Isolation and Identification of Antioxidant Flavonoids from the Seeds of *Cardiocrinum Giganteum* var. Yunnanense

**ABSTRACT**

One new biflavonoid, 3″-hydroxyrobustaflavone (1), together with four known compounds (2-5) were isolated from the seeds of *Cardiocrinum giganteum* var. yunnanense. The new structure was elucidated based on the extensive spectroscopic methods and the known compounds were identified by comparison with the literatures. In addition, all of these isolated compounds possessed good antioxidant capacities beyond that of L-ascorbic acid.

**KEYWORDS** *Cardiocrinum giganteum* var. yunnanense, biflavonoid, flavonoid, oxygen radical absorbance capacity

**INTRODUCTION**

Flavonoids are a group of prevalent secondary metabolites which were produced via the phenylpropanoid biosynthetic pathway in various plants. However, the dimers, biflavonoids are limited to exist in a few families, which were formed through phenol-oxidative coupling of different types of flavonoid monomers. It was reported that both the dimer and monomer types of flavonoids possess a variety of pharmacological properties, such as antioxidant, anti-inflammatory, anti-bacterial, etc.

*Cardiocrinum giganteum* var. yunnanense Leichtlin ex Elwes, belonging to the family of Liliaceae, is a perennial herb of *Cardiocrinum* (Endl.) Lindl. The seeds of this herb is commonly called “doulingzi” and used as the succedaneum of Aristolochia fruits in treating cough. So far, the chemical constituents and the pharmacological activities of *C. giganteum* have rarely been investigated. During our systematic investigation on the chemical constituents of 95% ethanol extract of the seeds of *Cardiocrinum giganteum* var. yunnanense, one new biflavonoid, 3″-hydroxyrobustaflavone (1), as well as four known ones, 3″-hydroxymentoflavone (2), quercetin (3), and apigenin (4), and kaempferol (5) were isolated. Herein, we reported the isolation, structural elucidation and antioxidant activities of these isolated compounds.

**MATERIALS AND METHODS**

**General**

Melting points were obtained on an X-5 melting point apparatus reported without correction. Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH solution at room temperature. HR-ESI-TOFMS was obtained on an Agilent 6210 ESI/TOF mass spectrometer. IR spectra (KBr) were recorded on a Jasco FT/IR-480 Plus Fourier transform infrared spectrometer. UV spectra (MeOH) solutions were tested on a Jasco V-550 UV/vis spectrophotometer. NMR spectra were recorded on a Bruker AV-300 or AV-400 spectrometer. Precoated silica gel GF254 plates (Yantai Institute of Chemical Industry, YanTai, China) were used for TLC analysis. Column chromatography was carried out on silica gel (200–400 mesh, Qingdao Marine Chemical Plant, Qingdao, China), reversed-phase C18 silica gel (Welch Materials, China), and Sephadex LH-20 (Pharmacia Biotec AB, Uppsala, Sweden). HPLC analysis was carried out on Agilent 1200 series.
High Performance Liquid Chromatography. Preparative HPLC was conducted on HPLC-LC100 (FIRICH). All solvents used in separation and analysis were of analytical grade (Shanghai Chemical Plant, Shanghai, P. R. China) and chromatographic grade (Fisher Scientific, NJ, USA), respectively.

**Plant material**

The seeds of *Cardiocrinum giganteum* var. yunnanense Leichtlin ex Elwes were collected at Teng-Chong city in Yun-Nan Province of China in December, 2014 and authenticated by Professor Pang-Chui Shaw using DNA identification methods. A specimen (No. 2014092001) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, P. R. China.

**Isolation**

The dried and powdered seeds of *Cardiocrinum giganteum* var. yunnanense Leichtlin ex Elwes (1 kg) were extracted under ultrasonic condition (25°C, 30 min) with 95% (V/V) ethanol three times. The solution was concentrated under reduced pressure to afford a crude extract (102.3 g), which was subsequently partitioned between EtOAc and H$_2$O. The EtOAc part (20.5 g) was subjected to preparative HPLC eluted by CH$_2$Cl$_2$ (1:1) to yield three flavones (Fr. 14A to Fr. 14J). Fr. 14G (2.8 g) was separated by Sephadex LH-20 and preparative HPLC eluted by CH$_3$CN-H$_2$O (40:60) to give compounds 3 (17 mg), 4 (12 mg) and 5 (8 mg).

3"-hydroxyrobusflavone (1): yellow powder; [α]$_D^{25}$ +3.7 (c = 1.0, MeOH); UV (MeOH) $\lambda_{max}$ (log e): 207 (2.94), 350 (3.15) nm; IR (KBr): $\nu$$_{max}$ 3423, 1613, 1172 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; HR-ESI-MS m/z: 553.0780 [M-H]$^-$ (calcd for [C$_{29}$H$_{30}$O$_{11}$-H]$^-$: 553.0771).

**Antioxidant assay**

The antioxidant activities of the flavonoids compounds were measured using an automated oxygen radical absorbance capacity (ORAC) assays by the method described previously$^{14}$. The reaction was initiated with the addition of compounds, fluorescein and 2, 2’-azobis (2-amidinopropane) dihydrochloride. The analysis was performed utilizing a GENios luciferase-based microplate reader (Tecan, Mannedorf, Switzerland) with excitation/emission filter pair of 485/527 nm. The results were calculated as the area under the fluorescence decay curve using Trolox as a standard and L-ascorbic acid as a positive control.

**RESULTS AND DISCUSSION**

The dried and powdered seeds of *Cardiocrinum giganteum* var. yunnanense Leichtlin ex Elwes were extracted under ultrasonic condition with 95% ethanol. The EtOAc partition of the total extract was separated using various chromatography methods, i.e. silica gel, ODS, and preparative HPLC to afford compounds 1-5 (Fig. 1).

