsequently, 150 μL of a 0.5 mM iodine solution (0.5 mM I_2 and 0.5 mM KI) was added and the absorbance was measured in an EPOCH 2 (BIOTEK ®) microplate reader at 580 nm. Inhibitory activity (%) was calculated according to the formula [18]:

Inhibition (%) = [(*AbS2 – AbS1)/(AbS4-AbS3*)] X 100

where *AbS1* is the absorbance of the incubated solution containing sample, starch and amylase, *AbS2* is the absorbance of incubated solution containing sample and starch, *AbS3* is the absorbance of incubated solution containing starch and amylase, and *AbS4* is the absorbance of incubated solution containing starch. IC_{50} values were calculated by curve fitting of data (GraphPad Prism 5.0).

α-Glucosidase inhibition assay: The α-glucosidase inhibitory activity was determined using a 96-well microtiter plate with *p*-nitrophenyl-α-D-glucopyranoside (PNPG, SIGMA N1377) as the substrate, according to the method described by Tao *et al*. [19], with minor modifications. Acarbose was used as the positive control. Tested sample solutions were prepared by dissolving 10 mg in 1 mL of MeOH-H_2O (1:1); several dilutions with PBS were made in case of complete enzyme inhibition. PBS (75 µL) was mixed with sample solution (5 µL) and 20 µL of the enzyme solution (SIGMA G5003, 0.15U/mL in PBS pH 7.4); subsequently, the mixture was preincubated at 37 °C for 5 min before initiating the reaction by adding the substrate. Then, PNPG (5mM in phosphate buffer, pH 7.4, 20 µL) was added and incubated at 37 °C. The amount of *p*-NP released was measured in an EPOCH 2 (BIOTEK ®) microplate reader at 405 nm for 60 min, recording the absorbance every 5 min. The results were expressed as inhibition percentage by means of the formula [20]:

Inhibition (%) = [(*Ao – As)/Ao*] X 100,

where *Ao* is the absorbance recorded for the enzymatic activity without inhibitor (control), and *As* is the absorbance recorded for the enzymatic activity in presence of the inhibitor (sample). IC_{50} values were calculated by the GraphPad Prism 5.0 software.

Acknowledgments – We acknowledge the support of a fellowship to A.I.S from the SENESCYT-PROMETEO program.

References

[1] World Health Organization (WHO) (Nov 22, **2016**) Diabetes. WHO website. Retrieved from: http://www.who.int/mediacentre/factsheets/fs312/en/

[2] (a) Elavarasi S, Saravanan K, Renuka C. (**2013**) A systematic review on medicinal plants used to treat diabetes. *International Journal of Pharmaceutical Chemical and Biological Sciences*, ***3***, 983-992; (b) Gaikwad SB, Krishna-Mohan G, Sandhya Rani M. (**2014**) Phytochemicals for diabetes management. *Pharmaceutical Crops*, ***5***, 11-28; (c) Kalita H, Boruah DC, Deori M, Hazarika A, Sarma R, Kumari S, Kandimalla R, Kotoky J, Devi R. (**2016**) Antidiabetic and antilipidemic effect of *Musa balbisiana* root extract: A potent agent for glucose homeostasis in streptozotocin-induced diabetic rats. *Frontiers in Pharmacology*, *7*, 102.

[3] (a) Bussmann RW, Malca G, Glenn A, Sharon D, Nilsen B, Parris B, Dubose D, Ruiz D, Saleda J, Martinez M, Carillo L, Walker K, Kuhlman A, Townesmith A. (**2011**) Toxicity of medicinal plants used in traditional medicine in Northern Peru. *Journal of Ethnopharmacology*, ***137***, 121–140; (b) Inzucchi SE. (**2002**) Oral antihyperglycemic therapy for type2 diabetes mellitus. Scientific review. *Journal of the American Medical Association*, ***287***, 360–372.

