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Hypoglycemic activity of *Chenopodium formosanum* Koidz. components using a glucose uptake assay with 3T3-L1 adipocytes

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ABSTRACT

Djulis (*Chenopodium formosanum* Koidz.) is a cereal plant native to Taiwan. In this study, the hypoglycemic activity of djulis was investigated using a glucose uptake assay with 3T3-L1 adipocytes. Dehusk seeds (SD) or husks (HD) of djulis were extracted using 6 solvents with different polarities (i.e., water, hexane, acetone, ethyl acetate, methanol and ethanol). The crude water extract of husk of djulis (WHD) showed the highest increase in glucose uptake with 3T3-L1 adipocytes. Thus, it was further separated by solvent partition, XAD-7 gel column chromatography, and HPLC. Two hypoglycemic components (P1 and P2) were isolated from WHD. They showed hypoglycemic activity at 5–10 ppm. P1 might have a synergistic effect with insulin. P2, however, did not enhance insulin-stimulated glucose uptake, which might mean it is an insulin mimic. The results suggested that djulis is a potential anti-diabetes functional food ingredient.

1. Introduction

Chenopodium formosanum Koidz. (djulis) is an indigenous cereal plant in Taiwan. It mainly grows in some aboriginal inhabited areas and has been consumed for more than 100 years by aborigines. Traditionally, djulis is cooked with taro, rice or other millets. It can also be used as an ingredient in wine brewing. The colorful spikes of djulis are attributed to its high content of betalains (indole-containing pigments). According to their structure, betalains are divided into betacyanins (red-violet color) and betaxanthins (yellow-orange color). Both are sensitive to heat, light, oxygen and pH (Tsai, Chen, Sheu, & Chen, 2011; Tsai, Sheu, Wu, & Sun, 2010). Djulis is rich in nutrients, the content of starch, protein and dietary fiber are about 50%, 14%, and 14%, respectively (Tsai, 2008). The total amount of dietary fiber in djulis is about 3 and 6 times higher than oat and sweet potatoes, respectively (Tsai, 2008). The amount of calcium in djulis is about 6 and 50 times higher than oat and rice, respectively (Tsai, 2008).

Djulis contains many functional phytochemicals such as betalains, phenolic acids and flavonoids. Betanin (one of the betacyanins) has been reported to suppress linoleate peroxidation and inhibit proliferation of melanoma cells (Kanner, Harel, & Granit, 2001; Tsai et al., 2011; Wu et al., 2006). Studies indicate that djulis is a rich source of phenolic acids and flavonoids (Chyau, Chu, Chen, & Duh, 2015; Hsu, Lin, Inbaraj, & Chen, 2017; Tsai et al., 2011). Phenolic acids are a group of secondary metabolites in plants that have strong antioxidative activities (Gruz, Ayaz, Torun, & Strnad, 2011; Lodovici, Guglielmi, Meoni, & Dolara, 2001). Flavonoids have many biological activities, such as anti-inflammation and reduction of the risk of cardiovascular diseases, and are ubiquitous in plants (Kao, Wu, Hong, Wu, & Chen, 2007; Terao, Kawai, & Murota, 2008). Hsu et al. (2017) developed an improved HPLC-DAD-ESI-MS/MS method for simultaneous determination of phenolic acids and flavonoids in djulis. Their results showed that hydroxyphenylacetic acid pentoside was the major phenolic acid (1860 μg/g) followed by vanillic acid (70.7 μg/g) and hydroxyphenylacetic acid (55.4 μg/g). The major flavonoid in dehusk djulis was rutin-*O*-pentoside (257 μg/g), followed by quercetin-acetyl-rutinoside hexoside glucuronide (72.8 μg/g) and quercetin-3-*O*-(coumaroyl)-rutinoside (70.7 μg/g).