<table>
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<th>$^{13}$C</th>
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<th>$^1$H</th>
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<td>5-OH</td>
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</table>

$^1$H and $^{13}$C NMR data of 1 (δ, in DMSO-d$_6$, J in Hz).
of hydroxyl and carbonyl groups. After a detailed analysis of the 1D and 2D spectra of \(1\), the existence of one flavone and one flavonol units was confirmed (rings A-C and D-F). The \(^1\)H NMR spectrum of \(1\) showed proton signals at \(\delta^H 13.07 (1\ H, s, 5-OH)\), \(6.16 (1\ H, d, J = 1.8\ Hz, H-6)\), and \(6.37 (1\ H, d, J = 1.8\ Hz, H-8)\) in a 1,3,4,5 tetra-substituted ring A, \(\delta^H 8.18 (1\ H, d, J = 2.4\ Hz, H-2')\), \(7.91 (1\ H, dd, J = 8.7, 2.4\ Hz, H-6')\) and \(6.96 (1\ H, d, J = 8.7\ Hz, H-5')\) in a 1,3,4 tri-substituted ring B, and \(\delta^H 6.79 (s)\) in ring C. These above evidences supported the existence of a flavone part A in \(1\) (Fig. 2). Accordingly, the proton signals at \(\delta^H 12.66 (1\ H, s, 5''-OH)\) and \(6.15 (1\ H, s, H-8'')\) for ring D, \(\delta^H 7.80 (2H, d, J = 8.8\ Hz, H-2'', H-6'')\) and \(6.58 (2H, d, J = 8.8\ Hz, H-3'', H-5'')\) for a para-substituted ring E were in accordance with a flavonol part B in \(1\) (Fig. 2). The \(^1^3\)C NMR spectrum showed thirty carbon signals, including nineteen aromatic quaternary carbons, and eleven aromatic methines, among which, carbon signals at \(\delta^C 181.5 (C-4'')\) and \(175.5 (C-4')\) were two typical flavonoid keto-carbonyl groups, confirming that \(1\) was biflavonoid type natural product containing two C6-C3-C6 units.

The \(^1\)H and \(^1^3\)C NMR signals of \(1\) were all assigned unambiguously based on the \(^1\)H-\(^1\)H COSY, HSQC and HMBC data (Table 1). In the HMBC spectrum, HMBC correlations between H-2' and H-6'', and between H-8'' and H-4'' indicated that the flavone unit was connected to the flavonol via a C3'-C6'' carbon-carbon bond. Thus, the structure of \(1\) was established to be 3''-hydroxyrobusta flavone, which was a robustaflavone type biflavonoid, consisting of apigenin (4) and kaempferol (5).

The antioxidant activities of these isolated compounds were evaluated using oxygen radical absorbance capacity antioxidant assay (ORAC) (Figs. 3 and 4). The results showed that either the monomers or the dimers exhibited much higher antioxidant activities than the positive control L-ascorbic acid. At the concentrations of hydroxyl and carbonyl groups. After a detailed analysis of the 1D and 2D spectra of 1, the existence of one flavone and one flavonol units was confirmed (rings A-C and D-F). The \(^1\)H NMR spectrum of 1 showed proton signals at \(\delta^H 13.07 (1\ H, s, 5-OH)\), \(6.16 (1\ H, d, J = 1.8\ Hz, H-6)\), and \(6.37 (1\ H, d, J = 1.8\ Hz, H-8)\) in a 1,3,4,5 tetra-substituted ring A, \(\delta^H 8.18 (1\ H, d, J = 2.4\ Hz, H-2')\), \(7.91 (1\ H, dd, J = 8.7, 2.4\ Hz, H-6')\) and \(6.96 (1\ H, d, J = 8.7\ Hz, H-5')\) in a 1,3,4 tri-substituted ring B, and \(\delta^H 6.79 (s)\) in ring C. These above evidences supported the existence of a flavone part A in 1 (Fig. 2). Accordingly, the proton signals at \(\delta^H 12.66 (1\ H, s, 5''-OH)\) and \(6.15 (1\ H, s, H-8'')\) for ring D, \(\delta^H 7.80 (2H, d, J = 8.8\ Hz, H-2'', H-6'')\) and \(6.58 (2H, d, J = 8.8\ Hz, H-3'', H-5'')\) for a para-substituted ring E were in accordance with a flavonol part B in 1 (Fig. 2). The \(^1^3\)C NMR spectrum showed thirty carbon signals, including nineteen aromatic quaternary carbons, and eleven aromatic methines, among which, carbon signals at \(\delta^C 181.5 (C-4'')\) and \(175.5 (C-4')\) were two typical flavonoid keto-carbonyl groups, confirming that 1 was biflavonoid type natural product containing two C6-C3-C6 units.

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of 4.0 μM, the dimers, 1 and 2 were 1.5 times more active than L-ascorbic acid at 40.0 μM. By contrast, the monomers, 3 and 4 at the same concentration of 4.0 μM showed 2.5 times of activities compared with L-ascorbic acid (40.0 μM). Compound 5, which was the most potent one, at a lower concentration of 1.0 μM are 4 times more active than L-ascorbic acid (40.0 μM). These above results indicated that although all compounds are shown to be potential antioxidant agents, the monomers are more favorable for the antioxidant activities.

CONCLUSION

Two flavonoid dimers (1–2) and three monomers (3–5) were identified from the seeds Cardiocrinum giganteum, which enhanced the understanding of the chemical connotation of Cardiocrinum giganteum. As a rich source of flavonoids (polyphenols) with potent antioxidant activities, Cardiocrinum giganteum might be developed to anti-oxidative products for anti-aging, relieving oxidative stress, etc.

REFERENCES