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Radical Scavenging Constituents of *Cyperus flaccidus*

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Abstract – In the course of screening for antioxidant compounds by measuring the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging effect and superoxide quenching activity, methanol extract of *Cyperus flaccidus* (Cyperaceae) was found to show potent antioxidant activities. Subsequent activity-guided fractionation of the methanolic extract led to the isolation of ten compounds, kaempferol-3,7-*O*-α-L-dirhamnopyranoside (**1**), caffeic acid (**2**), quercetin-3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (**3**), kaempferol-3-*O*-α-L-rhamnopyranoside (**4**), quercetin-3-*O*-α-L-rhamnopyranoside (**5**), luteoiln-7-*O*-β-D-glucopyranoside (**6**), kaempferol-3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (**7**), luteoiln (**8**), quercetin (**9**) and quercetin-3-*O*-β-D-glucuronide (**10**). Their structures were elucidated by spectroscopic studies. Compounds **1** - **10** were isolated for the first time from this plant. Among them, compounds **2**, **8** and **9** showed the significant radical scavenging effects on DPPH, and the potent xanthine-originated superoxide quenching activities.
Keywords – *Cyperus flaccidus*, Cyperaceae, DPPH, Superoxide quenching activity

Introduction

Cyperus flaccidus R. Br. (Cyperaceae) is widely distributed in Korea, Japan and China (Lee, 1996). Phytochemical and pharmacological studies of this plant have not been performed yet. In the course of searching for antioxidants from natural plants in Korea by measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol extract of the whole plant of *C. flaccidus* was found to show potent antioxidant activity. Subsequent activity-guided fractionation of the methanolic extract led to the isolation of ten compounds from the active ethyl acetate and *n*-butanol fractions. Among them, several compounds showed the significant antioxidative effects on DPPH and superoxide radicals. This paper deals with the isolation and structural characterization of those ten compounds and their scavenging activity of the stable DPPH free radical and xanthine-originated superoxide quenching activities.

Experimental

General experimental procedures – NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. Sephadex LH-20 was used for column chromatography (25 - 100 μm; GE Healthcare, Uppsala, Sweden). Prep-HPLC was carried out on a Jaigel GS310 column (Tokyo, Japan). TLC was carried out on Merck (Darmstadt, Germany) precoated silica gel F_{254} plates, and silica gel for column chromatography was Kiesel gel 60 (230 - 400 mesh, Merck). Spots were detected under UV and by spraying with 10% H_2SO_4 in ethanol followed by heating at 100 - 120 °C for 3 min. All other chemicals and solvents were of analytical grade and used without further purification. Ascorbic acid, butylated hydroxyanisole (BHA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. (St. Louis, USA). Absorbance of the result in solution was measured on a microplate reader (GENios, Tecan, Grödig, Austria).

Plant materials – The whole plants of *C. flaccidus* were collected in August 2007 at Jinahn, Jeonbuk, Korea. A voucher specimen was deposited in the herbarium of the College of Pharmacy, Woosuk University (WSU-10-005).

Extraction and isolation – The shade dried and powdered whole plants of *C. flaccidus* (230 g) was extracted three times with MeOH at 50 °C, and then filtered. The extracts were combined and evaporated *in vacuo* at 40 °C. The resultant methanolic extract (40 g) was successively partitioned to *n*-hexane (5.3 g), methylene chloride (2.6 g), ethyl acetate (1.2 g), *n*-butanol (3.6 g) and water soluble

