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Antioxidants and α-glucosidase inhibitors from *Ipomoea batatas* leaves identified by bioassay-guided approach and structure-activity relationships

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ABSTRACT

Sweet potato (*Ipomoea batatas*) leaf (SPL) is an underused commercial vegetable with considerable bioactivities. By means of DPPH· scavenging ability and α-glucosidase inhibitory oriented isolation, 9 and 7 compounds were isolated and identified, respectively. Among them, *trans*-*N*-(*p*-coumaroyl)tyramine (**1**), *trans*-*N*-feruloyltyramine (**2**), *cis*-*N*-feruloyltyramine (**3**), 4,5-feruloylcourmaoylquinic acid (**8**), caffeic acid ethyl ester (**10**), 7-hydroxy-5-methoxycoumarin (**11**), 7,3′-dimethylquercetin (**13**) and indole-3-carboxaldehyde (**15**), were firstly identified from SPL, and four of them (**1**, **2**, **3** and **10**) were firstly identified from genus *Ipomoea*. Phenethyl cinnamides and 3,4,5-triCQA exhibited the strongest α-glucosidase inhibition, while 3,4,5-triCQA and diCQAs were the dominant antioxidants. Structure-activity relationship revealed that higher caffeoylation of quinic acid and lower methoxylation of flavonols resulted in stronger antioxidant activity, and methylation and *cis*-configuration structure of phenethyl cinnamides weaken the α-glucosidase inhibition. Aforementioned results could help to explain the antioxidant activity and anti-diabetic activity of SPL, and provide theoretical basis for its further application.

1. Introduction

Diabetes mellitus (DM) is a global metabolic disorder characterized by high blood glucose levels. In 2013, 382 million people worldwide are living with DM, 90% of them were affected by type 2 diabetes, and the number is expected to rise to 592 million by 2035 with the development of urbanization, enhancement of living standard and changes of humans' diet habits (Ley, Hamdy, Mohan, & Hu, 2014 and Seuring, Archangelidi, & Suhrcke, 2015). DM also impose serious adverse effect on people's health, these with DM are more liable to risk for cardiovascular disease, kidney failure, frailty, depression, premature death, and cognitive decline, et al. (Gerstein et al., 2008). What's more, diabetes has become a major cause of death in people younger than 60 years, the death caused by DM accounts for nearly 9% of the total global deaths (Aguiree et al., 2013). Therefore, it is urgent to explore effective therapy method for DM.

A promising approach for the management of DM, particularly in non-insulin-dependent DM, is to decrease postprandial hyperglycemia by using α-glucosidase inhibitors to suppress carbohydrate digestion. α-Glucosidase is a carbohydrase located in the brush-border surface membrane of intestinal cells, which facilitates the absorption of glucose by small intestine through catalyzing the hydrolysis of oligosaccharide into monosaccharide (Lebovitz, 1997). Effective inhibitors can significantly delay intestinal glucose absorption, reduce fasting and postprandial hyperglycemia, and help manage diabetes and decrease the incidence of late diabetic complications (Göke et al., 1994). α-Glucosidase inhibitors have been recommended as a first line therapy by the American Association of Clinical Endocrinologists (AACE) and

Abbreviations: HPLC, high performance liquid chromatography; Semi-HPLC, semi-preparative high performance liquid chromatography; MPLC, medium pressure liquid chromatography; 1D NMR, one-dimensional nuclear magnetic resonance spectroscopy; 2D NMR, two-dimensional nuclear magnetic resonance spectroscopy; COSY, ^{1}H–^{1}H correlation spectroscopy; HSQC, heteronuclear single-quantum correlation spectroscopy; HMBC, heteronuclear multiple-bond correlation spectroscopy; IC_{50}, half maximal inhibitory concentration; SPL, sweet potato leaves; CF, chloroform fraction; EAF, ethyl acetate fraction; *n*BF, *n*-butanol fraction; WF, water fraction; triCQA, tricaffeoylquinic acid; diCQA, dicaffeoylquinic acid; DM, diabetes mellitus.