Type 2 diabetes, characterized by high blood glucose levels, is a major metabolic disease worldwide. Its prevalence is increasing yearly and the number of people with diabetes mellitus (DM) is expected to reach 360 million in 2030 (Alonso-Castro, Miranda-Torres, Gonzalez-Chavez, & Salazar-Olivo, 2008; Maritim, Sanders, & Watkins, 2003; Shim et al., 2003). Furthermore, DM is associated with several chronic

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complications, such as cardiovascular disease, and pathological changes of retina and kidney (Maritim et al., 2003; Shim et al., 2003). Currently, oral hypoglycemic drugs are the major treatment for type 2 diabetes. However, most of them can cause undesirable side effects (Alonso-Castro et al., 2008; Hui, Tang, & Go, 2009; Spiller & Sawyer, 2006). Thus, effective phytochemicals without undesirable side effect are needed. Some studies indicated that oxidative stress related closely to the high level of free radicals that have an important role in the pathogenesis of DM. In addition, high oxidative stress may also induce insulin resistance (Baynes & Thorpe, 1999; Ceriello, 2000; Maritim et al., 2003). Owing to its great protective ability against oxidative stress, djulis has a potential to be developed as a functional food ingredient for the prevention of diabetes (Chyau et al., 2015; Tsai et al., 2010, 2011). Thus, the objective of this study was to investigate the hypoglycemic activity of djulis using a glucose uptake assay with 3T3-L1 adipocytes.

2. Materials and methods

2.1. Materials

Chenopodium formosanum Koidz. (djulis) (including dehusk seeds (SD) and husks (HD)) was a gift from Dr. Pi-Jen Tsai, Department of Food Science, National Pingtung University of Science and Technology, Pingtung, Taiwan. After harvesting, the djulis were dried using a solar drying. SD were ground into powder using a rotor speed mill (Yuanmei Co., Taipei, Tawain) and packed into plastic bags. On the other hand, HD were not ground. Both SD powder and HD were stored at −20 °C for a maximum of 90 days. Bovine serum albumin (BSA, 96% purity), dexamethasone (DEX, 97% purity), *p*-anisaldehyde (99% purity), D-(+)-glucose (99% purity), insulin from porcine pancreas (≧27 USP units/mg), glycerol (99% purity), and 3-isobutyl-1-methylxanthine (99% purity) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Disodium hydrogen phosphate ($Na_2HPO_4{\cdot}2H_2O$, 99.5% purity), magnesium chloride ($MgCl_2{\cdot}6H_2O$, 99% purity), potassium chloride (KCl, 99.5% purity), sodium bicarbonate ($NaHCO_3$, 99% purity), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$, 99% purity) and sodium dodecyl sulfate (SDS, 99% purity) were purchased from Merck Co. (Darmstadt, Germany). 2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyl-D-glucose (2-NBDG) was purchased from Invitrogen (Eugene, OR, USA). Sulfuric acid (H_2SO_4) was purchased from OSAKA (Osaka, Japan).

The HPLC-grade solvents (including acetonitrile and methanol) were purchased from Macron Chemicals (Center Valley, PA, USA). Acetone, butanol, ethyl acetate, ethanol and hexane were purchased from Echo Chemical Co. (Taipei, Taiwan). Acetic acid was purchased from Riedel de Haen (Seelze, Germany). Formic acid was purchased from Showa Kako Co. (Tokyo, Japan). Dimethyl sulfoxide (DMSO) was purchased from Merck Co.

Dulbecco's modified Eagle's medium (DMEM) (including high glucose or low glucose), antibiotic-antimycotic solution (containing 10,000 units/mL of penicillin, 10,000 μg/mL of streptomycin and 25 μg/mL of Fungizone™), fetal bovine serum (FBS), bovine serum (BS), L-glutamine and 0.25% trypsin-EDTA were purchased from Gibco Co. (Grand Island, NY, USA).

2.2. Preparation of crude water extract of djulis

SD powder (10 g) or HD (5 g) samples were mixed with deionized water (Millipore Co., Billerica, MA, USA) (the ratio of sample to water was 1:50, g/mL) for 5 min. The mixture was then extracted using a reflux condenser at a temperature of 100 °C for 30 min and then filtered through filter paper (Whatman No. 4, Buckinghamshire, UK). The filtrates were freeze-dried using a freeze dryer (SFD-25, Chang Juing Macvhinert Co., Kaohsiung, Taiwan) and stored at −80 °C for a maximum of 90 days.

