Antioxidant Constituents from *Leonurus japonicus*

Guan-Zheng Qu¹, Chuan-Ling Si², and Myeong-Hyeon Wang¹,*

¹School of Biotechnology, Kangwon National University, Chuncheon 200-701, Korea
²Tianjin Key Laboratory of Pulp & Paper, Tianjin University of Science & Technology, Tianjin, 300222, China

Abstract – Two phenolic acids, gallic acid (1) and syringic acid (2), and five flavonoids, apigenin (3), luteolin (4), kaempferol (5), quercetin (6), and myricetin (7), were isolated from the aerial parts of *Leonurus japonicus*. Their structures were elucidated by chemical and spectral analysis. The antioxidant activities of the crude extracts, partitioned fractions and isolated compounds were evaluated by DDPH free radical-scavenging assay. Results suggested that the EtOAc partitioned fraction and compounds 1, 4, 5, 6, and 7 showed significantly high antioxidant potential compared with α-tocopherol and BHT, which were used as controls.

Keywords – Aerial parts, antioxidant, constituents, *Leonurus japonicus*

Introduction

Plants provide the pharmaceutical industry with some of the most important sources of components for the research of new medicines. In the last a few decades, much study has been directed at popular medicine, with the aim of identifying natural products with therapeutic properties (Hamburger and Hostetman, 1991; Weisburger *et al*., 1996). Moreover, the interest and need to identify new natural products from plants for use as safe and effective additives in the food industry are increasing (Sherwin, 1990; Wanasundara and Shahidi, 1998).

*Leonurus japonicus* Houtt. (syn L. Sweet) or Chinese motherwort (Lamiaceae) is an annual herb widely distributed in the pantropical regions where aerial parts extracts are commonly employed in folk medicines for their heart antiarrhythmic (Hotta *et al*., 2003), sedative (Widy-Tyszkiewicz and Schminda, 1997), antimicrobial (De Souza *et al*., 2004), anticoagulant (Lee *et al*., 1991), and antitumoral (Chinwala *et al*., 2002) properties. Phytochemical investigations of this species resulted in the isolation of iridoid glucosides, phenolic glycosides (Sugaya *et al*., 1998), alkaloids, phenolid acids, flavonoids, fatty acids, volatile essential oils (Ruan *et al*., 2003), melatonin (Chen *et al*., 2003), â-sitostenone (Hotta *et al*., 2003), and several diterpenes (Satoh *et al*., 2003; Giang *et al*., 2005; Roman *et al*., 2006).

Pursuing our research project for the study of active compounds from natural sources to develop medicines and food additives, we report the isolation of two phenolic acids (1-2) and five flavonoids (3-7) from the aerial parts of the plants and their antioxidant properties.

Experimental

General procedure – To determine the structures of the isolated compounds, ¹H- and ¹³C-NMR spectra data were obtained using a Bruker Avance D PX 400 MHz NMR spectrometer. For the determination of molecular weights of the isolated compounds, EI-MS was recorded on a Micromass Autospec M363. DMSO-­d₆ and MeOH-­d₄ were used as NMR solvents with TMS as an internal standard. A column was packed with Sephadex LH-20 using MeOH-H₂O (4 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 5, v/v) and EtOH-hexane (4 : 1, 3 : 1, 2 : 1, v/v) for elution. Eluents were collected using a Gilson FC 204 fraction collector. The columns were washed with acetone-H₂O (1 : 1, v/v) when the eluents were colorless. TLC was performed on 25 DC-Plastik-folien Cellulose F (Merk) plates and developed with t-BuOH-H₂O (3 : 1, 1 : 1, v/v/v) or acetic acid-water (3 : 47, v/v). Visualization was done by illuminating ultraviolet light (254 and 365 nm), by spraying 1% FeCl₃, and by heating. Two dimensional TLC was also tried to verify the purification of the isolated compounds. All solvents were routinely distilled prior to use.