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International Diabetes Federation (IDF) due to the characteristics of safety, efficacy, lack of hypoglycemia, tolerability benefits of cardiovascular (Garber et al., 2013; Group, 2014), and are termed the 'untapped diamonds' of diabetology (Lebovitz, 1997). However, synthetic α-glucosidase inhibitors have many undesirable adverse effects (Diaz-Gutierrez, Ladero, & Diaz-Rubio, 1998 and Lebovitz, 1997), as a consequence, great efforts have been made to search for effective, non-toxic and inexpensive α-glucosidase inhibitors from natural resources that can prevent the risk of type 2 diabetes and related complications.

Sweet potato leaf (SPL) is a byproduct of sweet potato products, and have been consumed as a leafy vegetable in many parts of the word. It has attracted researchers' attention due to considerable functions in the prevention of many diseases, such as inflammation, tumor, cancer, cardiovascular, hypertension, hyperglycemia and diabetes (Islam, 2006; Kurata, Adachi, Yamakawa, & Yoshimoto, 2007; Nagamine et al., 2014; Yoshimoto et al., 2002). It is reported that SPL showed much higher polyphenols than many other commonly consumed edible plants (Yang, Lin, & Kuo, 2008). The health benefits of SPL extract could be due to the properties of polyphenols as free radical scavengers, hydrogen-donating compounds, and/or singlet oxygen quenchers. Thu et al. (2004) revealed that SPL extract gave the highest antioxidant activity as compared to that of other 30 kinds of vegetables in Vietnam. However, to date, there is limit information regarding the exactly compounds contribute to the antioxidant activity, especially to the anti-diabetes effect, which provoke our great interests to conduct this research.

This study was carried out to discover the bio-active compounds in SPL responsible for the α-glucosidase inhibition and antioxidant activity using a bioassay-guided approach. Orange-fleshed SPL were selected to be the materials because of its higher antioxidant activity and polyphenol content than cream-fleshed SPL. α-Glucosidase inhibitory and DPPH radical (DPPH·) scavenging ability assays were used as the bio-active models. Crude extract of SPL was successively separated with chloroform, ethyl acetate and *n*-butanol to yield chloroform fraction (CFF), ethyl acetate fraction (EAF) and *n*-butanol fraction (*n*BuF). Following, the compounds with high radical scavenging ability or α-glucosidase inhibitory were further isolated and purified by using XAD-16 Amberlite resin, Sephadex LH-20, middle pressure liquid chromatography (MPLC) and semi-preparative high performance liquid chromatography (Semi-HPLC). During the separation, only the fractions/sub-fractions exhibited the strongest α-glucosidase inhibitory or DPPH· scavenging ability were chosen for further separation and purification. The structures of purified compounds were elucidated by analyzing the spectroscopic data including nuclear magnetic resonance (NMR) and electrospray ionization mass spectrometry (ESIMS).

2. Material and methods

2.1. General

All 1D nuclear magnetic resonance (^{1}H and ^{13}C NMR) and 2D NMR [^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond coherence (HMBC)] data were acquired either on a Bruker 300 MHz or a Varian 500 MHz instrument using tetramethylsilane (TMS) as a reference at room temperature. Deuterated methanol (CD_3OD) or dimethyl sulfoxide (DMSO-d_6) was used as the solvent for all of the NMR experiments. ESIMS data were acquired on a Q-Star Elite (Applied Biosystems MDS) mass spectrometer under positive and negative ion mode. Column chromatography was conducted over XAD-16 Amberlite resin, Sephadex LH-20 and MPLC with prepacked C18 columns. High performance liquid chromatography (HPLC) analysis were conducted on a Hitachi Elite Lachrom system (Pleasanton, CA, UAS) equipped with a L-2450 diode array detector, a L-2200 autosampler, a L-2130 Pump, EZChrom Elite software and a Luna C18(2) 100A (4.6 × 250 mm, 5 μm, Phenomenex) column at a flow rate of 0.75 mL/min. Semi-HPLC was performed on a SunFire™ Pre C18 (5 μm, 10 × 250 mm, Waters) column at a flow rate of 2.8 mL/min.

