Arch Pharm Res Vol 28, No 1, 22-27, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Antioxidant Activity of Anthraquinones and Flavonoids from Flower of *Reynoutria sachalinensis*

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(Received October 25, 2004)

Bioassay-guided fractionation of methanol extract of *Reynoutria sachalinensis* flower using DPPH assay has led to the isolation of three anthraquinones and three flavonoids. Their structures were identified as emodin (**1**), emodin-8-*O*-β-D-glucopyranoside (**2**), physcion-8-*O*-β-D-glucopyranoside (**3**), quercetin-3-*O*-α-L-arabinofuranoside (**4**), quercetin-3-*O*-β-D-galactopyranoside (**5**), and quercetin-3-*O*-β-D-glucuronopyranoside (**6**) by comparing their physicochemical and spectral data with those published in literatures. All isolated compounds were evaluated for antioxidant activities with free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging, superoxide radical scavenging and Cu^{2+}-mediated low density lipoprotein (LDL) oxidation assay. The results demonstrated that three flavonoids, **4**, **5**, and **6** had remarkable antioxidant activities with the IC_{50} values of 64.3, 54.7, and 46.2 μM (DPPH scavenging), the IC_{50} values of 6.0, 6.7, and 4.4 μM (superoxide radical scavenging) and the IC_{50} values of 3.8, 3.2, and 5.4 μM against LDL oxidation, respectively.

Key words: *Reynoutria sachalinensis*, Polygonaceae, Anthraquinones, Flavonoids, Antioxidant, DPPH, Superoxide radical, LDL oxidation

INTRODUCTION

Reynoutria sachalinensis (Fr. Schm.) Nakai (Polygonaceae) is a medicinal plant which mainly distribute in China, Korea and Japan. The root of genus *Reynoutria* has been used as a traditional Chinese medicine for treatment of arthralgia, jaundice caused by damp-heat, amenorrhea, cough with expectoration, scalds and burns, traumatic injuries, carbuncles and scores (Bae, 1999; Pharmacopia Commission of PRC, 1997). The early works have been reported that numbers of compounds were isolated from the genus *Reynoutria*, include anthraquinones (Kang and Woo, 1982a; Xiao *et al.*, 2002), stilbenes (Vastano *et al.*, 2000; Hua *et al.*, 2001; Xiao *et al.*, 2000), flavonoids (Kang and Woo, 1981; Kang and Woo, 1982b) and other phenolic compounds. However, there is no report on chemical constituents and biological activity of the flower of *R. sachalinensis*.

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In continuation of our screening program for antioxidant agents, we found that the MeOH extract of *R. sachalinensis* flower exhibited a considerable free radical scavenging activity on DPPH assay. The result suggested that the MeOH extract possess antioxidant compounds. Bioassay-guided fractionation had led to the isolation of six compounds, four from EtOAc fration and two from BuOH fraction. By comparing their physicochemical and spectral data with those published in literatures, they were identified as emodin (**1**), emodin-8-*O*-β-D-glucopyranoside (**2**), physcion-8-*O*-β-D-glucopyranoside (**3**), quercetin-3-*O*-α-L-arabinofuranoside (**4**), quercetin-3-*O*-β-D-galactopyranoside (**5**), quercetin-3-*O*-β-D-glucuronopyranoside (**6**). Moreover, three flavonoids **4**, **5**, and **6** showed significant antioxidant activities on both radical scavenging assays (DPPH and superoxide) and Cu^{2+}-mediated LDL oxidation. In this paper, the isolation, structure elucidation and biological evaluation were described and discussed.

MATERIALS AND METHODS

Reagents

1,1-Diphenyl-2-picrarylhydraryl (DPPH), xanthine, xanthine

oxidase, cupric sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), thiobarbituric acid (TBA), α-tocopherol, butylated hydroxyl toluene (BHT), nitro blue tetrazolium (NBT), were purchased from Sigma Chemical Co., USA. ethylenediaminetetraacetatic acid (EDTA), tricloroacetic acid (TCA), were obtained from Daejung Chemical, Ltd., Korea.

Plant material

The flower of *R.sachalinensis* was collected in Chungnam National University medicinal plant garden, and identified by one of authors, Prof. KiHwan Bae. A voucher specimen (CNU 482) has been deposited at the herbarium in the College of Pharmacy, Chungnam National University.

