

## Antioxidative Components from the Aerial Parts of *Lactuca scariola* L.

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The antioxidant activity of *Lactuca scariola* (Compositae) was investigated by measuring the radical scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The methanolic extract of the aerial parts of *Lactuca scariola* showed strong radical scavenging activity. The EtOAc soluble fraction exhibited a stronger activity than the others, and was purified by silica gel and Sephadex LH-20 column chromatography. Quercetin-3-O- $\beta$ -D-glucopyranoside, luteolin-7-O- $\beta$ -D-glucopyranoside, luteolin, quercetin and kaempferol, together with 11 $\beta$ ,13-dihydrolactucin were isolated from the EtOAc soluble fraction as active ingredients.

**Key words:** *Lactuca scariola*, Compositae, Antioxidant activity, Flavonoids

### INTRODUCTION

In search for antioxidative components from several Korean plants, the methanolic extract of the aerial parts of *Lactuca scariola* was found to exhibit significant antioxidative activity, based on the scavenging activity of the stable DPPH free radical. *L. scariola* L. (Compositae) is widely distributed in South Korea (Park, 1995). Although several plants of the *Lactuca* genus have been examined for their chemical constituents, *L. scariola* has not been investigated in detail on phytochemical analysis. Recently, several papers reported that seed oil of *L. scariola* contained normal fatty acids and epoxy acids (Ahmad et al., 1991; Ansari et al., 1987). And earlier investigations on the chemical constituents of the *Lactuca* genus mainly dealt with the isolation of sesquiterpenoids (Kisiel et al., 2000; Kisiel, 1998a; Kisiel et al., 1998b; Kisiel et al., 1998c; Kisiel et al., 1997a; Kisiel et al., 1997b; Kisiel et al., 1995; Kisiel et al., 1993). From the EtOAc soluble fraction of *L. scariola*, the active compounds were isolated and the structures of these compounds were determined by analysis of EI- and positive FAB-MS as well as <sup>1</sup>H- and <sup>13</sup>C-NMR spectra. This paper reports the isolation, structure elucidation, and the free radical scavenging property of the isolated compounds.

### MATERIALS AND METHODS

#### General

<sup>1</sup>H- and <sup>13</sup>C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. EI- and positive FAB-MS were determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). IR spectra were obtained on a JASCO FT/IR 410 spectrometer and UV spectra were recorded on Shimadzu UV-1601 UV-Visible spectrophotometer. TLC was carried out on Merck aluminium plates precoated with silica gel F<sub>254</sub> and column chromatography was carried out on silica gel (Kiesel gel 60, 230-400 mesh, Merck) and Sephadex LH-20 (25-100, Pharmacia). LPLC was carried out on Yamazen 540 pump with Merck Lichroprep Si 60 (Lobar A, 240-10 mm, 0.2 ml/min). Ascorbic acid and BHA (butylated hydroxyanisole) were obtained from Sigma Chemical Co. All other chemicals and solvents were analytical grade and used without further purification.

#### Plant materials

The aerial parts of *L. scariola* were collected in August 1999 at Wanju, Chonbuk, Korea. A voucher specimen (WSU-99-002) is deposited in the herbarium of College of Pharmacy, Woosuk University.

#### Extraction and isolation

The air-dried plant materials (1.2 Kg) was extracted

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three times at room temperature for 7 days with methanol. The resultant MeOH extract (130 g) followed by the successive solvent partition to give *n*-hexane (30 g), CH<sub>2</sub>Cl<sub>2</sub> (15 g), EtOAc (10 g), *n*-BuOH (30 g) and H<sub>2</sub>O soluble fractions. Each fraction was tested for the scavenging effect on DPPH radical. The EtOAc soluble fraction exhibited strong scavenging activity on DPPH radical (IC<sub>50</sub>=4.8 µg/ml), which was subjected to Sephadex LH-20 column and eluted with THF-MeOH (1:1) to afford 3 subfractions (E1-E3). Subfraction E2 (100 mg) was rechromatographed on Sephadex LH-20 column (MeOH) to give 5 subfractions (E21~E26). Subfraction E21 was further purified by silica gel Lobar-A column (CH<sub>2</sub>Cl<sub>2</sub>-EtOAc-MeOH, 3:1:1) to yield **1** (15 mg). Subfraction E22 was crystallized with MeOH to yield **2** (12 mg). Subfractions E23 and E24 were applied over silica gel column chromatography (CHCl<sub>3</sub>-MeOH, 8:1) to yield **3** (10 mg) and **4** (15 mg), respectively. Subfraction E25 was further purified by silica gel Lobar-A column (CH<sub>2</sub>Cl<sub>2</sub>-EtOAc-MeOH, 8:1:1) to yield **5** (12 mg). Column chromatography of subfraction E1 over silica gel with CHCl<sub>3</sub>-MeOH (8:1) yielded **6** (18 mg).