Plant material – Aerial parts of *L. japonicus* were collected in the field of Yantai, China, in August 2006, air-dried for two weeks at room temperature at laboratory and then ground to fine powders to be extracted.
Extraction and isolation – The L. japonicus powders (2.5 g) were extracted with acetone-water (7:3, v/v) three times at room temperature, then the solvent was decanted, filtered and evaporated. The combined extracts were successively partitioned with n-hexane, CH<sub>2</sub>Cl<sub>2</sub> and EtOAc using a separatory funnel. Each fraction was concentrated and freeze dried to get powder. 84.4 g of EtOAc partitioned powders were subjected to column chromatography over Sephadex LH-20 and eluted with MeOH-H<sub>2</sub>O (4:1, v/v/s) to give five subfractions designated as LJE-1 – LJE-5. Subfraction LJE-1 (1.57 g) was rechromatographed using MeOH-H<sub>2</sub>O as eluent to afford 103 mg of compound 2. Subfraction LJE-2 (3.43 g) was rechromatographed using MeOH-H<sub>2</sub>O (1:1, 1:3 and 1:5, v/v/s) to afford 103 mg of compound 2 and 17 mg of compound 7. Subfraction LJE-3 (1.78 g) was rechromatographed with repeated chromatography column eluting with MeOH-H<sub>2</sub>O (2:1 and 1:1, v/v/s) to give 88 mg of compound 3, 60 mg of compound 4, and 72 mg of compound 6.

**Compound 1** – Yellow amorphous powder; EI-MS: m/z [M]+ 170; <sup>1</sup>H-NMR (400 MHz, δ, MeOH-d<sub>4</sub>): 7.09 (2H, s, H-2,6); <sup>13</sup>C-NMR (100 MHz, δ, MeOH-d<sub>4</sub>): 109.85 (C-2,6), 122.36 (C-1), 138.42 (C-4), 145.69 (C-3,5), 170.67 (C-7).

**Compound 2** – Yellow amorphous powder; EI-MS: m/z [M]+ 198; <sup>1</sup>H-NMR (400 MHz, δ, MeOH-d<sub>4</sub>): 7.19 (2H, s, H-2,6), 3.78 (6H, s, H-8,9); <sup>13</sup>C-NMR (100 MHz, δ, MeOH-d<sub>4</sub>): 57.12 (C-8,9), 107.31 (C-2,6), 120.43 (C-1), 140.60 (C-4), 147.85 (C-3,5), 167.81 (C-7).

**Compound 3** – Yellow amorphous powder; EI-MS: m/z [M]+ 270; <sup>1</sup>H-NMR (400 MHz, δ, DMSO-d<sub>6</sub>): 6.18 (1H, d, J = 1.9 Hz, H-6), 6.48 (1H, d, J = 1.9 Hz, H-8), 6.72 (1H, s, H-3), 6.89 (2H, d, J = 8.5 Hz, H-3',5'), 7.88 (2H, d, J = 8.5 Hz, H-2',6'); <sup>13</sup>C-NMR (100 MHz, δ, DMSO-d<sub>6</sub>): 94.79 (C-8), 99.65 (C-6), 103.66 (C-3), 104.48 (C-10), 116.76 (C-3',5'), 121.93 (C-1'), 129.02 (C-2',6'), 158.08 (C-9), 161.94 (C-5), 161.99 (C-4'), 164.34 (C-2), 164.87 (C-7), 181.58 (C-7).

**Compound 4** – Yellow amorphous powder; EI-MS: m/z [M]+ 286; <sup>1</sup>H-NMR (400 MHz, δ, DMSO-d<sub>6</sub>): 6.26 (1H, d, J = 1.8 Hz, H-6), 6.51 (1H, d, J = 1.8 Hz, H-8), 6.68 (1H, s, H-3), 7.01 (1H, d, J = 8.4 Hz, H-5'), 7.47 (1H, d, J = 2.0 Hz and J = 8.4 Hz, H-6), 7.51 (2H, d, J = 2.0 Hz, H-2'); <sup>13</sup>C-NMR (100 MHz, δ, DMSO-d<sub>6</sub>): 94.71 (C-8), 99.71 (C-6), 104.21 (C-3), 105.46 (C-10), 114.15 (C-2'), 116.65 (C-5'), 120.12 (C-6'), 123.73 (C-1), 146.48 (C-3'), 130.13 (C-4'), 158.79 (C-9), 162.87 (C-5), 164.36 (C-2), 164.93 (C-7), 182.01 (C-4).