General procedures

Melting points were determined on a Kofler microhotstage; UV spectra were obtained with a Beckman Du-650 UV-VIS recording spectrophotometer; IR spectra were obtained on a Jasco Report-100 type spectrometer from KBr disc; MS were carried out with a JEOL JMS-HX/HX110A tandem mass spectrometer. ^{1}H-NMR (300 MHz, 400 MHz) and ^{13}C-NMR (75 MHz, 100 MHz) were recorded on a Bruker DRX300 and JEOL 400 spectrometers; The chemical shifts were referenced to δ using TMS as an internal standard. Analytical TLC were performed on precoated silica gel 60 F_{254} plates (Merck) or RP-18 F_{254}. Silica gel (Kieselgel 60, 70~230 mesh and 230-400 mesh, Merck) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography.

Extraction and isolation

Dried flower of *Reynoutria sachalinensis* (0.75 kg) was extracted three times with 10 L methanol. Methanol extract were combined and concentrated to obtain 118 g residue, then suspended the residue into water and partitioned with hexane, ethyl acetate (EtOAc) and buthanol (BuOH), respectively. The EtOAc fraction (21.3 g) was applied to silica gel column and eluted with CH_2Cl_2-MeOH mixtures of increasing polarity to yield 6 fractions (1-6). The fraction 2 was chromatographed on silica gel column (230-400 mesh) eluting with CH_2Cl_2-MeOH (30:1) to give compound **1** (35 mg); Fraction 4 was subjected to column chromatography using CH_2Cl_2-MeOH (20:1) as eluting solvent to afford three compounds **2** (26 mg), **3** (4 mg), **4** (10 mg). The BuOH fraction (28.8 g) was subjected to silica gel column and eluted with $CHCl_3$-MeOH (90:10→10:30) to give five subfractions (BuOH. 1-5). Fraction 3 was further chromatographed on silica gel column eluted with CH_2Cl_2-MeOH (9:1) to yield compound **5** (356 mg). Finally, fraction 4 was applied to Sephadex LH-20 column using MeOH-H_2O (3:1) as eluting solvent to give compound **6** (70 mg).

Emodin (1)

Orange needle; $FeCl_3$ positive; Bornträger positive; mp: 244-245°C; UV (MeOH) λ_{max} nm: 222.0, 288.0; IR (KBr) cm^{-1}: 3400 (OH), 1625 (C=O), 1478 (C=C); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.38 (3H, s, H-15), 6.55 (1H, d, J = 2.3 Hz, H-7), 7.05 (1H, d, J = 2.3 Hz, H-5), 7.09 (1H, s, H-2), 7.40 (1H, s, H-4), 11.95 (1H, s, OH), 12.03 (1H, s, OH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 21.4 (CH_3, C-15), 107.8 (C-7), 108.7 (C-5), 108.7 (C-12), 113.1 (C-13), 120.3 (C-4), 123.9 (C-2), 132.6 (C-14), 134.9 (C-11), 148.1 (C-3), 161.3 (C-1), 164.3 (C-8), 164.5 (C-6), 181.1 (C-10), 189.5 (C-9).

Emodin-8-*O*-β-D-glucopyranoside (2)

Yellow powder; Bornträger positive; mp: 219-220°C; UV (MeOH) λ_{max} nm: 222.0, 284.0, 420; IR (KBr) cm^{-1}: 3300 (OH), 1675 (C=O), 1625 (C=O), 1590, 1480 (C=C), 1040 (C-O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.41 (3H, s, H-15), 3.17~3.75 (m, sugar-H), 5.06 (1H, d, J = 7.5 Hz, H-1'), 7.02 (1H, d, J = 2.4 Hz, H-7), 7.16 (1H, *br* s, H-2), 7.30 (1H, d, J = 2.4 Hz, H-5), 7.47 (1H, *br* s, H-4), 11.21 (1H, s, β-OH), 13.16 (1H, s, α-OH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 22.3 (CH_3, C-15), 61.5 (C-6'), 70.3 (C-4'), 74.1 (C-2'), 77.2 (C-3'), 78.2 (C-5'), 101.7 (C-1'), 109.2 (C-5), 109.2 (C-7), 115.3 (C-12), 114.3 (C-13), 120.1 (C-4), 125.0 (C-2), 133.0 (C-14), 137.4 (C-11), 147.8 (C-3), 161.9 (C-1), 162.6 (C-8), 165.0 (C-6), 183.0 (C-10), 187.3 (C-9).

