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# Antioxidant Constituents from *Leonurus japonicus*

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**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 antiarrhythimc (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.

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## Experimental

**General procedure** – To determine the structures of the isolated compounds, $^1$H- and $^{13}$C-NMR spectra data were obtained using a Bruker Avance DPX 400 MHz NMR spectrometer. For the determination of molecular weights of the isolated compounds, EI-MS was recorded on a Micromass Autospec M363. DMSO-$d_6$ and MeOH-$d_4$ were used as NMR solvents with TMS as an internal standard. A column was packed with Sephadex LH-20 using MeOH-$H_2O$ (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-$H_2O$ (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-HOAc-$H_2O$ (3 : 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% $FeCl_3$, 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 kg) were extracted with acetone-water (7 : 3, v/v) three times at room temperature, then the solvent was decanted, filtered and evaporated. The combined extractives were successfully partitioned with *n*-hexane, $CH_2Cl_2$ and EtOAc using a separatory funnel. Each fraction was concentrated and freeze dried to get powder. 8.4 g of EtOAc partitioned powders were subjected to column chromatography over Sephadex LH-20 and eluted with MeOH-$H_2O$ (4 : 1, v/v) to give five subfractions designed as LJE-1 ~ LJE-5. Subfraction LJE-1 (1.57 g) was rechromatographed using MeOH-$H_2O$ (2 : 1 and 1 : 2, v/v) and EtOH-hexane (3 : 1) to give 24 mg of compound **1** and 75 mg of compound **5**. Subfraction LJE-2 (3.43 g) was rechromatographed with MeOH-$H_2O$ (1 : 1, 1 : 3 and 1 : 5, v/v) to afford 103 mg of compound **2** and 17 mg of compound **7**. Subfraction LJE-5 (1.78 g) was also purified with repeated chromatography column eluting with MeOH-$H_2O$ (2 : 1 and 1 : 3, v/v) followed by EtOH-hexane (2 : 1 and 1 : 1, v/v) to give 88 mg of compound **3**, 60 mg of compound **4**, and 72 mg of compound **6**.

**Compound 1** – Yellowish amorphous powder; EI-MS: *m/z* $[M]^+$ 170; $^1$H-NMR (400 MHz, δ, MeOH-$d_4$) : 7.09 (2H, s, H-2,6); $^{13}$C-NMR (100 MHz, δ, MeOH-$d_4$) : 109.85 (C-2,6), 122.36 (C-1), 138.42 (C-4), 145.69 (C-3,5), 170.67 (C-7).

**Compound 2** – Yellowish amorphous powder; EI-MS: *m/z* $[M]^+$ 198; $^1$H-NMR (400 MHz, δ, MeOH-$d_4$) : 7.19 (2H, s, H-2,6), 3.78 (6H, s, H-8,9); $^{13}$C-NMR (100 MHz, δ, MeOH-$d_4$): 57.12 (C-8,9), 107.31 (C-2,6), 120.43 (C-1), 140.60 (C-4), 147.83 (C-3,5), 167.81 (C-7).

**Compound 3** – Yellow amorphous powder; EI-MS: *m/z* $[M]^+$ 270; $^1$H-NMR (400 MHz, δ, DMSO-$d_6$): 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'); $^{13}$C-NMR (100 MHz, δ, DMSO-$d_6$): 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'), 164.36 (C-2), 164.87 (C-7), 181.58 (C-4).