**Compound 5** – Yellowish amorphous powder; EI-MS: m/z [M]+ 286; <sup>1</sup>H-NMR (400 MHz, δ, DMSO-d<sub>6</sub>): 6.31 (1H, d, J = 2.0 Hz, H-6), 6.55 (1H, d, J = 2.0 Hz, H-8), 7.06 (2H, d, J = 8.3 Hz, H-3',5'), 8.21 (2H, d, J = 8.3 Hz, H-2',6'); <sup>13</sup>C-NMR (100 MHz, δ, DMSO-d<sub>6</sub>): 94.61 (C-8), 99.21 (C-6), 104.21 (C-10), 116.44 (C-3',5'), 123.42 (C-1'), 130.49 (C-2',6'), 136.69 (C-3), 147.12 (C-2), 157.81 (C-9), 160.12 (C-5), 162.35 (C-4'), 165.02 (C-7), 176.77 (C-4).

**Compound 6** – Yellowish amorphous powder; EI-MS: m/z [M]+ 302; <sup>1</sup>H-NMR (400 MHz, δ, DMSO-d<sub>6</sub>): 6.31 (1H, d, J = 2.1 Hz, H-6), 6.53 (1H, d, J = 2.1 Hz, H-8), 7.04 (1H, d, J = 8.1 Hz, H-5'), 7.69 (1H, dd, J = 8.1 Hz and J = 2.2 Hz, H-6'), 7.79 (1H, d, J = 2.2 Hz, H-2'); <sup>13</sup>C-NMR (100 MHz, δ, DMSO-d<sub>6</sub>): 94.96 (C-8), 99.55 (C-6), 104.37 (C-10), 116.13 (C-2'), 115.59 (C-5'), 121.91 (C-1'), 123.82 (C-6'), 137.05 (C-3), 146.31 (C-3'), 147.88 (C-2), 148.78 (C-4'), 157.99 (C-9), 162.03 (C-5), 165.51 (C-7), 177.06 (C-4).

**Compound 7** – Yellowish amorphous powder; EI-MS: m/z [M]+ 318; <sup>1</sup>H-NMR (400 MHz, δ, MeOH-d<sub>4</sub>): 6.12 (1H, d, J = 2.2 Hz, H-6), 6.30 (1H, d, J = 2.2 Hz, H-8), 7.28 (2H, s, H-2',6'); <sup>13</sup>C-NMR (100 MHz, δ, MeOH-d<sub>4</sub>): 94.25 (C-8), 98.86 (C-6), 104.38 (C-10), 107.99 (C-1'), 123.82 (C-6'), 136.90 (C-3), 137.04 (C-3'), 146.51 (C-3',5'), 147.77 (C-2), 158.04 (C-9), 162.12 (C-5), 165.24 (C-7), 177.21 (C-4).

DPPH free radical scavenging assay – The antioxidant activity was determined on the basis of the scavenging activity of the stable DPPH free radical method introduced by Yoshida et al. (1989) with slight modification. MeOH solutions (4 ml) of samples at different concentrations (2–40 µg/ml) were added to a solution of DPPH (1.5 × 10<sup>-6</sup> M, 1 ml) in MeOH. After mixing gently and standing at room temperature for 30 min, the optical density was measured at 517 nm with a UV-visible spectrophotometer (Libra S32, Biochrom LTD). The results were calculated by taking the mean of all triplicated values. IC<sub>50</sub> values were obtained through extrapolation from concentration of sample necessary to scavenge 50% of the DPPH free radicals. BHT and α-tocopherol were used as controls.

Results and Discussion

Chromatographic separation of an EtOAc participated fraction of L. japonicus led to the isolation of six yellow amorphous compounds 1–7 (Fig. 1). Chemical structures of compounds 1–7 were determined as gallic acid (1) (Kashiwada et al., 1988; Satjo et al., 1990), syringic acid (2) (Cong et al., 2003), apigenin (3) (Markham and Chari,