Physcion-8-*O*-β-D-glucopyranoside (3)

Yellow needle crystal; Bornträger positive; mp: 241-242°C; UV (MeOH) λ_{max} nm: 223, 271, 419; IR (KBr) cm^{-1}: 3400 (OH), 1670 (α, β-unstaturated ketone), 1630 (C=O), 1595, 1485 (C=C), 1080 (C-O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.42 (3H, s, H-15), 3.17-3.77 (sugar-H), 3.97 (3H, s, OCH_3), 5.18 (1H, d, J = 7.5 Hz, H-1'), 7.19 (2H, d, J = 2.4 Hz, H-2, 7), 7.38 (1H, d, J = 2.4 Hz, H-5), 7.50 (1H, br s, H-4), 13.07 (1H, s, α-OH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 22.3 (C-15), 56.9 (-OCH_3), 61.7 (C-6'), 70.7 (C-4'), 74.1 (C-2'), 77.5 (C-3'), 78.4 (C-5'), 101.6 (C-1'), 107.4 (C-7), 108.3 (C-5), 115.4 (C-12,13), 120.2 (C-4), 125.1 (C-2), 133.0 (C-14), 137.2 (C-11), 148.0 (C-3), 161.6 (C-1), 162.6 (C-8), 165.6 (C-6), 182.8 (C-10), 187.3 (C-9).

Quercetin-3-*O*-α-L-arabinofuranoside (Avicularin) (4)

Yellow crystal; HCl-Mg positive; mp: 216-217°C; UV (MeOH) λ_{max} nm: 257, 357; IR (KBr) cm^{-1}: 3400 (OH), 1650 (α, β-unstaturated ketone), 1605, 1510 (C=C), 1110 (C-O); ^{1}H-NMR (300 MHz, methanol-d_4) δ (ppm): 3.32-3.92 (m, sugar-H), 4.35 (1H, dd, J = 3.0 Hz, H-5''), 5.49 (1H, d, J = 0.6 Hz, H-1''), 6.23 (1H, d, J = 2.1 Hz, H-6), 6.42 (1H, d, J = 2.1 Hz, H-8), 6.93 (1H, d, J = 8.4 Hz, H-

5'), 7.50 (1H, d, J = 2.1 Hz, H-2'), 7.55 (1H, dd, J = 6.9, 2.4 Hz, H-6'); ^{13}C-NMR (75 MHz, methanol-d_4) δ (ppm): 61.6 (C-5''), 77.7 (C-3''), 82.3 (C-2''), 87.1 (C-4''), 93.8 (C-8), 98.9 (C-6), 104.6 (C-10), 108.6 (C-1''), 115.4 (C-5'), 115.9 (C-2'), 121.9 (C-6'), 122.1 (C-1'), 133.9 (C-3), 145.4 (C-3'), 148.8 (C-4'), 157.6 (C-9), 158.3 (C-2), 162.1 (C-5), 165.0 (C-7), 179.0 (C-4).

Quercetin-3-*O*-β-D-galactopyranoside (Hyperin) (5)

Yellow powder; HCl-Mg positive; mp: 235-236°C; UV (MeOH) λ_{max} nm: 257, 358; IR (KBr) cm^{-1}: 3350 (OH), 1650 (α, β-unstaturated ketone), 1606, 1558, 1507 (C=C), 1070 (C-O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.17-3.67 (m, sugar-H), 5.39 (1H, d, J = 7.8 Hz, H-1''), 6.21 (1H, d, J = 1.8 Hz, H-6), 6.42 (1H, d, J = 1.8 Hz, H-8), 6.84 (1H, d, J = 8.4 Hz, H-5'), 7.54 (1H, d, J = 2.1 Hz, H-2'), 7.68 (1H, dd, J = 2.1, 2.1 Hz, H-6'), 12.60 (1H, C5-OH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 61.0 (C-6''), 68.8 (C-4''), 72.1 (C-2''), 74.1 (C-3''), 76.7 (C-5''), 94.4 (C-8), 99.5 (C-6), 102.7 (C-1''), 104.8 (C-10), 116.1 (C-2'), 116.8 (C-5'), 121.7 (C-1'), 122.9 (C-6'), 134.4 (C-3), 145.7 (C-3'), 149.3 (C-4'), 157.1 (C-2), 157.2 (C-9), 161.8 (C-5), 165.0 (C-7), 178.3 (C-4).

