Revista Brasileira de Farmacognosia xxx (2017) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

α-Glucosidase and pancreatic lipase inhibitory activities and glucose uptake stimulatory effect of phenolic compounds from *Dendrobium formosum*

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ARTICLE INFO

Article history:
Received 11 March 2017
Accepted 26 May 2017
Available online xxx

Keywords:
Dendrobium formosum
Glucose uptake
α-Glucosidase
Pancreatic lipase

ABSTRACT

A methanol extract from the whole plant of *Dendrobium formosum* Roxb. ex Lindl., Orchidaceae, showed inhibitory potential against α-glucosidase and pancreatic lipase enzymes. Chromatographic separation of the extract resulted in the isolation of twelve phenolic compounds. The structures of these compounds were determined through analysis of NMR and HR-ESI-MS data. All of the isolates were evaluated for their α-glucosidase and pancreatic lipase inhibitory activities, as well as glucose uptake stimulatory effect. Among the isolates, 5-methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone (**12**) showed the highest α-glucosidase and pancreatic lipase inhibitory effects with an IC_{50} values of 126.88 ± 0.66 μM and 69.45 ± 10.14 μM, respectively. An enzyme kinetics study was conducted by the Lineweaver-Burk plot method. The kinetics studies revealed that compound **12** was a non-competitive inhibitor of α-glucosidase and pancreatic lipase enzymes. Moreover, lusianthridin at 1 and 10 μg/ml and moscatilin at 100 μg/ml showed glucose uptake stimulatory effect without toxicity on L6 myotubes. This study is the first report on the phytochemical constituents and anti-diabetic and anti-obesity activities of *D. formosum*.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia that can cause further serious health problems such as neurological and cardiovascular complications (Peng et al., 2016). There are three main types of diabetes which are type I, type II, and gestational diabetes. Type II diabetes, which is due to insulin resistance, affects the majority (90–95%) of diabetic patients (American Diabetes Association, 2006; Rosak and Mertes, 2012). α-Glucosidase is a carbohydrate-hydrolyzing enzyme secreted from the intestinal chorionic epithelium. Inhibition of this enzyme is one of the therapeutic approaches for type II diabetes since it can cause retardation of carbohydrate digestion, which leads to the prevention of excess glucose absorption (You et al., 2012; Peng et al., 2016). Insulin plays a key role in reduction of the glucose level by stimulating the glucose transport from blood into skeletal muscle cells. It is well established that insulin-stimulated glucose uptake is impaired in type II diabetic patients (Yap et al., 2007; Choi et al., 2013). Therefore, searching for compounds that can enhance glucose uptake is an important approach to facilitate the development of new methods for insulin resistance treatment (Choi et al., 2013).

In addition, type II diabetes can be caused by the dysfunction of insulin-producing pancreatic β cells, which could be instigated by the excessive accumulation of lipids in the pancreas (Tushuizen et al., 2007; You et al., 2012). Pancreatic lipase is the key enzyme in lipid digestion, responsible for absorption of dietary fats through the breakdown of triacylglycerols into free fatty acids and monoacylglycerols in the intestinal lumen (Yang et al., 2014). Recently, inhibitors of pancreatic lipase have attracted much research interest due to their anti-obesity activity by delaying the lipolytic process. This action would lead to the decrease in lipid absorption and thus protect the pancreas, which will restore regular insulin production from the β cells (Tushuizen et al., 2007; You et al., 2012; Yang et al., 2014).

