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Article

Antioxidant Activity of Flavonoids and Their Glucosides from *Sonchus oleraceus L.*

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Eight compounds, including 2 flavones, luteolin (1) and apigenin (2), 2 flavonols, kaempferol (3) and quercetin (4), and 4 flavonoid glucosides, luteolin-7-*O*-β-D-glucoside (5), apigetrin (6), astragalin (7), and isoquercitrin (8), isolated from the whole herb of *Sonchus oleraceus L.* were analyzed on the basis of chemical and spectroscopic evidence. This was the first time to report compounds 3, 4, 6, 7 and 8 from the *Sonchus oleraceus L.* The antioxidant activities of the isolated flavonoids and their glucoside derivatives were evaluated by DPPH free radical-scavenging assay, showing that compounds 1, 3, 4 and 8 exhibited stronger antioxidant activities compared with α-tocopherol and curcumin. Flavonoids containing more hydroxyl groups exhibited better antioxidant activities. The antioxidant activity of flavonols was superior to their corresponding flavones, and that of aglycone are more potent than their glucoside derivatives.

Key words: *antioxidant activity, DPPH, flavonoids,* Sonchus oleraceus L.

Naturally occurring polyphenolic compounds, including flavonoids, phenolic acids and tannins, have gained much attention as dietary supplements for the prevention of many pathological diseases and for the improvement of human health conditions [Gutteridge and Halliwell, 1994; Halliwell, 1994; Weisburger *et al.*, 1996]. Food industry is attempting to develop new natural products from plants as safe and effective additives. [Wanasundara and Shahidi, 1998]. Therefore, comprehensive investigation for effective antioxidants from natural sources has been undertaken actively [Scalbert *et al.*, 2000].

Sonchus oleraceus L. (Compositae), a herb native to Europe, North Africa, and Asia, is widely found in moist areas of fields, orchards, roadsides, gardens, or cleared land [Holm *et al.*, 1977]. This plant has been used in folk medicines to treat diseases such as enteritis, diarrhea, pneumonia, hepatitis, appendicitis, chronic bronchopneumonia, icterus, throat swelling, haematemesis and uraemia [Jiangsu New Medical College, 1977]. Phytochemical investigations of this species resulted in the isolation of flavonoids (mainly luteolin and apigenin derivatives), fatty acids, phenolic acids, volatile essential oils and terpenes [Bai *et al.*, 2007; Hu *et al.*, 2005; Li *et al.*, 2005; Xu and Liang, 2005; Yin *et al.*, 2007]. In the present work, we report the isolation of 4 flavonoids (**1-4**) and 4 corresponding glucoside derivatives (**5-8**) from the *Sonchus oleraceus* and their structure-antioxidant activity relationship based on DPPH assay.

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Materials and Methods

General procedure. ^{1}H- and ^{13}C-NMR spectra data were recorded on a Bruker Avance DPX 400 MHz NMR spectrometer. EI- and positive FAB-MS were recorded using a Micromass Autospec M363. MeOH-d_4 was used as NMR solvents with TMS as an internal standard. A column was packed with Sephadex LH-20 using MeOH-H_2O and EtOH-hexane mixture 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 eluent was 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, Solvent A) or HOAc-H_2O (3 : 47, v/v, Solvent B). Visualization was done by illuminating ultraviolet light (254 and 365 nm), by spraying 1% ethanolic $FeCl_3$, or 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. The whole herb of *S. oleraceus* was

collected in Kangwon province in August, 2006, air-dried for two weeks at room temperature and then finely powdered to be extracted.

Extraction and isolation. Air-dried and ground *S. oleraceus* (3.5 kg) was extracted with acetone-H_2O (7 : 3). Removal of the solvent yielded a dark brown extract. The extract was subsequently fractionated with *n*-hexane, CH_2Cl_2 and EtOAc with a separatory funnel and then each fraction was freeze-dried to give 18.3 g of *n*-hexane, 26.1 g of CH_2Cl_2, 68 g of EtOAc and 402 g of H_2O partitioned fractions.