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fractions. Each fraction was tested for the radical scavenging effect on DPPH. Among these fractions, the ethyl acetate and *n*-butanol soluble fractions showed significant free radical scavenging effects on DPPH. Ethyl acetate soluble fraction was subjected to chromatography on a Sephadex LH-20 column (MeOH), and give ten fractions (E1-E10). Fraction E4 was chromatographed on silica gel column chromatography ($CHCl_3$-MeOH-H_2O, 50 : 15 : 1) as an eluent to give three subfractions (E41-E43). Subfraction E43 (56 mg) was further purified on a JAI-GS310 HPLC column (MeOH) to give compound **1** (5 mg). Fraction E5 (55 mg) was purified on a JAI-GS310 column (MeOH) to give compounds **2** (7 mg) and **3** (6 mg). Fraction E6 (440 mg) was chromatographed on silicagel column chromatography ($CHCl_3$-MeOH, 40:10) as an eluent to give nine subfractions (E61-E69). Subfractions E62 (35 mg), E63 (134 mg) and E65 (51 mg) were further purified on a JAI-GS310 HPLC column (MeOH) to give compounds **4** (4 mg), **5** (15 mg) and **6** (3 mg), respectively. Fraction E7 (71 mg) was purified on a Sephadex LH-20 column (MeOH) to give compounds **7** (17 mg) and **8** (10.3 mg). *n*-Butanol soluble fraction was subjected to chromatography on a Sephadex LH-20 column (MeOH), and give twelve fractions (B1-B12). Fraction B6 (169 mg) was chromatographed on silica gel column chromatography ($CHCl_3$-MeOH-H_2O, 40 : 10 : 1) to give compound **9** (12 mg). Compound **10** (55 mg) was obtained by crystallization of fraction B11 from methanol.

Kaempferol-3,7-*O*-α-L-dirhamnopyranoside (1) – ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.79 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.94 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.73 (1H, d, *J* = 2.4 Hz, H-8), 6.46 (1H, s, *J* = 2.4 Hz, H-6), 5.55 (1H, d, *J* = 2.0 Hz, H-1'''), 5.39 (1H, d, *J* = 2.0 Hz, H-1''), 1.29 (3H, d, *J* = 6.0 Hz, H-6'''), 0.92 (3H, d, *J* = 6.0 Hz, H-6''), ^{13}C-NMR (100 MHz, CD_3OD) (Table 1).

Caffeic acid (2) – ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.60 (1H, d, *J* = 15.6 Hz, H-7), 7.00 (1H, d, *J* = 2.2 Hz, H-2), 6.91 (1H, dd, *J* = 8.4, 2.2 Hz, H-6), 6.66 (1H, d, *J* = 8.4 Hz, H-5), 6.22 (1H, d, *J* = 15.6 Hz, H-8), ^{13}C-NMR (100 MHz, CD_3OD) δ: 128.1 (C-1), 115.2 (C-2), 146.5 (C-3), 150.3 (C-4), 116.6 (C-5), 122.8 (C-6), 146.8 (C-7), 116.7 (C-8), 168.4 (C-9).

Quercetin-3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (3) – ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.67 (1H, d, *J* = 2.0 Hz, H-2'), 7.63 (1H, dd, *J* = 8.8, 2.0 Hz, H-6'), 6.89 (1H, d, *J* = 8.8 Hz, H-5'), 6.37 (1H, d, *J* = 1.8 Hz, H-8), 6.19 (1H, d, *J* = 1.8 Hz, H-6), 5.08 (1H, d, *J* = 7.6 Hz, H-1''), 4.62 (1H, brs, H-1'''), 1.14 (3H, d, *J* = 6.4, H-6'''), ^{13}C-NMR (100 MHz, CD_3OD) (Table 1).

Kaempferol-3-*O*-α-L-rhamnopyranoside (4) – ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.75 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.92 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.36 (1H, d, *J* = 2.0 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6), 5.39 (1H, d, *J* = 1.4 Hz, H-1''). ^{13}C-NMR (100 MHz, CD_3OD) (Table 1).