**Compound 1** (Quercetin-3-O-β-D-glucopyranoside): yellow powder (MeOH), mp 233-234°C, positive FAB MS (m/z): 465[M<sup>+</sup>+1], UV λ max (MeOH) nm: 257, 264(sh), 295, 355, IR v max (KBr) cm<sup>-1</sup>: 3415 (-OH), 1655, 1605, 1205, <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD) δ: 7.84 (1H, d, J=2.1Hz, H-2'), 7.57 (1H, dd, J=8.4, 2.1Hz, H-6'), 6.89 (1H, d, J=8.4Hz, H-5'), 6.38 (1H, d, J=1.9Hz, H-8), 6.18 (1H, d, J=1.9Hz, H-6), 5.15 (1H, d, J=7.5Hz, anomeric H of glc.), 3.12 3.42 (sugar H), <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD): 179.6 (C-4'), 166.2 (C-7), 163.2 (C-5), 159.0 (C-9), 158.4 (C-2'), 149.9 (C-3'), 145.8 (C-4'), 135.6 (C-3), 99.2 (C-6), 123.0 (C-1'), 117.5 (C-5'), 116.1 (C-2'), 105.6 (C-10), 101.6 (C-1''), 99.6 (C-6), 94.7 (C-8), 78.4 (C-3''), 78.4 (C-5''), 75.7 (C-2''), 71.2 (C-4''), 62.6 (C-6'')

**Compound 2** (Luteolin-7-O-β-D-glucopyranoside): yellow powder (MeOH), mp 253-254, EIMS (m/z): 448 [M<sup>+</sup>], UV λ max (MeOH) nm: 256, 265(sh), 345, IR v max (KBr) cm<sup>-1</sup>: 3403 (-OH), 1650, 1605, 1510, <sup>1</sup>H-NMR (400MHz, DMSO-d<sub>6</sub>) δ: 12.98 (1H, brs, 5-OH), 7.43 (1H, dd, J=8.2, 1.9Hz, H-6'), 7.41 (1H, d, J=1.9Hz, H-2'), 6.89 (1H, d, J=8.2Hz, H-5'), 6.78 (1H, d, J=1.9Hz, H-8), 6.74 (1H, s, H-3), 6.43 (1H, d, J=1.9Hz, H-6), 5.07 (1H, d, J=7.1Hz, anomeric H of glc.), 3.16~3.80 (sugar H), <sup>13</sup>C-NMR (100MHz, DMSO-d<sub>6</sub>): 181.9 (C-4), 164.5 (C-2), 162.9 (C-7), 161.1 (C-5), 156.9 (C-9), 150.0 (C-4''), 145.8 (C-3), 121.4 (C-1), 119.2 (C-6), 116.0 (C-5), 113.5 (C-2), 105.4 (C-10), 103.2 (C-3), 99.9 (C-1''), 99.6 (C-6), 94.8 (C-8), 77.2 (C-5''), 76.4 (C-3''), 73.2 (C-2''), 69.6 (C-4''), 60.6 (C-6'')

**Compound 3** (Luteolin): yellow powder (MeOH), mp

29.-292°C, EIMS (m/z) 286 [M<sup>+</sup>], UV λ max (MeOH) nm: 255, 285(sh), 344, IR v max (KBr) cm<sup>-1</sup>: 3405 (-OH), 1663, 1603, 1510, <sup>1</sup>H-NMR (400MHz, DMSO-d<sub>6</sub>) δ: 12.94 (1H, s, 5-OH), 7.39 (1H, dd, J=8.0, 2.0Hz, H-6'), 7.37 (1H, d, J=2.0Hz, H-2'), 6.87 (1H, d, J=8.0Hz, H-5'), 6.66 (1H, s, H-3), 6.44 (2H, d, J=2.0, H-8), 6.18 (1H, d, J=2.0, H-6)

**Compound 4** (Quercetin): yellow powder (MeOH), mp 316-317°C, EIMS (m/z) 302 [M<sup>+</sup>], UV λ max (MeOH) nm: 253, 270(sh), 305(sh), 372, IR v max (KBr)cm<sup>-1</sup>: 3340 (-OH), 1683, 1615, 1560, <sup>1</sup>H-NMR (400MHz, CD<sub>3</sub>OD) δ: 7.63 (1H, d, J=2.1Hz, H-2'), 7.53 (1H, dd, J=8.4, 2.1 Hz, H-6'), 6.78 (1H, d, J=8.4Hz, H-5'), 6.28 (1H, d, J=1.8Hz, H-8), 6.07 (1H, d, J=1.8Hz, H-6)