A portion of EtOAc fraction powder (30.0 g) was chromatographed on a Sephadex LH-20 column eluted with MeOH-H_2O (4 : 1, v/v) to give 5 major fractions labeled as E_1 (1.06 g), E_2 (14.7 g), E_3 (6.8 g), E_4 (4.3 g) and E_5 (2.7 g). Fraction E_2 was reapplied on the column for further purification with MeOH-H_2O (3 : 1, v/v) affording 4 subfractions as E_{21}-E_{24}. E_{23} was recognized as compound **4** (15 mg). Subfraction E_{21} was retreated on the column for further purification with MeOH-H_2O (1 : 1 and 1 : 3, v/v) and EtOH-hexane (3 : 1 and 1 : 1, v/v), affording compounds **1** (98 mg), **3** (45 mg), **5** (61 mg) and **6** (72 mg). Fraction E_4 was resubjected on the column with MeOH-H_2O (1 : 1 and 1 : 3, v/v) and EtOH-hexane (2 : 1 and 1 : 1, v/v) in a stepwise manner to yield compounds **2** (106 mg), **7** (38 mg) and **8** (47 mg).

Compound 1. Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 0.58 (solvent A) and 0.07 (solvent B); EI-MS: Found $[M]^+$ *m/z* 286; ^{1}H-NMR (400 MHz, MeOH-d_4, δ): 6.31 (1H, d, J = 1.8 Hz, H-6), 6.62 (1H, d, J = 1.8 Hz, H-8), 6.59 (1H, s, H-3), 7.10 (1H, d, J = 8.2 Hz, H-5'), 7.51 (1H, dd, J = 2.1 Hz and J = 8.2 Hz, H-6'), 7.59 (1H, d, J = 2.1 Hz, H-2'); ^{13}C-NMR (100 MHz, MeOH-d_4, δ): 94.74 (C-8), 99.72 (C-6), 104.25 (C-3), 105.39 (C-10), 114.20 (C-2'), 116.66 (C-5'), 120.15 (C-6'), 123.79 (C-1'), 146.51 (C-3'), 150.16 (C-4'), 158.81 (C-9), 163.39 (C-5), 164.94 (C-2), 165.19 (C-7), 183.10 (C-4).

Compound 2. Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 0.79 (solvent A) and 0.05 (solvent B). EI-MS: Found $[M]^+$ *m/z* 270; ^{1}H-NMR (400 MHz, MeOH-d_4, δ): 6.23 (1H, d, J = 1.9 Hz, H-6), 6.53 (1H, d, J = 1.9 Hz, H-8), 6.82 (1H, s, H-3), 6.96 (2H, d, J = 8.1 Hz, H-3',5'), 7.95 (2H, d, J = 8.1 Hz, H-2',6'); ^{13}C-NMR (100 MHz, MeOH-d_4, δ): 94.84 (C-8), 99.71 (C-6), 103.69 (C-3), 104.54 (C-10), 116.85 (C-3',5'), 122.04 (C-1'), 129.37 (C-2',6'), 158.20 (C-9), 162.07 (C-5), 162.34 (C-4'), 164.63 (C-2), 165.07 (C-7), 181.64 (C-4).

Compound 3. Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 0.55 (solvent A) and 0.00 (solvent B); EI-MS: Found $[M]^+$ *m/z* 286; ^{1}H-NMR (400 MHz, MeOH-d_4, δ): 6.29 (1H, d, J = 2.0 Hz, H-6), 6.59 (1H, d, J = 2.0 Hz, H-8), 7.05 (2H, d, J = 8.3 Hz, H-3',5'), 8.19 (2H, d, J = 8.3 Hz, H-2',6'). ^{13}C-NMR (100 MHz, MeOH-d_4, δ): 94.55 (C-8), 99.21 (C-6), 104.21 (C-10), 116.37 (C-3',5'), 123.36 (C-1'), 130.50 (C-2',6'), 136.69 (C-3), 147.05 (C-2), 157.81 (C-9), 160.19 (C-5), 162.35 (C-4'), 165.02 (C-7), 176.64 (C-4).