Quercetin-3-*O*-β-D-glucuronopyranoside (6)

Yellow powder; HCl-Mg positive; mp: 219-221°C; UV (MeOH) λ_{max} nm: 257, 358; IR (KBr) cm^{-1}: 3340 (COOH), 1650 (α, β-unstaturated ketone), 1600, 1507 (C=C), 1080 (C-O). ^{1}H-NMR (400 MHz, methanol-d_4) δ (ppm): 3.16-3.38 (m, sugar-H), 5.19 (1H, d, J = 6.0 Hz, H-1''), 6.17 (1H, s, H-6), 6.37 (1H, s, H-8), 6.82 (1H, d, J = 8.4 Hz, H-5'), 7.31 (1H, d, J = 8.4 Hz, H-2', 6'). ^{13}C-NMR (100 MHz, Methanol-d_4) δ (ppm): 71.7 (C-4''), 73.8 (C-2''), 74.1 (C-3''), 76.6 (C-5''), 93.7 (C-8), 98.9 (C-6), 103.6 (C-10), 103.2 (C-1''), 115.3 (C-5'), 118.3 (C-2'), 120.3 (C-6'), 120.4 (C-1'), 133.9 (C-3), 144.6 (C-3'), 148.2 (C-4'), 156.4 (C-9), 157.7 (C-2), 160.8 (C-5), 165.7 (C-7), 171.9 (C-6''), 177.4 (C-4).

DPPH radical scavenging activity

The DPPH radical scavenging effect was measured as previously described method (Na *et al.*, 2003). Briefly, the sample solution which is dissolved in DMSO 5 μL was added to 195 μL of 150 μM DPPH solution in methanol in 96 well plates. The mixed solution was incubated at room temperature for 30 min, and then the absorbance of reaction mixture was read at 517 nm using a microplate reader and the remaining DPPH was calculated. The free radical scavenging activity was expressed as follow:

$$\text{DPPH scavenging activity (\%)} = \left(\frac{Ac-As}{Ac-Ab}\right)\times 100$$

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (MeOH). Each sample was assayed at five concentrations and four wells for each concentration. All experiments were carried out in triplicate. The IC_{50} values were defined as the concentration that could scavenge 50% DPPH free radical. Quercetin and á-tocopherol were used as positive control.

Xanthine/Xanthine Oxidase assay

The assay was carried out basically according to the method of Beauchamp and Fridovich (Paya *et al.*, 1992; Masaki *et al.*, 1995) with some modification. The 495 μL assay mixture consisted of 50 mM sodium carbonate buffer (pH 10.2), 0.1 mM xanthine and 25 μM nitro blue tetrazolium (NBT). The reaction was initiated by addition of 5 μL 20 nM xanthine oxidase in the presence or absence of each compound. The increase in absorbance at 560 nm was read after 5 min on spectrophotometer (Shimadzu UV-1240, Tokyo, Japan). Superoxide radical scavenging activity was expressed by the degree of NBT reduction decrease of test group in comparison with that of the control group and calculated by the following equation:

$$\text{Scavenging activity (\%)} = \left(\frac{Ac-As}{Ac-Ab}\right)\times 100$$

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (where xanthin oxidase was not added). The IC_{50} values were defined as the concentration that cause 50% decrease in NBT reduction.

Preparation of low density lipoprotein

Plasma was obtained from fasted healthy normalipidemic individual. Low density lipoprotein was isolated according to standard procedure with slight modification (Kerry and Abbey, 1998) Plasma was centrifuged at 43800 rpm for 20 h at 4°C in Beckman T8-M ultracentrifuge and then chylomicron and very low density lipoprotein floated to the top of tube was removed. Other infranatants was collected, adjusted to d = 1.063 g/mL using NaBr and centrifuged at 43800 rpm for 28 h at 4°C. The top layer of LDL was collected and dialyzed against 10 mM phosphate saline buffer (pH 7.4). LDL protein was determined by bicinchoninic acid method using bovine serum albumin as standard (Andrew and Edward, 1995).