**Compound 5** (Kaempferol): yellow power (MeOH), mp 274-275°C, EIMS (m/z) 286 [M<sup>+</sup>], UV λ max (MeOH) nm: 265, 323(sh), 368, IR v max (KBr) cm<sup>-1</sup>: 3328 (-OH), 1653, 1615, 1510, <sup>1</sup>H-NMR (400MHz, CD<sub>3</sub>OD) δ: 7.99 (2H, d, J=8.8Hz, H-2', 6'), 6.80 (2H, d, J=8.8Hz, H-3', 5'), 6.29 (1H, d, J=1.8Hz, H-8), 6.08 (1H, d, J=1.8Hz, H-6)

**Compound 6** (11,13-Dihydrolactucin): whit powder (MeOH), mp 90-91°C, EIMS (m/z) 278 [M<sup>+</sup>], IR v max (KBr) cm<sup>-1</sup>: 3380 (-OH), 1765 (lactone C=O), <sup>1</sup>H-NMR (400MHz, CDCl<sub>3</sub>) δ: 6.44 (1H, s, H-3), 4.87 (1H, d, J=17.6Hz, H-15α), 4.56 (1H, d, J=17.6Hz, H-15β), 3.76 (1H, m, H-8), 3.66 (1H, m, H-6), 3.57 (1H, d, J=10.0 Hz, H-5), 2.81 (1H, m, H-9α), 2.59 (1H, m, H-11), 2.46 (3H, s, H-14), 2.39 (1H, m, H-9β), 2.15 (1H, m, H-7), 1.46 (3H, d, J=7.2Hz, H-13), <sup>13</sup>C-NMR (100MHz, CDCl<sub>3</sub>) δ: 194.5 (C-2), 177.0 (C-12), 171.3 (C-4), 146.6 (C-10), 133.3 (C-3), 132.1 (C-1), 80.5 (C-6), 69.5 (C-8), 62.6 (C-15)\*, 61.2 (C-7)\*, 49.3 (C-5)\*\*, 48.6 (C-9)\*\*, 41.6 (C-11), 21.9 (C-14), 15.5 (C-13). \*\*\*Assignments may be reversed.

### DPPH radical scavenging effect

The antioxidant activity of compounds **1-6** and each

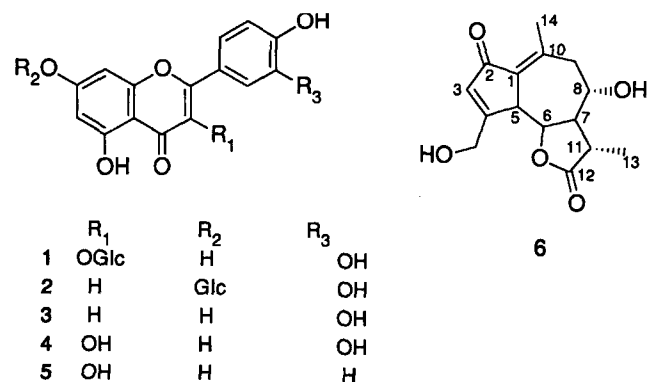


Fig. 1. Structures of compounds **1-6**

solvent fraction was assessed on the basis of the scavenging activity of the stable DPPH free radical (Choi *et al.*, 1993; Yoshida *et al.*, 1989). MeOH solutions (4 ml) of samples at various concentrations were added to a solution of DPPH in MeOH ( $1.5 \times 10^{-4}$  M, 1 ml) and the reaction mixture were shaken vigorously. After storing these mixtures for 30 min at room temperature, the remaining amounts of DPPH were determined by colorimetry at 520 nm. And the radical scavenging activity of each compound was expressed by the ratio of the lowering of the DPPH solution in the absence of compounds. The mean values were obtained from triplicate experiments.

## RESULTS AND DISCUSSION

Activity-guided fractionation of the EtOAc soluble fraction of the aerial parts of *L. scariola* led to the isolation of five flavonoids and a sesquiterpene. The structures of five flavonoids were identified as quercetin-3-O- $\beta$ -D-glucopyranoside (**1**, Kim *et al.*, 1999; Choi *et al.*, 1998), luteolin-7-O- $\beta$ -D-glucopyranoside (**2**, Markham *et al.*, 1994; Agrawal *et al.*, 1989) luteolin (**3**, Lee *et al.*, 1998), quercetin (**4**, Shen *et al.*, 1993) and kaempferol (**5**, Lee *et al.*, 2000).

