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Phenolic compounds and antioxidant activities of edible flowers of *Pyrus pashia*

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ABSTRACT

Pyrus pashia flowers are used as edible and medicinal materials in Yunnan Province, China. The antioxidant activities of 85% ethanolic extract of *P. pashia* flowers and derived soluble fractions were evaluated for the first time using four different test systems. The total phenolic and total flavonoid contents of each fraction were also determined. It was found that the ethyl acetate fraction exhibited the strongest antioxidant effect. An investigation of this flower's chemical constituents was carried out and led to the isolation of a new phenolic glycoside, 4-*O*-*Z*-coumaroylarbutin (**1**), along with twenty-seven other phenolic compounds (**2–28**). Hydroquinone (**13**) showed the highest content and powerful activity, which implied that the compound plays an important role in the antioxidant activity of this flower. Thus, *P. pashia* flowers could serve as a valuable natural antioxidant source for potential application in functional foods.

1. Introduction

Edible flowers, which have been used in the culinary arts for thousands of years worldwide, are receiving renewed interest because of their beneficial health effects on the immune system, cardiovascular disease, and certain cancers (Oueslati et al., 2012; Yang & Walters, 1992). These properties are generally attributed to high amounts of bioactive compounds such as phenolics, which have been reported to exhibit various physiological effects in humans, including inhibiting platelet aggregation, reducing the risk of coronary heart disease and cancer, and preventing oxidative damage to lipids and low-density lipoproteins (Kaur, Alam, Jabbar, Javed, & Athar, 2006;

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Chemical compounds: 4-hydroxybenzaldehyde (PubChem CID: 126); 3,4-dihydroxybenzaldehyde (PubChem CID: 8768); 4-methoxybenzoic acid (PubChem CID: 7478); *p*-hydroxyacetophenone (PubChem CID: 7469); Hydroquinone (PubChem CID: 785); Arbutin (PubChem CID: 440936); Gastrodin (PubChem CID: 115067); Apigenin (PubChem CID: 5280443); Apigenin 7-*O*-*β*-D-glucopyranoside (PubChem CID: 5280746).

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Shui & Leong, 2006; Zeng, Zhao, & Peng, 2008). Phenolics have strong *in vitro* and *in vivo* antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate prooxidant metal ions (Kaisoon, Siriamornpun, Weerapreeyakul, & Meeso, 2011; Wang et al., 2014). The increased consumption of phenolics has been correlated with anti-inflammatory activity and reduced risk of cardiovascular disease and certain cancers (Oliveras-López, Berná, Jurado-Ruiz, López-García de la Serrana, & Martín, 2014).

Pyrus pashia Buch.-Ham. ex D. Don (Rosaceae) is a type of tree that is distributed in the wild in Yunnan, Sichuan, Guizhou and Gansu Provinces in China at an altitude of 650–3000 m (Institute of Botany, Chinese Academy of Sciences, Institute of Materia Medica, & Chinese Academy of Medical Sciences, 1979). Various parts of *P. pashia* are used as herbal products in Chinese traditional medicine (CTM). For example, its fruits are effective in the treatment of dyspepsia and dysmenorrhoea, whereas its branches and leaves are effective in the treatment of diarrhoea (Khandelwal, Paliwal, Chauhan, & Siddiqui, 2008). In particular, *P. pashia* flowers have been used not only as an herbal medicine for lowering blood lipids but also as one of the most common edible flowers in Yunnan Province, China. Conventionally, after being soaked in water for approximately 24 h, *P. pashia* flowers are cooked with chicken, eggs or meat to treat cough, emesis and diarrhoea. This plant is a potential source of functional food and is worth developing and popularizing worldwide. Nevertheless, a systematic study on the bioactive ingredients of *P. pashia* flowers, which may play an important role in developing and popularizing this edible flower on a global scale, has not yet been performed. As part of our continuous work on the discovery of bioactive ingredients from different parts of *P. pashia* (Cai et al., 2014; Zhao et al., 2013), we have carried out an extensive investigation on *P. pashia* flowers, which led to the isolation of thirteen compounds from the less polar fraction during our preliminary study (Liu, Lin, Wang, Chen, & Yang, 2009). In the present study, the antioxidant activities of 85% ethanolic extract from *P. pashia* flowers and derived soluble fractions were evaluated in four different antioxidant models, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), β-carotene bleaching assay (BC), and inhibition of lipid peroxidation in mouse tissues (LPO). Subsequently, an investigation of the extract's chemical constituents was carried out and led to the isolation of a new phenolic glycoside, 4-*O*-*Z*-coumaroylarbutin (**1**), along with twenty-seven other phenolics (**2–28**) (Fig. 1). Moreover, the antioxidant activities and contents of some isolated compounds were evaluated.