Table 1. ^{13}C-NMR spectral data of isolated compounds from *Cyperus flaccidus*

C	**1**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**7**[b]	**8**[b]	**9**[a]	**10**[b]
2	158.1	158.5	158.2	159.2	164.5	155.5	163.8	147.6	156.5
3	136.5	135.7	136.2	136.2	103.2	133.4	102.8	135.7	133.9
4	179.8	179.4	179.5	179.5	182.0	177.5	181.6	175.7	177.4
5	163.0	163.0	163.1	163.1	162.2	160.7	161.5	160.6	160.9
6	100.6	100.0	99.8	99.8	100.0	98.8	98.8	98.1	99.1
7	163.6	166.0	165.8	165.7	163.0	164.1	164.2	163.7	165.5
8	95.6	94.7	94.8	94.7	94.9	94.1	93.9	93.3	93.9
9	159.8	159.2	159.2	158.4	157.0	156.8	157.3	156.1	157.4
10	107.6	105.8	105.9	105.9	105.4	104.1	103.6	103.0	103.3
1'	122.4	123.4	122.6	122.9	121.5	121.2	121.4	121.8	120.5
2'	132.0	116.2	132.3	116.9	113.6	131.0	113.3	115.1	115.4
3'	116.6	145.8	116.5	146.3	146.0	115.3	145.7	146.7	144.8
4'	161.8	150.2	161.5	149.7	149.9	159.7	149.8	145.0	148.4
5'	116.6	117.9	116.5	116.3	116.0	115.3	116.0	115.5	118.1
6'	132.0	123.7	132.3	122.9	120.0	131.0	119.0	119.8	120.6
1''	103.5	104.7	103.5	103.5	99.9	101.1			102.9
2''	72.1	75.6	72.0	72.0	73.1	74.4			71.8
3''	72.1	78.2	72.1	72.1	76.3	76.5			76.7
4''	73.6	71.5	73.2	73.2	69.5	70.7			73.9
5''	71.7	77.7	71.9	71.7	77.1	75.7			74.3
6''	17.7	68.9	17.6	17.6	60.5	67.0			172.3
1'''	99.9	102.6				101.0			
2'''	71.9	72.3				70.5			
3'''	72.1	72.1				70.0			
4'''	73.2	74.1				71.6			
5'''	71.3	69.8				68.4			
6'''	18.1	17.8				17.7			

[a]Recorded at 100 MHz in CD_3OD
[b]Recorded at 100 MHz in DMSO-d_6

Quercetin-3-*O*-α-L-rhamnopyranoside (5) – ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.28 (1H, d, *J* = 2.0 Hz, H-2'), 7.26 (1H, d, *J* = 8.4 Hz, H-6'), 6.85 (1H, dd, *J* = 8.4, 2.0 Hz, H-5'), 6.27 (1H, d, *J* = 1.8 Hz, H-8), 6.14 (1H, d, *J* = 1.8 Hz, H-6), 5.29 (1H, d, *J* = 1.6 H-1''). ^{13}C-NMR (100 MHz, CD_3OD) (Table 1).

Luteoiln-7-*O*-β-D-glucopyranoside (6) – ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.72 (1H, s, H-3), 6.48 (1H, d, *J* = 1.9 Hz, H-6), 6.77 (1H, d, *J* = 1.9 Hz, H-8), 7.48 (1H, d, *J* = 2.0 Hz, H-2'), 6.94 (1H, d, *J* = 8.2 Hz, H-5'), 7.53 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 5.08 (1H, d, *J* = 7.2 Hz, H-1''), ^{13}C-NMR (100 MHz, DMSO-d_6) (Table 1).

Kaempferol-3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (7) – ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.98 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.80 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.23 (1H, d, *J* = 1.8 Hz, H-6), 5.01 (1H, d, *J* = 7.0 Hz, H-1''), 4.42 (1H, brs, H-1'''), ^{13}C-NMR (100 MHz, DMSO-d_6) (Table 1).

Luteolin (8) – ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.43

(1H, d, J = 2.4 Hz, H-2'), 7.41 (1H, dd, J = 8.8, 2.4 Hz, H-6'), 6.89 (1H, d, J = 8.8 Hz, H-5'), 6.68 (1H, s, H-3), 6.45 (1H, d, J = 2.4 Hz, H-8), 6.19 (1H, J = 2.4 Hz, H-6), ^{13}C-NMR (100 MHz, DMSO-d_6) (Table 1).

Quercetin (9) – ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.69 (1H, d, J = 2.4 Hz, H-2'), 7.56 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 6.90 (1H, d, J = 8.4 Hz, H-5'), 6.43 (1H, d, J = 2.0 Hz, H-8), 6.19 (1H, d, J = 2.0 Hz, H-6). ^{13}C-NMR (100 MHz, DMSO-d_6) (Table 1).

