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RESEARCH ARTICLE

In comparison with vitamin C and butylated hydroxytoluene, the antioxidant capacity of aqueous extracts from buds and flowers of Lonicera japonica Thunb.

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Abstract

OBJECTIVE: To assess the antioxidant capacity of aqueous extracts of buds and flowers of Lonicera japonica Thunb. (BLJ and FLJ) using *in vitro* assays.

METHODS: We assessed the *in vitro* antioxidant activities of aqueous extracts of BLJ and FLJ and compared with that of classical antioxidants vitamin C and butylated hydroxytoluene, using several well-established methods including the 1,1-diphenyl-2-picryl-hydrazyl assay, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) assay, reducing power assay, fluorescence recovery after photobleaching assay, β-carotene bleaching assay, ferric thiocyanate assay, and thiobarbituric acid method.

RESULTS: The aqueous extracts of both BLJ and FLJ had similarly potent antioxidant capacity. There were no significance differences between BLJ and FLJ in all the assays.

CONCLUSION: The aqueous extracts of both BLJ and FLJ have antioxidant activity with comparable efficacy. These findings suggest that both BLJ and FLJ may have the potential as natural antioxidants.

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Keywords: Buds; Flowers; Lonicera Japonica; Free radical scavengers; Lipid peroxidation

INTRODUCTION

Many medicinal plants have antioxidant properties.[1] These plants have been traditionally used in folk medicine as natural remedies for their preventive and/or therapeutic effects in cardiovascular diseases, inflammatory disorders, and cancer. These plants have also been utilized as food additives based on the preservative effects of their antioxidant and antimicrobial properties.[2]

Oxidation provides the energy for the human body for numerous activities such as exercising, breathing, digestion, metabolism, and blood circulation. In fact, a certain degree of oxidation can provide the necessary energy to maintain health and the production and elimination of free radicals within the bodies should be maintained at an equilibrium because excessive oxidation may be hazardous.

Free radicals are unstable molecules that can damage the cells of the human body. The oxidative stress is the result of an imbalance between the production of free radicals and the body's antioxidant defense system.[3] The excessive free radicals in the human body could increase lipid peroxides, which are believed to contribute

to the pathogenesis of a variety of degenerative diseases. Antioxidants can regulate various oxidative reactions that naturally occur in tissues to help maintain redox balance.[4]

Lonicera japonica Thunb., also known as Jinyinhua or Rendong, is commonly used in the Traditional Chinese Medicine (TCM). Buds of Lonicera japonica Thunb. have been used for the treatment of exopathogenic wind-heat, epidemic febrile diseases, sores, carbuncles, furuncles, and infectious diseases. It is also used as additives in food, cosmetics, and soft beverages for its specific activities.[5]

In the Pharmacopoeia of the People's Republic of China, Jinyinhua is recorded as a caprifoliaceae honeysuckle, Lonicera japonica Thunb.. While the dried buds of Lonicera japonica Thunb. (BLJ) is the form of Lonicera japonica Thunb. commonly used in the TCM, the opened flowers of Lonicera japonica Thunb. (FLJ) have not been tested for their efficacy in the treatment of specific conditions and its pharmacological effects are unclear.

In the present study, we comprehensively evaluated the antioxidant activity of the aqueous extracts of BLJ and FLJ using several well-established *in vitro* assays.

MATERIALS AND METHODS

Drugs and reagents

Reference standards cynaroside and chlorogenic acid (both with purity > 98%) were purchased from Chengdu MUST Bio-Technology Co., Ltd. (Sichuan, China). HPLC grade methanol and acetonitrile were from Honeywell Burdick & Jackson (Morristown, NJ, USA). Phosphoric acid was from Dikma Technologies Inc. (Beijing, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Vitamin C was from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Linoleic acid was from Alfa Aesar (Ward Hill, MA). 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), 2-thiobarbituric acid (TBA), trichloroacetic acid, potassium persulfate, 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), and β-carotene were from Sigma. Butylated hydroxyltoluene (BHT) was from the National Medicine Group Chemical Reagent Co., Ltd. (Beijing, China). All other chemicals used in this study were of analytical grade.

Plant materials and sample pretreatment

BLJ and FLJ were obtained from Fengqiu country, Henan province, China and they were authenticated by Professor Lidong Zhou at Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College. A voucher specimen (No. 20160001) was deposited in the National Compound Bank of Traditional Chinese Academy of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing.

The BLJ (10 g) and FLG (10 g) were extracted three times in boiling water for 30 min respectively. The filtrate was first evaporated via a rotary evaporator to a volume of about 500 mL, and then 500 mL of absolute ethanol was added. The resulting solution was filtrated and then evaporated to a volume of about 30 mL prior to lyophilization.

Preparation of standards and samples for ultra performance liquid chromatography (UPLC) analysis

The standards were dissolved in 50% methanol to make the final standard solution of cynaroside (4.75 μg/mL) and chlorogenic acid (55 μg/mL). Corresponding to 100 mg materials, the extracts of FLJ (46.0 mg) and BLJ (31.4 mg) were dissolved in 50% methanol in an ultrasonic bath at room temperature for 30 min. The solutions of BLJ and FLJ were filtered through 0.22 μm membrane. A 3 μL aliquot of the filtrate and standard solution were injected into the UPLC system for analysis.

UPLC analysis

Waters Acquity UPLC™ system (Waters, MA, USA) was equipped with a PDA detector and an auto sampler. An ACQUITY UPLC HSS T3 column (2.1 × 150 mm, 1.8 μm) was used for the separation at 30 ℃. The mobile phase consisted of A (water containing 0.4% phosphoric acid, v/v) and B (acetonitrile). The flow rate was 0.20 mL/min and the detection wavelength was 350 nm. The conditions of the linear gradient elution were as follows: 0-2 min, 5%-16% B; 2-4 min, 16%-20% B; 4-8 min, 20%-50% B; and 8-10 min, 50%-100% B.

DPPH radical scavenging activity assay

The DPPH radical scavenging effect was analyzed as described elsewhere[6] Equal volumes (1 mL) of the extract solution (in 65% ethanol) at various concentrations and 0.2 mM DPPH (in 65% ethanol) were mixed. After incubation for 1 h at room temperature in the dark, the absorbance value was measured at wavelength 517 nm using a microplate reader spectrophotometer (Bio Tek, VT, USA). The concentrations of BHT and VC (as positive controls) were identical to those of the experimental samples. Radical scavenging activity was determined using the following equation: [(As-Ai)/As] × 100% (As = Absorbance of DPPH alone, Ai = Absorbance of DPPH in the extract solution at various concentrations).

ABTS radical scavenging activity assay

The ABTS radical scavenging activity of the BLJ and FLJ extracts were determined as described elsewhere.[7] A solution of ABTS radical cation was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) at room temperature in the dark for 16 h. The mixture was then diluted with deionized water to

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