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Original article

Antihyperglycemic and antioxidant activities of twig extract from *Cinnamomum osmophloeum*

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ABSTRACT

This is the first report concerning the α-glucosidase, α-amylase and protein tyrosine phosphatase 1B (PTP1B) inhibitory activities of cinnamon twig extracts. Comparing the antihyperglycemic activity of renewable plant parts, indigenous cinnamon (*Cinnamomum osmophloeum*; 土肉桂 tǔ ròu guì) twig extracts (CoTE) showed better α-glucosidase and α-amylase activities than leaf, 2-cm branch and 5-cm branch extracts. Chemotype of *C. osmophloeum* has no influence on the antihyperglycemic activities and proanthocyanidin contents of CoTE. Among four soluble fractions obtained from CoTE by following bioactivity-guided fractionation procedure, the *n*-butanol soluble fraction (BSF) with abundant proanthocyanidins and condensed tannins, exhibited the best antihyperglycemic and PTP1B inhibitory activities. In addition, the BSF displayed the excellent DPPH free-radical scavenging and ferrous ion-chelating activities. The antihyperglycemic and antioxidant activities of all four soluble fractions from CoTE showed high correlation coefficient with their proanthocyanidin and condensed tannin contents. Furthermore, CoTE had no toxicity on 3T3-L1 preadiocytes. Results obtained demonstrated that CoTE has excellent antihyperglycemic, antioxidant and PTP1B inhibitory activities, and thus has great potential as a source for natural health products.

1. Introduction

The incidence and prevalence of type 2 diabetes have acutely increased since 1990. A dramatic 64% growth between 2010 and 2025 is predicted, influencing 53.1 million people and posing an extremely huge medical and societal cost. The incidence of type 2 diabetes is highly related to age, inheritance, diet, lifestyle and environmental pressure.[1] Obesity showed high correlation with risk of type 2 diabetes, and aggravated insulin resistance of type 2 diabetes. Excessive nutrients lead to energy overload, thus affecting the metabolic function of adipocytes. The dysfunction of adipocytes would cause generation of reactive oxygen species (ROS), change secretion of adipokines, increase release of fatty acids and inflammatory factors. Overload of free fatty acids would result in lipotoxicity and dyslipidemia, which in turn affect uptake of glucose and insulin sensitivity.[2,3]

Besides injection of insulin, there are three kinds of hypoglycemic drugs, namely insulin secretagogues, insulin sensitizers and α-glucosidase inhibitors for maintaining glucose homeostasis in type 2 diabetes patients.[4] These commercial hypoglycemic drugs also have many side effects.[5] The antihyperglycemic assays, such as α-glucosidase inhibition, α-amylase inhibition, protein tyrosine phosphatase 1B (PTP1B) inhibition and glucose uptake in cells are commonly used to estimate the beneficial effects on the treatment of type 2 diabetes. Over 400 plant extracts have been estimated for the treatment of diabetes throughout the world. Several phytochemicals in plant extracts were evaluated by antihyperglycemic assays and might have multiple benefits on type 2 diabetes to avoid side effects.[6] Aqueous extract from *Cinnamomum burmannii* bark is rich in proanthocyanidins.[7] It was also reported that extracts of *C. burmannii* contained proanthocyanidins with A-type linkage and had insulin-like biological activity.[8] Furthermore, four water extracts of *Cinnamomun* bark were reported to have significant inhibitory activities of glucose metabolic enzymes, and might be

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potential substitution of commercial α-glucosidase inhibitory drugs.[9]

Studies have showed that the coumarin contents in commercial cinnamons, such as *Cinnamomum cassia* and *C. burmannii*, are high. The average coumarin content in *C. cassia* barks and twigs was 5790 mg/kg of sample, and coumarin contents in *C. burmannii* bark were between 2140 and 9300 mg/kg of sample.[10,11] Coumarin was reported that have hepatotoxic and carcinogenic effects in animals.[12,13] The results of no observed-adverse-effect level for liver toxicity in the most sensitive animal species led the European Food Safety Authority to establish a tolerable daily intake of 0.1 mg of coumarin/kg body weight.

Cinnamomum osmophloeum (Lauraceae) (土肉桂 tǔ ròu guì) is an endemic species in Taiwan and its leaves have high cinnamaldehyde and low coumarin contents.[14] Meanwhile, coumarin was not detected in the essential oil of *C. osmophloeum* twig.[15] However, studies on the antihyperglycemic activities of *C. osmophloeum* twig are lacking, hence its inhibitory activities of glucose metabolic enzymes and active components merit investigation.

With the consideration for sustainability and non-destructive utilization, twigs and branches of *C. osmophloeum* were used in this study instead of the bark. The antihyperglycemic activities were evaluated by α-glucosidase and α-amylase inhibitory assay. Inhibition of these two glucose metabolic enzymes could decrease the absorption rate of glucose to prevent acute rise of postprandial blood glucose of type 2 diabetes.[16] PTP1B inhibitory activities of cinnamon twigs have not been investigated yet. PTP1B has been known to play an important role in inhibiting signaling pathways of insulin and leptin receptors. PTP1B-knockout animals need lower insulin to activate glucose uptake of cells and decreased weight.[17] Therefore, benefit effect on insulin and leptin sensitivities were evaluated by PTP1B inhibitory assay. DPPH free-radical scavenging and ferrous ion-chelating activity were utilized to estimate the antioxidant activities. The active components were presumed by the correlation analysis between different phenolic contents and bioactivities. Finally, the viability of 3T3-L1 preadipocytes was examined for the toxicity of extracts. With the above-mentioned assays, the antihyperglycemic potency of *C. osmophloeum* twigs for nature health products could be elucidated.