Qurcetin-3-*O*-β-D-glucuronide (10) – ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.27 (1H, d, J = 2.4 Hz, H-2'), 7.33 (2H, dd, J = 8.4, 2.4 Hz, H-6'), 6.81 (2H, d, J = 8.4 Hz, H-5'), 6.31 (1H, d, J = 2.0 Hz, H-8), 6.12 (1H, d, J = 2.0 Hz, H-6), 5.27 (2H, d, J = 6.8 Hz, H-1''), ^{13}C-NMR (100 MHz, DMSO-d_6) (Table 1).

DPPH radical scavenging effect – Ethanol solutions of test samples at various concentrations (0.1 - 100 μg/mL) were added to a solution of DPPH in methanol (1.5×10^{-4} M) in 96 well plates. After storing these mixtures for 30 minutes at room temperature, the remaining amounts of DPPH were determined by colorimetry at 520 nm on a microplate reader (Yoshida *et al*., 1989). 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.

Xanthine-originated superoxide scavenging activity – Superoxide radicals were generated by xanthine/xanthine oxidase and measured by previously reported method (Thuong *et al*., 2007). In brief, test samples were mixed with 20 mM phosphate buffer (pH7.8) containing 0.48 mM NBT (nitro blue tetrazolin) and 1.6 mM xanthine. After 5 min, xanthine oxidase (0.05 U/mL) 100 μL was added. The absorbance of reaction mixture was read at 570 nm after 30 min incubation at 37 ºC. Superoxide radical scavenging activity was expressed by the degree of NBT reduction of a test group in comparison to that of control.

Rseults and Discussion

In the course of our screening for antioxidative components from natural plants, methanolic extract of the whole plants of *C. flaccidus* was found to show scavenging activity on DPPH radical (Fig. 1). Subsequent activity-guided fractionation of methanolic extract led to the isolation of ten phenolic compounds (Fig. 2).

Compounds **1**, **4** and **7** were positive to $FeCl_3$ reagent suggesting that these compounds contained phenol groups, and have similar patterns in their NMR spectra. In the ^{1}H-NMR spectra of compounds **1**, **4** and **7**, two *meta*-coupled doublets to be assignable to H-6 and H-8 of A-ring, and two *ortho*-coupled doublets protons to be assignable to H-2', 6' and H-3', 5' of B-ring were observed respectively, based on the J value, indicating the presence of kaempferol skeleton. Main differences among three compounds were the sugar moieties of those chemical structures. Structure characterization of these compounds was carried out by interpretation of their spectral data and comparison with the data reported in the literature. Consequently, compounds **1**, **4** and **7** were identified as kaempferol-3,7-*O*-α-L-dirhamnopyranoside (**1**) (Moreno *et al*., 2002), kaempferol-3-*O*-α-L-rhamnopyranoside (**4**) (Hur *et al*., 2001; Jang *et al*., 2002), and kaempferol-3-*O*-[α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside] (**7**) (Hong *et al*., 2008), respectively.

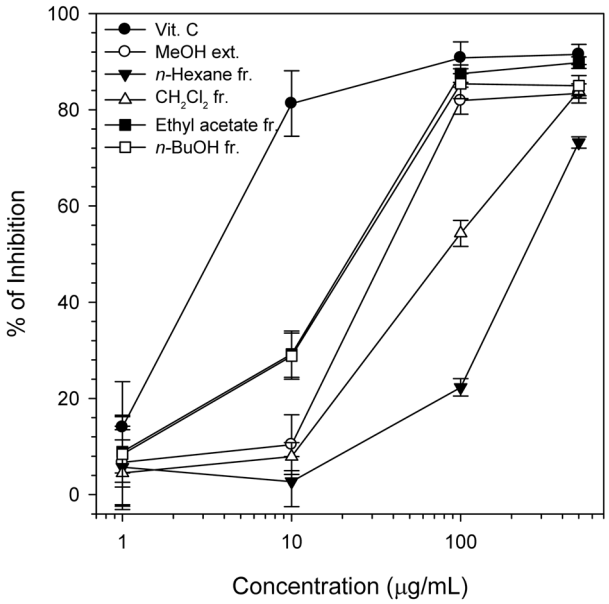
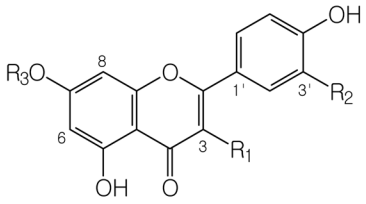


Fig. 1. Radical scavenging effects on DPPH radical of the fractions from *Cyperus flaccidus*.