**Compound 4** – Yellow amorphous powder; EI-MS: *m/z* $[M]^+$ 286; $^1$H-NMR (400 MHz, δ, DMSO-$d_6$): 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'); $^{13}$C-NMR (100 MHz, δ, DMSO-$d_6$): 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'), 150.13 (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; $^1$H-NMR (400 MHz, δ, DMSO-$d_6$) : 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'). $^{13}$C-NMR (100 MHz, δ, DMSO-$d_6$): 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), 160.122 (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; $^1$H-NMR (400 MHz, δ, DMSO-$d_6$): 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'); $^{13}$C-NMR (100 MHz, δ, DMSO-$d_6$): 94.96 (C-8), 99.55 (C-6), 104.37 (C-10), 116.13 (C-2'), 116.59 (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; $^1$H-NMR (400 MHz, δ, MeOH-$d_4$): 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'); $^{13}$C-NMR (100 MHz, δ, MeOH-$d_4$): 94.25 (C-8), 98.86 (C-6), 104.38 (C-10), 107.99.15 (C-2',6'), 122.90 (C-1'), 136.51 (C-4'), 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 ($1.5 \times 10^{-4}$ 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. $IC_{50}$ 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; Saijo *et al.*, 1990), syringic acid (**2**) (Cong *et al.*, 2003), apigenin (**3**) (Markham and Chari,

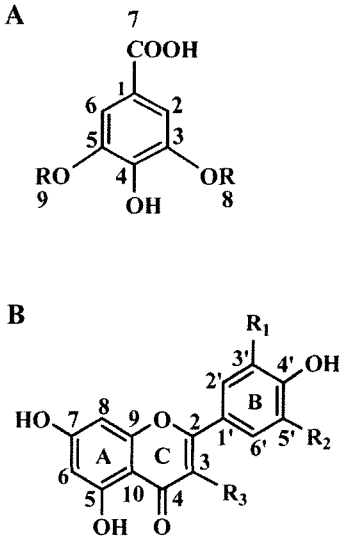


**Fig. 1** Structures of compounds isolated from *L. japonicus*.
**A**, Structures of compounds **1**, **2**
R = H: gallic acid (**1**), R = $CH_3$: syringic acid (**2**)
**B**, Structures of compounds **3-7**
$R_1 = R_2 = R_3 = H$: apigenin (**3**), $R_1$ = OH, $R_2 = R_3 = H$: luteolin (**4**)
$R_1 = R_2 = H$, $R_3$ = OH: kaempferol (**5**), $R_1 = R_3$ = OH, $R_2 = H$: quercetin (**6**)
$R_1 = R_2 = R_3$ = OH: myricetin (**7**)

1982; The Wiley Registry of Mass Spectral Data, 1996), luteolin (**4**) (Agrawal, 1989; Watanabe, 1999), kaempferol (**5**) (Markham *et al.*, 1978; Okuyama *et al.*, 1978), quercetin (**6**) (Ternai and Markham, 1976; Wenkert and Gottlieb, 1977), and myricetin (**7**) (Sakushima *et al.*, 1983), respectively, by comparing the $^1H$ and $^{13}C$-NMR spectral data with those of reported in literature and a verification of their MS values. Though compounds **2, 3, 5** and **6** have been previously purified from *L. japonicus* (Cong *et al.*, 2003; Ruan *et al.*, 2006), to our knowledge, this was the first time of isolation compounds **1, 4,** and **7** from this species.

The antioxidant activities of the crude extracts, participated fractions and the six isolated compounds were determined by DDPH assay. The results were summarized in Table 1. Among them, the EtOAc participated fraction, gallic acid (**1**), luteolin (**4**) kaempferol (**5**), quercetin (**6**) and myricetin (**7**) exhibited potent antioxidant activities comparable to α-tocopherol and BHT, which were used as controls, while the rest compounds, participated fractions and crude extracts showed negligible activities. The $IC_{50}$ values for gallic acid (**1**), luteolin (**4**) kaempferol (**5**), quercetin (**6**), myricetin (**7**) were 12 μg/ml, 16 μg/ml, 13 μg/ml, 11 μg/ml and 9 μg/ml, respectively, which suggested that the catechol and pyrogallol structure