2.2. Chemicals and materials

All solvents used for HPLC analysis were HPLC grade, others were analytical grade, and were obtained from Pharmco-AAPER through Wilkem Scientific (Rhode Island, USA). Sephadex LH-20, XAD-16 Amberlite resin, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteau reagent were purchased from Sigma-Aldrich (Missouri, USA). α-Glucosidase powder were from MP Biomedicals (Illkirch, France).

Fresh orange-fleshed SPL (Jishu No. 16), were manually collected from the farm in Yichun (Oct., 2013, Jiangxi, China), and identified by Guan-xian Liu in Jiangxi Academy of Agricultural Science. The leaves were washed with running tap water, dried at 45 °C, grounded into powders and sieved through a 200 mash mesh screen. The obtained powders were then stored in a sealed polyethylene bag and kept in 4 °C until use.

2.3. Extraction and isolation of compounds

For activity-guided fractionation, dried SPL powders (1.0 kg) were extracted with dynamic high pressure microfluidization-assisted extraction following previously optimized parameters (Huang et al., 2013). The suspension was then centrifuged and evaporated to dryness with a rotary evaporator *in vacuo* at 40 °C to obtain 244.0 g crude extract (CE). Then, 100.0 g CE was suspended in 100 mL water and successively partitioned with chloroform (1.0 L × 3), ethyl acetate (1.0 L × 3) and *n*-butanol (1.0 L × 3) to obtain four fractions. Ethyl acetate fraction (EAF, 21.5 g) was applied to XAD-16 Amberlite resin and eluted successively with 40% ~ 90% MeOH/H_2O (46%, v/v) at an increment of 10% (1.0 L for each gradient) to afford six sub-fractions (EA1 ~ EA 6) based on HPLC spectra. Following, fraction EA-1 and EA-4 were separately chromatographed over a Sephadex LH-20 with MeOH as eluent collecting with each tube of 8.0 mL. According to the HPLC spectra of elutes, sub-fractions EA-1a ~ EA-1c were obtained from fraction EA-1, sub-fractions EA-4a ~ EA-4c and compound **4** (103.2 mg) were obtained from fraction EA-4. Fraction EA-1a was future purified by using Semi-HPLC eluting with 34% MeOH/0.1% TFA in water (v/v) to afford compound **9** (20.3 mg, t_R = 14.21 min). Fraction EA-1b was purified by Semi-HPLC eluting with 38% MeOH/0.1% TFA in water (v/v) to afford compounds **5** (8.1 mg, t_R = 18.61 min), **6** (7.14 mg, t_R = 19.85 min) and **12** (7.3 mg, t_R = 30.19 min). Fraction EA-1c was separated with MPLC (20–60% MeOH, v/v) followed by purification with Semi-HPLC to give compounds **7** (2.9 mg, t_R = 11.58 min), **8** (6.0 mg, t_R = 16.27 min), **13** (2.3 mg, t_R = 19.13 min), **14** (5.8 mg, t_R = 22.08 min) and **4** (5.7 mg, t_R = 24.71 min). Fraction EA-4a was separated by Semi-HPLC and eluted with 43% MeOH/0.1% TFA in water to yield compound **3** (1.8 mg, t_R = 19.15 min). Fraction EA-4b was further purified by Semi-HPLC eluted with 48–50% MeOH/0.1% TFA in water to obtain compounds **1** (7.6 mg, t_R = 17.85 min), **2** (28.2 mg, t_R = 19.04 min), **10** (27.7 mg, t_R = 26.87 min), **11** (3.6 mg, t_R = 12.71 min) and **15** (2.8 mg, t_R = 14.98 min). The flow chart was given in Fig. 1.