Compound 4. Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 0.58 (solvent A) and 0.00 (solvent B); EI-MS: Found $[M]^+$ *m/z* 302; ^{1}H-NMR (400 MHz, MeOH-d_4, δ): 6.29 (1H, d, J = 1.8 Hz, H-6), 6.57 (1H, d, J = 1.8 Hz, H-8), 7.04 (1H, d, J = 8.2 Hz, H-5'), 7.70 (1H, dd, J = 8.2 Hz and J = 2.0 Hz, H-6'), 7.80 (1H, d, J = 2.0 Hz, H-2'); ^{13}C-NMR (100 MHz, MeOH-d_4, δ): 94.94 (C-8), 99.56 (C-6), 104.37 (C-10), 116.12 (C-2'), 116.61 (C-5'), 121.89 (C-1'), 123.84 (C-6'), 137.04 (C-3), 146.26 (C-3'), 147.89 (C-2), 148.78 (C-4'), 158.03 (C-9), 162.04 (C-5), 165.49 (C-7), 177.03 (C-4).

Compound 5. Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 0.49 (solvent A) and 0.09 (solvent B); Positive FAB-MS: Found $[M+H]^+$ *m/z* 449; ^{1}H-NMR (400 MHz, MeOH-d_4, δ): 3.1~3.6 (5H, m, H-2",3",4",5",6"), 5.13 (1H, d, J = 8.0 Hz, H-1"), 6.52 (1H, br. s, 6-H), 6.79 (1H, s, 3-H), 6.93 (1H, br. s, 8-H), 6.93 (1H, d, J = 8.2 Hz, 5-H), 7.42 (1H, dd, J = 8.2 and 1.8 Hz, 6'-H), 7.36 (1H, d, J = 1.8 Hz, 2-H); ^{13}C-NMR (100 MHz, MeOH-d_4, δ): 62.11 (C-6), 71.84 (C-4), 74.63 (C-2), 78.60 (C-5), 78.89 (C-3), 96.32 (C-8), 101.30 (C-6), 101.79 (C-1), 104.62 (C-3), 107.12 (C-10), 115.41 (C-2), 118.09 (C-5), 121.21 (C-6), 123.48 (C-1), 147.22 (C-3), 152.05 (C-4), 159.20 (C-9), 163.22 (C-5), 165.31 (C-7), 166.48 (C-2), 184.21 (C-4).

Compound 6. Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 0.51 (solvent A) and 0.59 (solvent B); Positive FAB-MS: Found $[M+H]^+$ *m/z* 433; ^{1}H-NMR (400 MHz, MeOH-d_4, δ): 3.1~3.6 (5H, m, H-2",3",4",5",6"), 5.0 (1H, d, J = 7.1 Hz, H-1"), 6.2 (1H, d, J = 1.9 Hz, H-6), 6.6 (1H, d, J = 1.9 Hz, H-8), 6.6 (1H, s, H-3), 6.8 (2H, d, J = 8.5 Hz, H-3',5'), 7.7 (1H, d, J = 8.5 Hz, H-2',6'); ^{13}C-NMR (100 MHz, MeOH-d_4, δ): 62.48 (C-6"), 71.29 (C-4"), 74.76 (C-2"), 77.87 (C-5"), 78.41 (C-3"), 96.10 (C-8), 101.21 (C-6), 101.63 (C-1"), 104.14 (C-3), 107.11 (C-10), 117.08 (C-3',5'), 123.08 (C-1'), 129.69 (C-2',6'), 158.98 (C-9), 162.88 (C-4'), 162.96 (C-5), 164.82 (C-7), 166.79 (C-2), 184.11 (C-4).

