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Original Article

Antihyperlipidemic activity of *Allium chinense* bulbs

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ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form
5 January 2016
Accepted 21 January 2016
Available online 12 April 2016

Keywords:
Allium chinense
antihyperlipidemic
antioxidant
essential oil
flavonoid

ABSTRACT

Allium chinense is a medicinal plant and nutritional food commonly used in Eastern Asia. In this study, we investigated the *in vitro* antioxidant activity (scavenging of α,α-diphenyl-β-picrylhydrazyl free radical, total phenol content, reducing power, and total antioxidant activity) and constituents of various extracts from *A. chinense*. Moreover, we also studied the *in vivo* hypolipidemic effects of extracts on high-fat-diet Wistar rats. Ethanol extracts from *A. chinense* showed notable antioxidant activity, and its high-dose essential-oil extract both significantly reduced serum and hepatic total cholesterol, triglyceride, and low-density lipoprotein levels and increased serum high-density lipoprotein levels in high-fat-diet Wistar rats compared with those observed following treatment with the control drug probucol. Additionally, visceral fat in high-fat-diet Wistar rats was reduced. Furthermore, groups with high doses of essential-oil and residue extracts showed protective effects associated with histopathological liver alteration. These results suggested that *A. chinense* is a valuable plant worthy of further investigation as a potential dietary supplement or botanical drug.

1. Introduction

Both reactive oxygen species (ROS) and oxidative stress play a central role in the pathology and progression of many related human diseases. Therefore, enormous efforts to scientifically investigate potential endogenous and exogenous antioxidants have arisen [1]. For estimating actual oxidative defense or stress status, a selected group of nutrients and antioxidant biomarkers, such as superoxide dismutase, catalase, glutathione peroxidase, and NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, are measured. In general, appropriate ROS levels result in transmission of intracellular and defense signals against pathogens. However, increasing ROS levels lead to the development of several human diseases, including cancer, diabetes, atherosclerosis, ischemia, and endocrine dysfunction. Therefore, moderating ROS levels and lowering the amount of serum lipids are important in disease treatment.

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http://dx.doi.org/10.1016/j.jfda.2016.01.010

Distributed in Eastern Asia, *Allium chinense* (synonyms: *Allium bakeri*; common name: rakkyo, Chinese onion) is a perennial herb native to China that is usually harvested from March to May. *A. chinense* is commonly cultivated for nutritional and medicinal purposes, and is often pickled and served as a side dish or as a nutritional supplement. The plant is traditionally used to treat stenocardia, heart asthma, and antiplatelet aggregation [2]. Its naturally occurring sulfur-containing compounds responsible for its onion-like flavor were proven to influence plasma cholesterol and atherosclerosis *in vitro* [3,4]. Another study also reported the ability of its steroidal constituents to prevent cardiac injuries induced by oxidative stress. Although more than 20 different steroidal compounds have been identified, including furostanol saponins [5], spirostanol saponins [6], and xiebai-saponins [7], only one study investigated the antioxidant activity of *A. chinense* [8]. Because its antihyperlipidemic activity has not been previously studied, we investigated the target activity and chemical constituents of *A. chinense*.

In this study, *A. chinense* bulbs cultivated in Taiwan were investigated for their principal components and organic acids. The amount of quercetin and rutin in extracts was analyzed by high-performance liquid chromatography (HPLC), while volatile oil compounds were analyzed by gas chromatography mass spectrometry (GC-MS). Moreover, extracts of *A. chinense* bulbs were tested in a series of *in vitro* antioxidation assays, as well as in an *in vivo* antihyperlipidemic rat model. Evidence reported herein for antioxidative and antihyperlipidemic effects of *A. chinense* extracts show that the plant material is a promising candidate for counteracting oxidative stress-related diseases and atherosclerosis.