**Table 1.** Antixoidant activities ($IC_{50}$ values) of the crude extracts, participated fractions and isolated compounds from *L. japonicus*

| | samples | $IC_{50}$ (μg/ml) |
|---|---|---|
| controls | α-tocopherol | 26 |
| | BHT | 30 |
| partitioned fractions | crude extracts | 76 |
| | *n*-hexane participated fraction | 131 |
| | $CH_2Cl_2$ participated fraction | 114 |
| | EtOAc participated fraction | 25 |
| | $H_2O$ participated fraction | 42 |
| isolated compounds | gallic acid (**1**) | 12 |
| | syringic acid (**2**) | 57 |
| | apigenin (**3**) | 39 |
| | luteolin (**4**) | 16 |
| | kaempferol (**5**) | 13 |
| | quercetin (**6**) | 11 |
| | myricetin (**7**) | 9 |

play an important role in the antioxidant function as exhibited in flavonoids and phenolic acids (Pietta, 2000; Akdemir *et al.*, 2001). The results also suggested that gallic acid, kaempferol, quercetin and myricetin could be mainly responsible for the potent antioxidant effect of the EtOAc participated fraction of *L. japonicus*. This fact indicated that the extracts of *L. japonicus* had high antioxidant potential and can be used as a useful source for antioxidants.

## References

Agrawal, P.K., *Carbon-13 NMR of flavanoids*. Elsevier, 1989.

Akdemir, A.S., Tati, I.I., Saraccoglu, I., Ismailoglu, U.B., Sahin-Erdemli, I., and Dalis, I., Polyphenolic compounds from Geranium pratense and their free radical scavenging activities. *Phytochemistry* **56**, 189-193 (2001).

Chen, H., Huo, Y., Tan, D.X., Liang, Z., Zhang, W., and Zhang, Y., Melatonin in Chinese medicinal herbs. *Life Sci.* **73**, 19-26 (2003).

Chinwala, M.G., Gao, M., Dai, J., and Shao, J., *In vitro* anticancer activities of *Leonurus heterophyllus* Sweet (Chinese motherwort herb). *J. Chinese Med. Mat.* **25**, 71-72 (2002).

Cong, Y., Wang, J.X., Guo, H.R., and Li, X., Isolation and identification of the chemical constituents of *Leonurus japonicus* Houtt. II. *Chinese J. Med. Chem.* **13**, 349-352 (2003).

De Souza, C., Haas, A.P., Von Poser, G.L., Schapobal, E.E., and Elisabetsky, E., Ethnoparmacological studies of antimicrobial remedies in the south of Brazil. *J. Ethnopharmacol.* **90**, 135-143 (2004).

Giang, P.M., Son, P.T., Matsunami, K., and Otsuka, H., New bissirolabdane-type diterpenoids from *Leonurus heterophyllus* Sw. *Chem. Pharm. Bull.* **53**, 1475-1479 (2005).

Hamburger, M. and Hostetman, K., Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry* **30**, 3864-3884 (1991).

Hotta, K., Noguchi, Y., Matsunaga, M., Nishibe, K., Uchida, K., Shimizu, K., Tetsuya, K., and Akinobu, S., *Leonurus heterophyllus extracts and β-sitosterone as antiarrhythmics*. Jpn. Kokai. Tokkyo Koho, 2003.

Kashiwada, Y., Nonaka, G., and Nishoka, I., Galloylsucroses from *Rhubarbs. Phytochemistry* **27**, 1469-1472 (1988)

Lee, C.M., Jiang, L.M., Shang, H.S., Hon, P.M., He, Y., and Womg, H.N., Prehispanolone, a novel platelet-activating factor receptor antagonist from *Leonurus heterophyllus. Br. J. Pharmacol.* **103**, 1719-1724 (1991).

Markham, K.R. and Chari, V.M., Carbon-13 NMR spectroscopy of flavonoids, in Harboren J.B., and Mabry T. (Eds.), *The Flavonoids: Advances in research*, Chapman and Hall Ltd., London, UK, 1982.