Compound **2** was positive to $FeCl_3$ reagent test. In the ^{1}H-NMR, two olefinic protons having *trans*-configuration were observed at δ 7.60 (1H, dd, J = 15.6 Hz, H-7) and 6.22 (1H, d, J = 15.6 Hz, H-8). Typical signals for 1,3,4-trisubsituted benzene were detected at δ 7.00 (1H, d, J = 2.2 Hz, H-2), 6.91 (1H, dd, J = 8.4, 2.2 Hz, H-6), and 6.66 (1H, d, J = 8.4 Hz, H-5). In the ^{13}C-NMR, 9 carbons were detected including a carbonyl carbon at δ 168.4. On the basis of these observations and the direct comparison of the data with those previously published (Wu *et al*., 1999), the structure of compound **2** was identified as caffeic acid.

Compounds **3**, **5**, **9** and **10** were positive to $FeCl_3$ reagent suggesting that these compounds contained



	R_1	R_2	R_3
1	*O*-α-L-Rha	H	α-L-Rha
3	*O*-α-L-Rha(1→6)-β-D-Glc	OH	H
4	*O*-α-L-Rha	H	H
5	*O*-α-L-Rha	OH	H
6	H	OH	β-D-Glc
7	*O*-α-L-Rha(1→6)-β-D-Glc	H	H
8	H	OH	H
9	OH	OH	H
10	*O*-β-D-GlcUA	OH	H

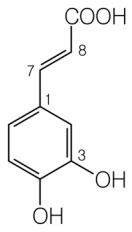


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Fig. 2. Structures of compounds **1** - **10** isolated from *Cyperus flaccidus*.

phenol groups, and have similar patterns in their NMR spectra. In the ^{1}H-NMR spectra of compounds **3**, **5**, **9** and **10**, two *meta*-coupled doublets to be assignable to H-6 and H-8 of A-ring, and typical 1,3,4-trisubstituted benzene signals appeared to be assignable to H-2' H-5' and 6' of B-ring were observed from each compounds, based on the *J* value, indicating the presence of quercetin skeleton. Main differences among four compounds were the sugar moieties of those chemical structures. Structure characterization of these compounds was carried out by interpretation of their spectral data and comparison with the data reported in the literature. Compounds **3**, **5**, **9** and **10** were identified as quercetin-3-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**3**), (Kim *et al.*, 1992; Hasan *et al.*, 1995), quercetin-3-*O*-α-L-rhamnopyranoside (**5**) (Lee *et al.*, 2007), quercetin (**9**) (Lee *et al.*, 1997; Lee *et al.*, 2007), and quercetin-3-*O*-β-D-glucuronide (**10**) (Moon *et al.*, 2001; Bouktaib *et al.*, 2002), respectively.

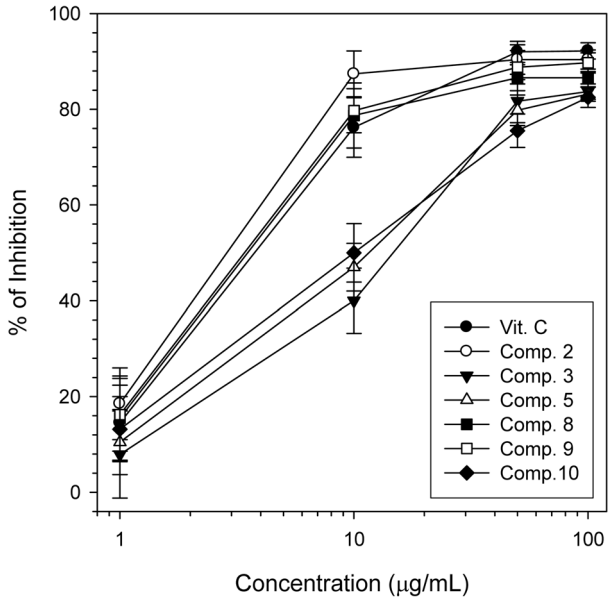

Fig. 3. Radical scavenging effects on DPPH radical of the isolated compounds from *Cyperus flaccidus*.