Compound **6** was obtained as a colorless prism. Its IR spectrum showed the presence of hydroxyl ( $3380\text{ cm}^{-1}$ ), and  $\nu$ -lactone ( $1765\text{ cm}^{-1}$ ) groups. The  $^1\text{H-NMR}$  spectrum showed two secondary methyl groups at  $\delta$  2.46 (3H, s) and 1.46 (3H, d,  $J=7.2\text{ Hz}$ ), four carbinol protons at 4.87 (1H, d,  $J=17.6\text{ Hz}$ ), 4.56 (1H, d,  $J=17.6.2\text{ Hz}$ ), 3.76 (1H, m) and 3.66 (1H, m), and an olefinic proton at  $\delta$  6.44 (1H, s). The  $^{13}\text{C-NMR}$  spectrum indicated the presence of fifteen carbon signals that contained two carbonyl carbons at 194.5, 177.0, four olefinic carbons at  $\delta$  171.3, 146.6, 133.3 and 132.1, and three oxygenated carbons 80.5, 69.5 and 62.6. The spectral data suggested that **6** was a guaiane sesquiterpene with two carbonyl groups and two alcohol groups. Based on the available chemical structures of the sesquiterpene (Seto *et al.*, 1988) and the NMR spectral data, the structure of **6** was determined to be 11 $\beta$ , 13-dihydrolactucin (8,15-dihydroxy-2-oxo-1(10), 3-guaiadien-12,6-olide). The NMR spectral and physical data of **6** were in good agreement with the literature (Sarg *et al.*, 1982).

The DPPH radical scavenging effect of the methanolic extract and its solvent partitioned fractions from *L. scariola* are shown in Table I. The  $\text{IC}_{50}$  values of the methanolic extract, and EtOAc, and n-BuOH fractions obtained from the methanolic extract were calculated as a concentration of 10.6, 4.8 and 18.4  $\mu\text{g/ml}$ , respectively, while the n-hexane,  $\text{CH}_2\text{Cl}_2$ , and  $\text{H}_2\text{O}$  soluble fractions showed a poor activity ( $>40.0\text{ }\mu\text{g/ml}$ ). The radical scavenging effect of six compounds obtained from *L. scariola* was also shown in Table II. Among six isolated compounds, the *ortho*-dihydroxylated aromatic components **1**, **2**, **3**, and **4** exhibited higher scavenging activity on

**Table I.** Scavenging effects of solvent fractions from the aerial parts of *L. scariola* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

Fractions	$\text{IC}_{50}$ ( $\mu\text{g/ml}$ )*
MeOH ext.	10.6
n-Hexane fr.	> 80.0
$\text{CH}_2\text{Cl}_2$ fr.	> 40.0
EtOAc fr.	4.8
n-BuOH fr.	18.4
$\text{H}_2\text{O}$	> 40.0

\*The values indicate 50% decrease of DPPH radical and are the means of triplicate data.

**Table II.** Antioxidative activities of compounds isolated from the aerial parts of *L. scariola*

Fractions $\text{IC}_{50}$	( $\mu\text{g/ml}$ )*
Quercetin-3-O- $\beta$ -D-glucopyranoside ( <b>1</b> )	14.8
Luteolin-7-O- $\beta$ -D-glucopyranoside ( <b>2</b> )	15.5
Luteolin ( <b>3</b> )	7.2
Quercetin ( <b>4</b> )	4.5
Kaempferol ( <b>5</b> )	27.6
11 $\beta$ ,13-Dihydrolactucin ( <b>6</b> )	> 120
Ascorbic acid	6.6
BHA	12.6

\*The values indicate 50% decrease of DPPH radical and are the means of triplicate data.

DPPH with  $\text{IC}_{50}$  values of 14.8, 15.5, 7.2 and 4.5  $\mu\text{g/ml}$ , respectively. However, **5** and **6** showed moderate or poor activities in comparison with reference antioxidants such as ascorbic acid and BHA. These results suggest that the radical scavenging effect in the original methanolic extract of *L. scariola* was partially attributable to **1**, **2**, **3**, and **4**. Compounds **1**, **2**, **3**, and **4** having catechol moiety, naturally occurring phenolic compounds are reported as antioxidative agents on reactive oxygen species (Yoshida *et al.*, 1989). The present study indicates that the methanolic extract of *L. scariola*, fractions of EtOAc and n-BuOH, and isolated flavonoid components may be useful for the treatment of various oxidative damage.

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