2. Experimental

2.1. Chemicals and equipment

DPPH, 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), and β-carotene were obtained from J&K Scientific Ltd (Beijing, China). Rutin, gallic acid, butylated hydroxyanisole (BHA) were purchased from Aladdin-Reagent (Shanghai, China). $CDCl_3$, CD_3OH and DMSO were obtained from Sigma-Aldrich (Shanghai, China). The thiobarbituric acid-malondialdehyde (TBA-MDA) detection kits were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Deionized water (resistivity ≥ 18.25 MΩ·cm^{-1}) was purified with a You Pu purity water system (You Pu, Chengdu, China). All other chemicals used were of analytical grade.

Optical rotation was measured with a Jasco P-1020 digital polarimeter (JASCO, Tokyo, Japan). A Shimadzu UV–Vis 2550 spectrometer (Shimadzu, Kyoto, Japan) was used for colorimetric measurements and collection of UV spectra. NMR spectra were acquired with either a Bruker AM-500 spectrometer (Bruker, Karlsruhe, Germany) or a Bruker DRX-600 spectrometer (Bruker) using TMS as the internal reference. A Nicolet Magna-IR 550 spectrometer (Thermo Nicolet, Madison, WI, USA) was used for scanning IR spectroscopy with KBr pellets. Melting points were determined on a XRC-1 Melting Point Apparatus (Sichuan University Science Instrument, Chengdu, China) and were not corrected. ESI-MS analyses were recorded with an Agilent G3250AA (Agilent, Santa Clara, CA, USA) and Auto Spec Premier P776 spectrometer (Waters, Milford, CT, USA). Silica gel (200–300 mesh and 300–400 mesh; Qingdao Marine, Qingdao, China) and Sephadex LH-20 (GE Healthcare, Fairfield, CT, USA) were used for column chromatography (CC). GF254 plates (Qingdao Marine, Qingdao, China) were used for thin layer chromatography, and spots were visualized under UV light or by spraying with 10% H_2SO_4 in ethanol followed by heating.

2.2. Plant material

P. pashia flowers were collected from Kunming City in Yunnan Province, China, in February 2009. The samples were identified by professor Shu-gang Lu at the School of Life Sciences at Yunnan University in Kunming City, China. A voucher specimen is deposited at the Key Laboratory of Medicinal Chemistry for Natural Resources, Ministry of Education in Kunming City of China (No. CLT-1).

2.3. Extraction and isolation

Air-dried and powdered flowers of *P. pashia* (8.0 kg) were extracted with 85% ethanol at 50 °C (3 × 15 L, each extraction 48 h). Removal of the solvent under reduced pressure afforded the crude extract (1.1 kg), which was partitioned successively with petroleum ether (PE), EtOAc, and BuOH to yield soluble fractions in PE (105 g), EtOAc (210 g), BuOH (250 g), and water (aqueous) (110 g), respectively. Five gram samples were collected from each fraction for the antioxidant experiments. The remainders were used to study the fractions' chemical components.

The EtOAc fraction was subjected to silica gel CC elution with a $CHCl_3$–CH_3OH gradient system (200:1 to 4:1, *v*/*v*) to give ten fractions (Fr.A1–Fr.A10). Fr.A4 (3.4 g) was subjected to silica gel CC (PE–acetone, 20:1 to 2:1, *v*/*v*) and Sephadex LH-20 (CH_3OH) to provide **5** (15 mg), **6** (9 mg) and **7** (10 mg). Fr.A6 (3.7 g) was subjected to silica gel CC ($CHCl_3$–CH_3OH, 100:1 to 30:1, *v*/*v*) to yield **8** (30 mg), **11** (30 mg), **12** (11 mg), **13** (500 mg), and **26** (10 mg). Fr.A7 (1.4 g) was subjected to silica gel CC ($CHCl_3$–CH_3OH, 50:1 to 10:1, *v*/*v*) and Sephadex LH-20 (CH_3OH) to provide **4** (50 mg) and **14** (10 mg). Fr.A8 (5.0 g) was subjected to silica gel CC ($CHCl_3$–CH_3OH, 20:1 to 1:1, *v*/*v*) to yield **2** (10 mg), **9** (15 mg), **15** (4 mg), **16** (5 mg), and **19** (41 mg). Fr.A9 (7.0 g) was