2. Materials and methods

2.1. Chemicals

Analytical grade solvents for extraction and chromatography were purchased from Echo Chemical Co. (Taiwan). α-Glucosidase from *Bacillus stearothermophilus*, α-amylase from porcine pancreas, 3,5-dinitrosalicylic acid (DNS), 4-nitrophenyl phosphate disodium salt hexahydrate (*p*NPP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (Ferrozine), trizma hydrochloride (Tris–HCl), DL-dithiothreitol (DTT), (+)-catechin hydrate, acarbose and rutin hydrate were purchased from Sigma Chemical Co. (USA). *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG), $KNaC_4H_4O_6 \cdot 4H_2O$, quercetin dihydrate, ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2), $FeCl_2 \cdot 4H_2O$, $NH_4Fe(SO_4)_2 \cdot 12H_2O$ and thiazolyl blue tetrazolium bromide (MTT) were purchased from Acros (Belgium). Ursolic acid, citric acid, vanillin and $AlCl_3 \cdot 6H_2O$ were purchased from Merck (Germany). PTP1B from human was purchased from Enzo Life Sciences (Switzerland). Dulbecco's modified Eagle's medium (DMEM) and newborn calf serum (NCS) were purchased from Gibco BRL (USA). Melacacidin was separated and purified from *Acacia confusa* root according to Lin and Chang.[18] All other unlabelled chemicals and reagents were purchased from Sigma Chemical Co. (USA).

2.2. Sampling of plant materials

The leaf, twig (diameter < 0.5 cm), 2-cm and 5-cm branch of cinnamaldehyde type and the other two chemotypes of twig, including mixed and linalool types of *C. osmophloeum* (土肉桂 tǔ ròu guì) were collected in the July of 2012 from the Hsin-Sheng Nursery (24.841532°N, 121.533524°E) in New Taipei city, and the trees were about 30 years. The species was identified by Mr. Yen-Ray Hsui (Taiwan Forestry Research Institute) and the materials were deposited at the laboratory of Wood chemistry (School of Forestry and Resource Conservation, National Taiwan University).

2.3. Extraction and isolation

Those dried samples were grounded into powder and soaked in 70% acetone at ambient temperature for seven days. The antihyperglycemic twig crude extracts were then extracted successively with *n*-hexane, ethyl acetate, *n*-butanol, and water to yield the *n*-hexane soluble fraction (HSF, 4.0%), ethyl acetate soluble fraction (EASF, 4.7%), *n*-butanol soluble fraction (BSF, 55.4%), and water soluble fraction (WSF, 35.9%). Each fraction was tested by the various assays including α-glucosidase inhibition, α-amylase inhibition, PTP1B inhibition, DPPH free-radical scavenging activity, and ferrous ion-chelating activity to determine the best active fraction.

2.4. Inhibitory assay for α-glucosidase

The inhibitory activity of α-glucosidase was estimated according to Lin and Lee.[19] Briefly, 20 μL of ddH_2O, 10 μL of extracts/50% methanol with different concentrations (1–100 μg/mL) and 60 μL of 0.25 unit/mL α-glucosidase/0.1 M phosphate buffer (pH 7.0) were mixed together. The mixture was incubated at 37 °C for 10 min, and then the reaction was initiated by the addition of 10 μL of 20 mM *p*NPG/0.1 M phosphate buffer for 10 min incubation and the absorbance was measured at 405 nm. Acarbose was used as a positive control.

2.5. Inhibitory assay for α-amylase

The inhibitory activity of α-amylase was estimated according to Apostolidis et al[20] with slight modifications. Briefly, 50 μL of extracts/50% methanol with different concentrations (25–1000 μg/mL) were mixed with 50 μL of 0.5 mg/mL α-amylase/20 mM phosphate buffer (pH 6.9). The mixture was incubated at 37 °C for 10 min, and then added 50 μL of 1% starch/20 mM phosphate buffer. After 10 min of incubation at 37 °C, added 100 μL of reagent (1% DNS in 0.4 M NaOH/ddH_2O of 12% $KNaC_4H_4O_6 \cdot 4H_2O$/ddH_2O) and incubated at 100 °C for 15 min. Finally, total volume was made up to 1.25 mL with ddH_2O and the absorbance was measured at 405 nm. Acarbose was used as a positive control.

2.6. Inhibitory assay for PTP1B

Method of this assay was carried out according to Na et al[21] with slight modification. Briefly, 10 μL of extract/10% DMSO with different concentrations (0.1–100 μg/mL), 10 μL of 2 mM *p*NPP/50 mM citrate buffer (pH 6.0, 0.1 M NaCl, 1 mM EDTA-Na_2 and 1 mM DTT) and 100 μL of 1 μg/mL PTP1B/50 mM citrate buffer were mixed together. The mixture was incubated at 37 °C for 30 min, and then added 100 μL of 1 N NaOH/ddH_2O to stop the reaction. The absorbance was measured at 405 nm. Ursolic acid was used as a positive control.