[4] World Health Organization (WHO) (Nov 22, **2016**) Diabetes country profiles 2016. WHO website. Retrieved from: http://www.who.int/diabetes/country-profiles/en/

[5] Graf BL, Rojas-Silva P, Baldeón ME. (**2016**) Discovering the pharmacological potential of Ecuadorian market plants using a Screens-to-Nature participatory approach. *Journal of Biodiversity Bioprospecting and Development*, ***3***, 1-9.

[6] Krenisky JM, Luo J, Reed MJ, Carney JR. (**1999**) Isolation and antihyperglycemic activity of bakuchiol from *Otholobium pubescens* (Fabaceae), a Peruvian medicinal plant used for the treatment of diabetes. *Biological & Pharmaceutical Bulletin*, ***22***, 1137-1140.

[7] Solgorre EJ. (**2005**) Efecto del extracto hidroalcohólico de hojas y flores de *Otholobium pubescens* en la hiperglicemia experimental en Rattus norvergicus var. Albinus. Universidad Nacional Mayor de San Marcos, Lima, Perú.

[8] Carraz M, Lavergne C, Jullian V, Wright M, Gairin JE, de la Cruz MG, Geneviève Bourdy G. (**2015**) Antiproliferative activity and phenotypic modification induced by selected Peruvian medicinal plants on human hepatocellular carcinoma Hep3B cells. *Journal of Ethnopharmacology*, ***166***, 185-199.

[9] Bhutkar MA, Bhise SB. (**2012**) *In vitro* assay of alpha amylases inhibitory activity of some indigenous plants. *International Journal of Chemical Sciences*, ***10***, 457-462.

[10] Labbé C, Faini F, Call J, Connolly JD. (**1996**) Bakuchiol derivatives from the leaves of *Psoralea glandulosa*. *Phytochemistry*, ***42***, 1299-1303.

[11] Xu J, Feng S, Wang Q, Zhang M, Zhang C. (**2014**) A new flavonoid glycoside from the rhizomes and roots of *Smilax scobinicaulis. Natural Product Research*, ***28***, 517-521.

[12] Lewis P, Kaltia S, Wähälä K. (**1998**) The phase transfer catalysed synthesis of isoflavone-*O*-glucosides. *Journal of Chemical Society*, ***1***, 2481-2484.

[13] Oh KY, Lee JH, Curtis-Long MJ, Cho JK, Kim JY, Lee WS, Park KH. (**2010**) Glycosidase inhibitory phenolic compounds from the seed of *Psoralea corylifolia. Food Chemistry*, ***121***, 940–945.

[14] Park M-H, Ju J-W, Park M-J, Han J-S. (**2013**) Daidzein inhibits carbohydrate digestive enzymes in vitro and alleviates postprandial hyperglycemia in diabetic mice. *European Journal of Pharmacology*, ***712***, 48-52.

[15] Lee D-S and Lee S-H. (**2001**) Genistein, a soy isoflavone, is a potent α-glucosidase inhibitor. *FEBS Letters*, ***501***, 84-86.

[16] Gilardoni G, Chiriboga X, Vita Finzi P, Vidari G. (**2015**) New 3,4-secocycloartane and 3,4-secodammarane triterpenes from the Ecuadorian plant *Coussarea macrophylla*. *Chemistry & Biodiversity*, ***12***, 946-954.

[17] Xiao Z, Storms R, Tsang A. (**2006**) A quantitative starch–iodine method for measuring alpha-amylase and glucoamylase activities. *Analytical Biochemistry*, ***351***, 146-148.

[18] Kusano R, Ogawa S, Matsuo Y, Tanaka T, Yazaki Y, Kouno I. (**2011**) α-Amylase and lipase inhibitory activity and structural characterization of *Acacia* bark proanthocyanidins. *Journal of Natural Products*, ***74***, 119-128.

[19] Tao Y. Zhang Y, Cheng Y, Wang Y. (**2013**) Rapid screening and identification of α-glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. *Biomedical Chromatography*, ***27***, 148-155.

[20] Choi CI, Lee SR, Kim KH. (**2015**) Antioxidant and α-glucosidase inhibitory activities of constituents from *Euonymus alatus* twigs. *Industrial Crops and Products*, ***76***, 1055-1060.