Dendrobium is a large genus in the Orchidaceae family which include about 1100 species, and 150 species have been identified

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http://dx.doi.org/10.1016/j.bjp.2017.05.005

Please cite this article in press as: Inthongkaew, P., et al. α-Glucosidase and pancreatic lipase inhibitory activities and glucose uptake stimulatory effect of phenolic compounds from *Dendrobium formosum*. Revista Brasileira de Farmacognosia (2017), http://dx.doi.org/10.1016/j.bjp.2017.05.005

in Thailand (Lam et al., 2015; Limpanit et al., 2016). Several *Dendrobium* species are well-known for their traditional medicinal properties. The stems of several species of *Dendrobium* have been used in folk Chinese medicine called "Shi-Hu" as sources of tonic, antipyretic, astringent, and anti-inflammatory substances (Hu et al., 2008; Lam et al., 2015). Previous studies revealed that *Dendrobium* plants contain diverse groups of secondary metabolites and possess various biological activities, including cytotoxic, antioxidant, anticancer, antimalarial, antifibrotic, hypoglycemic, and neuroprotective activities (Lam et al., 2015). A number of phenolic compounds from *Dendrobium tortile*, such as 3,4-dihydroxy-5,4′-dimethoxybibenzyl, (2*S*)-eriodictyol and dendrofalconerol A, showed strong α-glucosidase inhibitory activity in our recent investigation (Limpanit et al., 2016).

Dendrobium formosum Roxb. ex Lindl. is a rare orchid native to Himalayas and Indochina. It has one of the largest flowers among the dendrobes (Dohling et al., 2008). An earlier report of *D. formosum* described potential antitumor activity of its ethanolic extract (Prasad and Koch, 2014). However, prior to this study, there have been no reports of the phytochemical constituents and anti-diabetic and anti-obesity activities of this plant. As part of our ongoing research on bioactive constituents from *Dendrobium* species (Sukphan et al., 2014; Klongkumnuankarn et al., 2015), a methanol extract from the whole plant of *D. formosum* at a concentration of 50 μg/ml was evaluated and found to exhibit 95% inhibition against both α-glucosidase and pancreatic lipase enzymes. In this communication, we wish to report the first study on the chemical constituents of *D. formosum* and their α-glucosidase and pancreatic lipase inhibitory activities, as well as their glucose uptake stimulatory potential.

Material and methods

General

Mass spectra were recorded on a Bruker micro TOF mass spectrometer (ESI-MS). NMR spectra were recorded on a Bruker Avance DPX-300 FT-NMR spectrometer Microtiter plate reading was performed on a Perkin-Elmer Victor™ 1420 multilabel counter. Vacuum-liquid column chromatography (VLC) and column chromatography (CC) were performed on silica gel 60 (Merck, Kieselgel 60, 70–320 μm), silica gel 60 (Merck, Kieselgel 60, 230–400 μm) and Sephadex LH-20 (25–100 μm, GE Healthcare).

Chemicals

Alpha minimal essential medium (α-MEM), fetal bovine serum (FBS) and penicillin-streptomycin (10000 IU/ml) were purchased from Thermo Fisher Scientific (Grand Island, NY, USA). Glucose Oxidase (GO) assay kit, sodium dodecyl sulfate (SDS), 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), α-glucosidase from *Saccharomyces cerevisiae*, lipase from porcine pancreas, 4-methylumbelliferyl oleate (4MUO), *p*-nitrophenyl-α-D-glucopyranoside (pNPG), acarbose and orlistat were obtained from Sigma Aldrich (St Louis, MO, USA). Insulin (100 IU/ml) was obtained from Biocon (Bangalore, India). All other chemicals used were of analytical grade.

Cell lines and culture medium

L6 (Rat skeletal muscle, ATCC® CRL-1458) cell culture was purchased from the American Type Culture Collection (Manassas, VA, USA). Stock cells of L6 were cultured in α-MEM supplemented with 10% FBS, 1% penicillin-streptomycin, the growth medium, at 37 °C under 5% CO_2.