Compounds **6** and **8** were positive to $FeCl_3$ reagent suggesting that these compounds contained phenol groups, and have similar patterns in their NMR spectra except for the existence of glucose moiety. In the ^{1}H-NMR spectra of compounds **6** and **8**, two *meta*-coupled doublets to be assignable to H-6 and H-8 of A-ring, and typical 1,3,4-trisubstituted benzene signals appeared to be assignable to H-2' H-5' and 6' of B-ring were observed from each compounds, based on the *J* value. And the characteristic singlet signals at δ 6.72 (6) and 6.68 (8) were observed due to the C-3 position indicating flavone skeleton. Final structure characterization of these compounds was carried out by interpretation of their spectral data and direct comparison with the data reported in the literature. Compounds **6** and **8** were identified as luteolin-7-*O*-β-D-glucopyranoside (**6**) (Lee *et al.*, 2011) and luteolin (**8**) (Lee *et al*., 2007), respectively. To the best of our knowledge, these ten compounds were isolated for the first time from this plant in the present study.

The DPPH radical scavenging effects of compounds obtained from *C. flaccidus* were shown in Fig. 3. The positive control vitamin C showed the DPPH radical scavenging effect with the IC_{50} value of 3.9 μg/mL. Compound **2** exhibited the highest scavenging activity dose-dependently on DPPH with IC_{50} value of 2.8 μg/mL. Compounds **8** (3.3 μg/mL) and **9** (3.2 μg/mL) showed similar activities in comparison with ascorbic acid. To

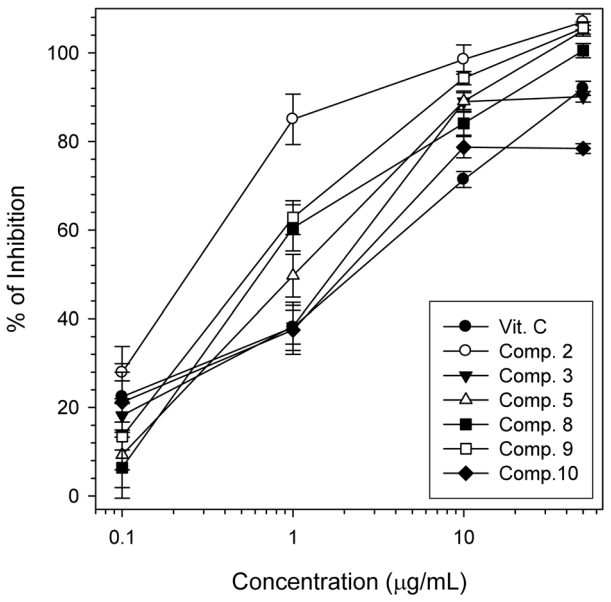

Fig. 4. Superoxide quenching activities of the isolated compounds from *Cyperus flaccidus*.

verify additional antioxidant effects of the isolated compounds, superoxide quenching activities were measured. Fig. 4 shows the superoxide quenching activities of the isolated compounds, as measured by the xanthine-NBT-xanthine oxidase system. Compound **2** exhibited the formation of the blue formazane in a dose-dependent manner with IC_{50} value of 0.22 μg/mL, while the positive control vitamin C showed the superoxide quenching activity with the IC_{50} value of 1.96 μg/mL. Compounds **8** (0.63 μg/mL) and **9** (0.54 μg/mL) showed similar potent superoxide quenching activities.

Superoxide radical produced as byproducts of metabolic processes is known to be very harmful to cellular components as a precursor of more reactive oxygen species, and the harmful action of the free radicals can be blocked by antioxidant substances (Kumaran and Karunakaran, 2006; Devi *et al*., 2008). The results from free radical scavenging systems revealed that the ethyl acetate and *n*-butanol soluble fractions of the whole plants of *C. flaccidus,* and compounds **2**, **8** and **9** had significant antioxidant activities. Among them, compound **2** showed the most significant antioxidative effects on those two antioxidant tests. In conclusion, the methanol extract of *C. flaccidus* including isolated compounds could be a good source of natural antioxidants.