Compound 7. Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 77 (solvent A), 0.25 (solvent B); positive FAB-MS: Found $[M+H]^+$ *m/z* 449; ^{1}H-NMR (400 MHz, MeOH-d_4, δ): 3.20~3.43 (4H, m, H-2",3",4",5"), 3.54 (1H, dd, J = 5.5 and 11.9 Hz, Hb-6"), 3.70 (1H, dd, J = 2.2 and 11.9 Hz, Ha-6"), 5.24 (1H, d, J = 7.3 Hz, H-1"), 6.19 (1H, d, J = 2.0 Hz, H-6), 6.38 (1H, d, J = 2.0 Hz, H-8), 6.88 (2H, d, J = 8.9 Hz, H-3',5'), 8.05 (2H, d, J = 8.91 Hz,

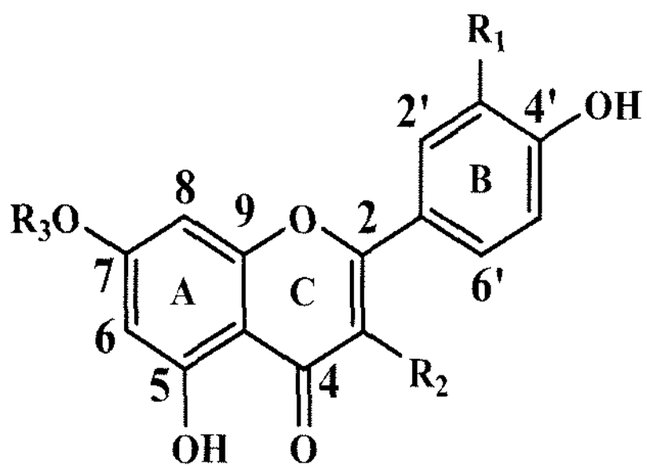

Fig. 1 Structures of compounds isolated from *S. oleraceus*. R_1=OH, R_2=R_3=H: luteolin (1), R_1=R_2=R_3=H: apigenin (2), R_1=R_3=H, R_2=OH: kaempferol (3), R_1=R_2=OH, R_3=H: quercetin (4), R_1=OH, R_2=H, R_3=-β-D-glucoside: luteolin-7-*O*-β-D-glucoside (5), R_1=H, R_2=H, R_3=-β-D-glucoside: apigetrin (6), R_1=H, R_2=-*O*-β-D-glucoside, R_3=H: astragalin (7), R_1=OH, R_2=-*O*-β-D-glucoside, R_3=H: isoquercitrin (8).

H-2',6'); ^{13}C-NMR (100 MHz, MeOH-d_4, δ): 62.65 (C-6''), 71.36 (C-4''), 75.77 (C-2''), 78.06 (C-3''), 78.43 (C-5''), 94.79 (C-8), 99.91 (C-6), 104.14 (C-1''), 105.76 (C-10), 116.10 (C-3',5'), 122.80 (C-1'), 132.33 (C-2',6'), 135.49 (C-3), 158.50 (C-9), 159.10 (C-2), 161.60 (C-4'), 163.07 (C-5), 165.98 (C-7), 179.53 (C-4).

Compound 8. Yellowish amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f: 0.64 (solvent A), 0.07 (solvent B); Positive FAB-MS: Found $[M+H]^+$ *m/z* 465; ^{1}H-NMR (400 MHz, MeOH-d_4, δ): 3.23~3.73 (4H, m, H-2'',3'',4'',6''), 5.25 (1H, d, *J* = 7.4 Hz, H-1''), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.86 (1H, d, *J* = 8.5 Hz, H-5'), 7.58 (1H, dd, *J* = 2.1 and 8.5 Hz, H-6'), 7.71 (1H, d, *J* = 2.1 Hz, H-2'); ^{13}C-NMR (100 MHz, MeOH-d_4, δ): 62.58 (C-6''), 71.24 (C-4''), 75.75 (C-2''), 78.14 (C-5''), 78.41 (C-3''), 99.91 (C-6), 104.35 (C-1''), 105.72 (C-10), 116.03 (C-2'), 117.60 (C-5''), 123.21 (C-6), 123.23 (C-1'), 135.65 (C-3), 145.92 (C-3'), 149.87 (C-4'), 158.48 (C-9), 159.04 (C-2), 163.06 (C-5), 166.01 (C-7), 179.51 (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^{-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. The results were calculated by taking the mean of all triplicate values. IC_{50} values were obtained through extrapolation from concentration of sample necessary to scavenge 50% of the DPPH free radicals. Curcumin and α-tocopherol were used as positive controls.