LDL oxidation assay

Copper ion-induced lipid peroxidation of human plasma LDL was carried out using a slight modification of the established method (Wang *et al.*, 2004; Kerry and Abbey, 1998). In brief, LDL (100 mg/mL) was incubated with 5 mM Cu^{2+} in 0.2 mL of 10 mM PBS buffer (pH 7.4) in the absence

or presence of each compound at various concentration at 37°C for 3 h. The oxidation was terminated by adding EDTA and BHT (1 mM and 500 mM final concentration, respectively). Upon incubation 0.2 mL aliquot was withdrawn and 0.5 mL TCA (20%) was added followed by 0.5 mL TBA (0.67%). The mixture were heated at 95~100°C for 15 min, cooled in ice and centrifuged at 5000 rpm for 5 min. Then the absorbance of supernatant was read at 532 nm. Lipid peroxidation inhibitory was calculated by the following equation:

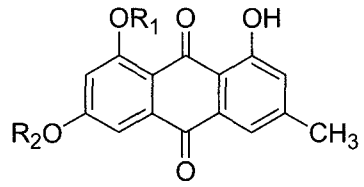
$$\text{Antioxidant activity (\%)} = \left(\frac{Ac-As}{Ac-Ab}\right) \times 100$$

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank (without sample and 5 mM Cu^{2+}). The IC_{50} was defined as a samples concentration that reduces 50% in lipid peroxidation comparison with a control.

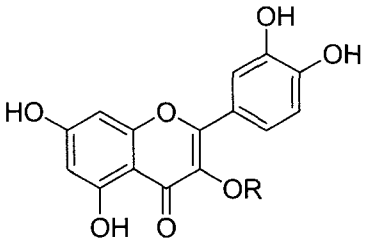
RESULTS AND DISCUSSION

The dried flower of *R. sachalinensis* was extracted exhaustively with hot MeOH. The MeOH extract, after concentration in vacuum to a dry mass, was suspended in water. The water suspension was partitioned against hexane, ethyl acetate, buthanol and then exhaustively evaporated to yield hexane, EtOAc, BuOH, and H_2O fractions, respectively. Then, fractions bioassay revealed that the active compounds of MeOH extract was almost located in EtOAc and BuOH fractions. Repeated chromatography on silica gel and LH-20 column led to the isolation of six compounds. Chemical structures of compounds **1~6** were identified as emodin (Cohen and Towers, 1995), emodin-8-*O*-β-D-glucopyranoside (Xiang *et al.*, 2001; Demirezer *et al.*, 2001), physcion-8-*O*-β-D-glucopyranoside (Li *et al.*, 2000; Coskun *et al.*, 1990), quercetin-3-*O*-α-L-arabinofuranoside (Lu and Foo, 1997; Wang *et al.*, 2003), quercetin-3-*O*-β-D-galactopyranoside (Lu and Foo, 1997; Wang *et al.*, 2003), and quercetin-3-*O*-β-D-glucuronopyranoside (Bouktaib *et al.*, 2002; Price *et al.*, 1998; Moon *et al.*, 2001), respectively, by comparing the physical (melting point) and spectral (UV, IR, ^{1}H-NMR, ^{13}C-NMR and DEPT) data with the values reported in literatures (Fig. 1). Among them, compounds **5** and **6** had been isolated previously from *Pyrola atropurpurea* (Lu and Foo, 1997; Wang *et al.*, 2003) and *Phaseolus vulgaris* (Price *et al.*, 1998), but this is the first report from *R. sachalinensis*.

For screening of extract and compounds, *R. sachalinensis* MeOH extract and compounds **1~6** were tested for their antioxidant activities using DPPH radical scavenging activity. As the results shown in Table I, MeOH extract of *R. sachalinensis* exhibited a prominent inhibitory activity with IC_{50} value of 47.2 μg/mL. The result suggested that



	R_1	R_2
Emodin (**1**)	H	H
Emodin-8-*O*-β-D-glucopyranoside (**2**)	Glucose	H
Physcion-8-*O*-β-D-glucopyranoside (**3**)	Glucose	CH_3



	R
Quercetin-3-*O*-α-L-arabinofuranoside (**4**)	Arabinose
Quercetin-3-*O*-β-D-galactopyranoside (**5**)	Galactose
Quercetin-3-*O*-β-D-glucuronopyranoside (**6**)	Glucuronic acid

Fig. 1 *Chemical structures of compounds* **1~6** *isolated from the flowers of R. sachalinensis*