2. Materials and methods

2.1. Chemicals

α,α-Diphenyl-β-picrylhydrazyl free radical (DPPH), linoleic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene, α-tocopherol, bovine serum albumin, thiobarbituric acid, ferrozine, lecithin, SDS (sodium dodecyl sulfate), ammonium thiocyanate, ferric chloride, KH_2PO_4, and K_2HPO_4 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium dihydrogen phosphate, disodium hydrogen phosphate, NaBr, and trichloacetic acid were obtained from Merck & Co. Inc. (Kenilworth, JN, USA). Tween 20 was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). HCl, NaCl, and copper sulfate were purchased from the Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). EDTA was purchased from Mallinckrodt Pharmaceuticals (Raleigh, NC, USA). Ferrous chloride, Coomassie brilliant blue G-250, *n*-butanol, and phosphotungstic acid were bought from Avantor Performance Materials (Baker analyzed reagents; Center Valley, PA, USA).

2.2. Plant material and the preparation of the extracts

A. chinense was bought and cultured from Puli Town in Nantou County, Taiwan (April 2013). The bulb portion of the plant was washed and dried in the shade. The water extract of *A. chinense* bulb was prepared by a plant-to-water ratio of (1:4), and then a blender was used to smash the bulb. Finally, the Likens-Nickerson apparatus was used to heat the extract with solvent (*n*-hexane-to-ether ratio: 1:1) for 3 hours. Later, nitrogen gas was used to dry the extract and obtain the essential oil (ABO). The remaining residue was filtered and freeze dried to obtain the supernatant (ABW) and residue (ABR) layers.

2.3. Analysis of principal components

2.3.1. Determination of water content, ash content, crude-fat content, crude-protein content, crude-fiber content, and carbohydrate content

The targeting components indicated were detected using methods approved by the Association of Official Agricultural Chemists.

2.3.2. Determination of organic acid

Sample powder (500 mg) was extracted with 50 mL 80% ethanol. This suspension was shaken for 45 minutes at room temperature and filtered through Whatman No. 4 filter paper. The residue was re-extracted five times with an additional 25 mL 80% ethanol. The combined filtrate was then rotary evaporated at 40°C and redissolved in deionized water to a final volume of 10 mL. The aqueous extract was filtered using a 0.45-mm polyvinylidene fluoride membrane filter (Millipore, Billerica, MA, USA) and analyzed using high-performance liquid chromatography (HPLC). The HPLC system consisted of a Hitachi L-2130 pump (Tokyo, Japan), a Rheodyne 7725i injector (Rohnert Park, CA, USA), a 20-mL sample loop, a Hitachi L-2400 UV detector, and an RP-18 GP250 column (4.6 mm × 250 mm; Mightysil, Kanto Chemical Co., Tokyo, Japan). The mobile phase was acetonitrile/deionizer water [75:25 (v/v)] at a flow rate of 0.8 mL/min, and UV detection was at 300 nm. Each organic acid was identified using the authentic organic acid (all from Sigma-Aldrich) and quantified by its respective calibration curve.

2.4. Flavonoids determination

2.4.1. Determination of the content of quercetin and rutin

One gram of the sample was extracted for 24 hours with 20 mL of ethanol in a 35°C water bath. The solution was filtered and prepared for further HPLC analysis. A Hitachi HPLC system with L-2130 pump, L-2200 autosampler, and UV-detector (214 nm and 350 nm, L-2400 UV detector) was used. A RP-18 GP250 column (250 mm × 4.6mm inner diameter, 5 μm; Mightysil, Kanto Chemical Co.) was used, with a mobile phase of phosphate buffer (H_2O: 85% phosphoric acid 99.7:0.3, v/v)/acetonitrile/methanol at a flow rate of 1.0 mL/min. The injection volume of the sample was 20 μL. The analytic condition was performed using Fuleki's method [9]. First, the standard products of quercetin and rutin were dissolved in methanol for establishment of a calibration curve, then calibration curves were constructed using the peak area (Y axis) and the concentration (mg/mL; X axis) of quercetin or rutin standards. The application of HPLC analysis of *A. chinense* extracts was performed, and the content of quercetin and rutin was determined under different processes associated with *A. chinense*.