Table 1. Antioxidant activities (IC_{50} values) of the isolated compounds from *S. oleraceus*

	Samples	IC_{50} (μg/mL)	IC_{50} (μM)
Controls	α-tocopherol	24	56
	Curcumin	28	76
Isolated compounds	Luteolin (**1**)	12	42
	Apigenin (**2**)	39	144
	Kaempferol (**3**)	13	45
	Quercetin (**4**)	11	36
	Luteolin-7-*O*-β-D-glucoside (**5**)	61	131
	Apigetrin (**6**)	89	206
	Astragalin (**7**)	45	100
	Isoquercitrin (**8**)	22	47

Results and Discussion

Extraction and isolation. Chromagographic separation of an EtOAc fraction of *S. oleraceus* led to the isolation of 8 yellow amorphous compounds **1-8** (Fig. 1). Chemical structures of compounds **1-8** were determined as luteolin (**1**), apigenin (**2**), kaempferol (**3**), quercetin (4), luteolin-7-*O*-β-D-glucoside (**5**), apigetrin (**6**), astragalin (**7**), and isoquercitrin (**8**) by comparing the ^{1}H and ^{13}-NMR spectral data with those of reported in literature and a verification of their MS values [Agrawal, 1989; Markham and Chari, 1982; Seidel *et al.*, 2000; Si *et al.*, 2006; Ternai and Markham, 1976; Wenkert and Gottlieb, 1977]. Though compound **1, 2** and **5** have been purified from *S. oleraceus* [Bai *et al.*, 2007; Hu *et al.*, 2005; Li *et al.*, 2005; Xu and Liang, 2005], to our knowledge, this was the first time of isolation compounds **3, 4, 6, 7** and **8** from *S. oleraceus*.

DPPH free radical scavenging assay. The antioxidant activities of the 8 isolated compounds were determined by DDPH assay. The results were presented in Table 1. Among those, luteolin (**1**), kaempferol (**3**), quercetin (**4**) and isoquercitrin (**8**) exhibited stronger antioxidant activities comparable to α-tocopherol and curcumin, which were used as positive controls, while the rest compounds showed weak activities. The DPPH results also indicated that compounds **1**, **3**, **4** and **8** could be mainly responsible for the potent antioxidant effect of the EtOAc soluble fraction of *S. oleraceus* [Yin *et al.*, 2007]. This fact suggested that the extracts of *S. oleraceus* had high antioxidant potential and can be used as a useful source for antioxidants.

The antioxidant activity also suggested that the flavonoids with free hydroxyl groups acted as free radical-scavengers, and the hydroxyl group on the β-ring

of flavonoids enhanced their antioxidant activities [Akdemir *et al.*, 2001; Pietta, 2000]: Comparing Fig. 1 with Table 1, for flavones, luteolin (12 μg/mL) > apigenin (39 μg/mL) and for flavonols, quercetin (11 μg/mL) > kaempferol (13 μg/mL), meaning that flavonols showed higher antioxidant activities than the corresponding flavones (quercetin (11 μg/mL) > lueolin (12 μg/mL), and kaempferol (13 μg/mL) > apigenin (39 μg/mL)). Apigenin, luteolin, kaempferol and quercetin showed comparatively higher antioxidant activity than corresponding glucosides, indicating that the free radical scavenging activities of glycosidic derivatives were significantly reduced when compared with their original flavonoid aglycones.

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