Table I. The free radical scavenging activities of compounds **1~6**

Compounds	IC_{50} (μM) [a]	
	DPPH radical scavenging	Superoxide radical scavenging
R. sachalinensis MeOH extract	47.2 (μg/mL)	-
Emodin (**1**)	>100	>100
Emodin-8-*O*-β-D-glucopyranoside (**2**)	>100	>100
Physcion-8-*O*-β-D-glucopyranoside (**3**)	>100	>100
Quercetin-3-*O*-α-L-arabinofuranoside (**4**)	64.3	6.0
Quercetin-3-*O*-β-D-galactopyranoside (**5**)	54.7	6.7
Quercetin-3-*O*-β-D-glucuronopyranoside (**6**)	46.2	4.4
Quercetin [b]	26.8	5.2
α-Tocopherol [b]	46.4	–[c]

[a] The IC_{50} values were the 50% inhibition concentration and were calculated from regression lines using six different concentrations in triplicate experiments and repeated at least three times; [b] The compounds were used as positive control; [c] inactive.

compounds present in MeOH extract might possess antioxidant activity. Among isolated compounds, **4~6** were found having moderate inhibition effects with the IC_{50} value of 46.2, 64.3, and 54.7 μM, respectivly. Furthermore,

compound **6** showed the higher activity than both compounds **4** and **5**. Comparing to the positive control, compound **4** exhibited equipotent with α-tocopherol (IC_{50} = 46.4 μM), but all three compounds were less potent than quercetin (IC_{50} = 26.8 μM). In contrast, three anthraquinones **1~3** showed negligible activities.

The results in superoxide radical scavenging assay were showed in Table I and demonstrated that compounds **4**, **5** and **6** inhibited against generation of superoxide anion in a concentration-dependent manner, with the IC_{50} values 6.0, 6.7 and 4.4 μM, respectively. It is noteworthy that compound **6** showed more potent activity than quercetin (IC_{50} = 5.2 μM), which was used as positive control. Meanwhile, α-tocopherol together with all three anthraquinones failed to express the inhibitory effect.

Previous studies have been reported that certain flavonoids could protect LDL from being oxidized (Fuhrman and Aviram, 2001). Therefore, we examined the antioxidant effects of compounds **4~6** on human low-density lipoprotein activated by Cu^{2+}. The results were evaluated and showed in Table II. All flavonoids **4**, **5**, and **6** reduced the formation of TBARS, with the IC_{50} values of 3.8, 3.2, and 5.4 μM, respectively. The results demonstrated that all three compounds significant exhibited LDL oxidation, comparison to that of α-tocopherol (IC_{50} = 21.3 μM), but less potent antioxidant activity than quercetin (IC_{50} = 1.4 μM). The oxidation of LDL was recognized to play an important role in atherosclerosis and the modified LDL was found to accumulate in atherosclerotic lesions and induce cholesterol to accumulate in human foam cells (Diaz *et al.*, 1997; Lusis, 2000). So, the results suggest that the flavonoids could be beneficial in preventing LDL oxidation and atherosclerosis disease.

In addition, our results clearly showed that three flavonoids possess much potent antioxidative effects than those of anthraquinones on all three assays. The antioxidant activities may relevant to the C-ring double bond and the *ortho*-dihydroxyl of flavonoids and a number of hydroxyl groups (Hou *et al.*, 2004). These functional groups in the compounds **4-6** may be important role for the activity against Cu^{2+}-induced LDL peroxidation.

Table II. Antioxidant activity of compounds **1~6** against Cu^{2+}-mediated in LDL

Compounds	IC_{50} (μM) [a]
Quercetin-3-*O*-α-L-arabinofuranoside (**4**)	3.8
Quercetin-3-*O*-β-D-galactopyranoside (**5**)	3.2
Quercetin-3-*O*-β-D-glucuronopyranoside (**6**)	5.4
Quercetin [b]	1.4
α-Tocopherol [b]	21.3

[a]The IC_{50} values were the 50% inhibition concentration and were calculated from regression lines using six different concentrations in triplicate experiments; [b]The compounds were used as positive control.

ACKNOWLEDGEMENTS

The research was supported by a grant of the Korea Health 21 R & D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG6-01NA01-0002). We are grateful to Korea Basic Science Institute (KBSI) for supplying the NMR spectra, and also grateful to Dr. HyeongKyu Lee at Immunomodulator Research Laboratory in KRIBB for supplying LDL.

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