Plant material

The whole plant of *Dendrobium formosum* Roxb. ex Lindl., Orchidaceae, was purchased from Jatujak market, Bangkok, Thailand in September 2015. It was collected from Mae Sot district, Tak province, Thailand. Plant identification was performed by Prof. Thatree Phadungcharoen (Faculty of Pharmacy, Rangsit University). A voucher specimen (BS-DF-092558) is deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Extraction and isolation

The dried and powdered whole plant of *D. formosum* (2 kg) was extracted with MeOH (3 × 10 l) at room temperature to give a viscous mass of dried extract (115 g) after removal of the solvent. This material was suspended in water and then partitioned with EtOAc and *n*-butanol to give an EtOAc extract (57 g), a butanol extract (25 g) and an aqueous extract (30 g), respectively, after evaporation of the solvent. The EtOAc extract was subjected to vacuum liquid chromatography (silica gel, EtOAc-hexane, gradient) to give eight fractions (A-H). Fraction F (6.8 g) was fractionated on a silica gel column (EtOAc-hexane, gradient) to give nine fractions (FI-FIX). Fraction FII (271 mg) was separated by column chromatography (CC) over silica gel, eluted with a CH_2Cl_2-hexane gradient to give four fractions (FII1-FII4). Confusarin (**1**) (3 mg) was obtained from fraction FII2. Hircinol (**2**) (15 mg) was obtained from fraction FII4 (21 mg) after purification on Sephadex LH-20 (MeOH). Purification of fraction FIII (85 mg) on Sephadex LH-20 (MeOH) gave erianthridin (**3**) (45 mg). Fraction FV (648 mg) was separated by CC (silica gel, CH_2Cl_2-hexane, gradient) to give six fractions (FV1-FV6). Gigantol (**4**) (94 mg) was obtained from fraction FV3. Fraction FV2 (17 mg) was further purified on Sephadex LH-20 (MeOH) to afford nudol (**5**) (10 mg). Fraction FV5 (193 mg) was subjected to CC (silica gel, EtOAc-hexane, gradient) and then purified on Sephadex LH-20 (MeOH) to yield lusianthridin (**6**) (8 mg). Fraction FVI (361 mg) was fractionated by CC (silica gel, CH_2Cl_2-hexane, gradient) to give six fractions (FVI1-FVI6). Coelonin (**7**) (75 mg) was obtained from fraction FVI2. Dihydroconiferyl dihydro-*p*-coumarate (**8**) (25 mg) and batatasin III (**9**) (18 mg) were yielded from fractions FVI4 (57 mg) and FVI5 (29 mg), respectively, after purification on Sephadex LH-20 (MeOH). Fraction FVIII (272 mg) was separated by CC (silica gel, EtOAc-CH_2Cl_2, gradient) and then purified on Sephadex LH-20 (MeOH) to afford 2,5,7-trihydroxy-4-methoxy-9,10-dihydrophenanthrene (**10**) (22 mg). Fraction G (10 g) was fractionated by CC over silica gel, eluted with an EtOAc-hexane gradient to give seven fractions (GI-GVII). Fraction GIII (508 mg) was separated on Sephadex LH-20 (MeOH) to give eight fractions (GIII1-GIII8). Fraction GIII2 (51 mg) was further purified by CC (silica gel, CH_2Cl_2-hexane, gradient) to yield moscatilin (**11**) (5 mg). 5-Methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone (**12**) (11 mg) was obtained from fraction GIII4 (52 mg) after purification by CC (silica gel, MeOH-CH_2Cl_2, gradient).

Confusarin (**1**): yellow amorphous solid; $C_{17}H_{16}O_5$; HR-ESI-MS *m/z* 299.0919 $[M-H]^-$ (calc. for $C_{17}H_{15}O_5$ requires 299.0919). Its structure was identified by comparison of NMR data with published values (Majumder and Kar, 1987).

Hircinol (**2**): yellow amorphous solid; $C_{15}H_{14}O_3$; HR-ESI-MS *m/z* 265.0847 $[M+Na]^+$ (calc. for $C_{15}H_{14}O_3Na$ requires 265.0840). Its structure was identified by comparison of NMR data with published values (Fisch et al., 1973).

Erianthridin (**3**): yellow amorphous solid; $C_{16}H_{16}O_4$; HR-ESI-MS *m/z* 295.0949 $[M+Na]^+$ (calc. for $C_{16}H_{16}O_4Na$ requires 295.0946). Its structure was identified by comparison of NMR data with published values (Majumder and Joardar, 1985).

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