Acknowledgement

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References

Bouktaib, M., Atmani, A., and Rolando, C., Regio- and steroselective synthesis of the major metabolite of quercetin, quercetin-3-*O*-β-D-glucuronide. *Tetrahedron Lett*. **43**, 6263-6266 (2002).

Devi, K.R., Suganthy, N., Kesika, P., and Pandian, S.K., Bioprotective properties of seaweeds: In vitro evaluation of antioxidant activity and antimicrobial cativity against food borne bacteria in relation to polyphenolic content. *BMC Complement. Altern. Med*. **8**, 1-11 (2008).

Hasan, A., Ahmed, K., Jay, M., and Voirin, V., Flavonoid glycosides and anthraquinone from *Rumex chalepensis. Phytochemistry* **39**, 1211-1213 (1995).

Hong, H.K., Kwak, J.H., Kang, S.C., Lee, J.W., Park, J.H., Ahn, J.W., Kang, H.S., Choung, E.S., and Zee, O.P., Antioxidative Constituents from the Whole Plants of *Euphorbia supina. Kor. J. Pharmacogn*. **39**, 260-264 (2008).

Hur, J.M., Park, J.C., and Hwang, Y.H., Aromatic Acid and Flavonoids from the Leaves of *Zanthoxylum piperitum*. *Nat. Prod. Sci.* **7**, 23-26 (2001).

Jang, K.G., Oh, H., Ko, E.K., Kang, K.H., Park, S.E., Oh, M.H., and Kim, Y.C., Free Radical Scavengers from the Leaves of *Albizzia julibrissin. Kor. J. Pharmacogn*. **33**, 28-30 (2002).

Kim, S.H., Hwang, K.T., and Park, J.C., Isolation of flavonoids and determination of rutin from the leaves of *Ulmus parviflora*. *Kor. J. Pharmacogn*. **23**, 229-233 (1992).

Kumaran, A. and Karunakaran, R.J., Antioxidant and free radical scavenging activity of and aqueous extract of *Coleus aromaticus*. *Food Chem*. **97**, 109-114 (2006).

Lee, H.J., Lee, S.K., Choi, Y.J., Jo, H.J., Kang, H.Y., and Choi, D.H., Extractives from the bark of *Platycarya strobilacea. Journal of Korean Forest Society* **96**, 408-413 (2007).

Lee, S., Han, S., Kim, H.M., Lee, J.M., Mok, S.Y., and Lee, S., Isolation and identification of phytochemical constituents from *Taraxacum coreanum*. *J. Korean Soc. Appl. Biol. Chem*. **54**, 73-78 (2011).

Lee, S.K., Park, W.G., and Bae, Y.S., Procyanidins from *Lindera obtusiloba* bark. *Mokchae konghak* **25**, 110-116 (1997).

Lee, W.T., *Coloured standard illustrations of Korean plants*, Academy Pub. Co., Seoul, pp. 468, 1996.

Moon, J.H., Tsushida, T., Kakahara, K., and Terao, J., Identification of quercetin 3-*O*-β-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. *Free Radic. Biol. Med*. **30**, 1274-1285 (2001).

Moreno, A., Martin-Cordero, C., Iglesias-Guerra, F., and Toro, M.V., Flavonoids from *Dorycnium rectum. Biochem. Sys. Eco*. **30**, 73-74 (2002).

Thuong, P.T., Kang, H.J., Na, M.K., Jin, W.Y., Youn, U.J., and Seong, Y.H., Anti-oxidant constituents from *Sedum takesimense*. *Phytochemistry* **68**, 2432-2438 (2007).

Wu, Z.J., Ouyang, M.A., and Yang, C.R., Polyphenolic constituents of *Salvia onchifolia*. *Acta Bot. Yunnan* **21**, 393-398 (1999).

Yoshida, T., Mori, K., Hatano, T., Okumura, T., Uehara, L., Komagoe, K., Fujita, Y., and Okuda, T., Studies 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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