Markham, K.R., Ternai, B., Stanly, R., Geiger, H., and Mabry, T.J., Carbon-13 NMR studies of flavonoids-III: Naturally occurring flavonoid glycosides and their acetylated derivatives. *Tetrahedron* **34**, 1389-1397 (1978).

Okuyama, T., Hosoyama, K., Hiragam, Y., Kurono, G., and Takemoto, T., The constituents of *Osmunda spp.*: A new flavonol glycoside of *Osmunda asiatica. Chem. Pharm. Bull.* **26**, 3071-3074 (1978).

Pietta, P.G., Flavonoids as antioxidants. *J. Nat. Prod.* **63**, 1035-1042 (2000).

Roman, R., Romero-Gonzalez, Jorge, L,, Avila-Nunez, Lianne, A., Miguel, E., and Alonso-Amelot, Labdane diterpenes from *Leonurus japonicus* leaves. *Phytochemistry* **67**, 965-970 (2006).

Ruan, J.L., Du, J.R., Zeng, Q.Z., and Qian, Z.M., Advance in studies on chemical components, harmacological effect and clinical application of *Leonurus japonicus. Chinese Traditional and Herbal Drugs* **34**, 15-18 (2003).

Saijo, R., Nonaka, G.I., and Nishioka, I,. Gallic acid esters of bergenin and norbergenin from *Mallotus japonicus. Phytochemistry* **29**, 267-270 (1990).

Sakushima, A.M., Cosakun, S.H., and Nishibe, S., Flavonoids from *Rhamnus pallasii. Phytochemistry* **22**, 1677-1678 (1983).

Satoh, M., Satoh, Y., Isobe, K., and Fujimoto, Y., Studies on the constituents of *Leonurus sibiricus* L. *Chem. Pharm. Bull.* **52**, 341-342 (2003).

Sherwin, E.R., Antioxidant, in Branen, A.L., Davidson, P.M., and Salminin, S. (Eds), *Food additives*, New York, 1990.

Sugaya, K., Hashimoto, F., Ono, M., Ito, Y., Masuoka, C., and Nohara, T., Antioxidative constituents from Leonurii Herba (*Leonurus japonicus*). *Food Sci. Technol. Int. Tokyo* **4**, 278-281 (1998).

Ternai, B. and Markham, K.R., Carbon-13 NMR studies of flavonoids-I: flavones and flavonols. *Tetrahedron* **32**, 565-569 (1976).

*The Wiley Registry of Mass Spectral Data*, sixth Ed. Wiley, New York, 1996.

Wanasundara, U.N. and Shahidi, F., Antioxidant and prooxidant activity of green tea extracts in maine oils. *Food Chem.* **63**, 335-342 (1998).

Watanabe, M., Antioxidant phenolic compounds from Japanese barnyard millet (echinochloa utilis) grains. *J. Agric. Food Chem.* **47**, 500-4505 (1999).

Weisburger, J.H., Hara, Y., Sulan, L., Luo, F.Q., Pittman, B., and Zang, E., Tea polyphenols as inhibitors of mutigenicity of major classes carcinogens. *Mutat. Res.* **371**, 57-63 (1996).

Wenkert, E. and Gottlieb, H.E., Carbon-13 nuclear magnetic resonance spectroscopy of flavonoid and isoflavonoid compounds. *Phytochemistry* **16**, 1811-1816 (1977).

Widy-Tyszkiewicz, E. and Schminda, R., A randomized double blind study of sedative effects of phytoterapeutic containing valerian, hops, balm, and motherwort versus placebo. *Herba. Pol.* **43**, 154-159 (1997).

Yoshida, T., Mori, K., Hatano, T., Okumura, T., Uehara, I., Komagoe, K., Fujita, Y., and Okuda, T., Study on inhibition mechanism of autooxidation by tannins and flavonoids. V. Radical scavenging effects of tannins and related polyphenols on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem. Pharm. Bull.* **37**, 1919-1921 (1989).

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