2.3. Preparation of djulis crude extracts with different solvents

Extraction solvents included hexane, acetone, ethyl acetate, methanol and ethanol. SD powder (10 g) or HD (5 g) samples were mixed with different solvents (the ratio of sample to solvent was 1:20, g/mL) for all samples. The mixtures were extracted with stirring at room temperature (25 ± 5 °C) for 24 h and then filtered through filter paper (Whatman No. 4). The filtered residues were collected and extracted again. The two extracts were combined from two replicates, concentrated under vacuum and stored at −80 °C for a maximum of 90 days.

2.4. Solvent partition of crude water extract of husks of djulis (WHD)

A 30 g sample of WHD was dissolved in 600 mL of deionized water (the ratio of sample to water was 1:20, g/mL) and then mixed with 600 mL of ethyl acetate followed by shaking by hand (1 min) and standing to allow layers to separate. The ethyl acetate layer (WHD-EA) was collected and the partitioning step repeated twice. The WHD-EA of three replicates were combined. Next, the water layer was further mixed with butanol (the ratio of water to butanol was 1:1, v/v) followed by shaking by hand (1 min) and standing until the layers separated. The butanol layer of WHD (WHD-Bu) was collected and the partitioning step repeated twice. The WHD-Bu of three replicates were combined, as well as the water layer (WHD-W) were collected. All sample solutions were dried under vacuum and stored at −80 °C for a maximum of 90 days.

2.5. Ethanol partition of WHD-W

A 10 g sample of WHD-W was dissolved in 100 mL of deionized water and then mixed with 95% ethanol (400 mL). Next, the mixture was centrifuged (6080 ×*g*, 4 °C, Avanti J-26 XP, Beckman Coulter Co., Brea, CA, USA) for 10 min. Both the ethanol soluble layer (WHD-W-ET) and precipitate (WHD-W-ET-P) were collected. WHD-W-ET was dried under vacuum and WHD-W-ET-P was freeze-dried. The samples were stored at −80 °C for a maximum of 90 days.

2.6. XAD-7 gel column chromatography of WHD-W-ET

The WHD-W-ET was dissolved in 50% methanol and poured into a XAD-7 gel column (600 × 40 mm I.D.) previously activated with 50% methanol (1200 mL). The column was eluted sequentially using different proportions of methanol in water (i.e., 50%, 60%, 70%, 80%, 90% and 100% methanol) with a flow rate of 15 mL/min. The volume of each mixture was 1200 mL. The eluates were collected in 200 mL portions.

2.7. Thin layer chromatography (TLC) analysis of WHD-W-ET

The fractions of WHD-W-ET were assayed using TLC (Silica gel 60 F_{254}, Merck Co.). After spotting, the TLC plate was placed in an elution chamber with a solution of ethyl acetate: ethanol:H_2O: formic acid = 5:3:1:0.1 (v/v/v/v). The TLC plate was then scanned at 254 and 366 nm (SJ1031A, ATTO, Tokyo, Japan). Next, the TLC plate was soaked in a *p*-anisaldehyde/H_2SO_4 solution containing 15 mL of *p*-anisaldehyde, 10 mL of acetic acid, 350 mL of ethanol and 10 mL of H_2SO_4, dried and then colorized on a hot plate (about 100 °C).

2.8. HPLC analysis of fraction 2 of WHD-W-ET (WHD-W-ET-F2)

A method based on Tsai, Wu, and Cheng (2008) was modified to separate samples. The sample (WHD-W-ET-F2) was dissolved in 50% methanol (2×10^4 μg/mL). The solution was filtered through a 0.22 μm membrane filter (Chrom Tech, Inc., Apple Valley, MN, USA) and then 20 μL of the solution was injected into an HPLC for analysis. The HPLC