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Article

Rhodiosin, an Antioxidant Flavonol Glycoside from *Rhodiola rosea*

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The EtOAc fraction of *Rhodiola rosea* ethanolic extracts showed a strong antioxidant activity. Through activity-guided fractionation and purification, we isolated two flavonol glycosides, which were identified as the well known flavonoids, rhodionin (1) and rhodiosin (2). To compare their antioxidant activities, we used an authentic aglycone compound, herbacetin (3). Among the compounds tested, rhodiosin (2) exhibited strong antioxidant activity, with IC_{50} values of 0.21 and 0.15 μM against ·OH and $\cdot O^-_2$, respectively. Rhodiosin (2) (100 mg/kg) reduced MDA content in the liver induced by irradiation when given prior to exposure of γ-radiation.

Key words: *antioxidant, γ-irradiation,* Rhodiola rosea*, rhodiosin, UWLA*

Reactive oxygen species (ROS) often exist in the form of a superoxide anion, hydrogen peroxide, or a hydroxyl radical. All of these forms retain surplus non-repair electrons formed as a result of respiration in aerobic organisms. Uncontrolled ROS are very unstable, and react rapidly with other substances, including DNA, membrane lipids and proteins. ROS are thought to be involved with many human health disorders, such as diabetes mellitus, cancer, Alzheimer's disease, and inflammatory diseases [Sussman and Bulkley, 1990; Debashis *et al*., 1997; Vajragupta *et al*., 2000; Butterfield *et al*., 2002]. Furthermore, ROS are the predominant cause of food decay, which leads to rancidity, toxicity and destruction of biomolecules important in physiological metabolism [Heo *et al*., 2005].

Commonly used synthetic antioxidants, such as buthylated hydroxyanisole (BHA) and buthylated hydroxyl toluene (BHT), are restricted by legislative rules because they are suspected to have certain toxic effects and are possible carcinogenesis. Therefore, there is a growing interest in naturally occurring antioxidants. Polyphenols, including flavonoids, coumarins, xanthones, and phenolic acid, which are widely distributed in plants and food, scavenged free radicals and prevent or reduce oxidative stress.

Rhodiola rosea L., also known as "golden root" or "highland ginseng", belongs to the plant family Crassulaceae. It is widely distributed at high altitudes in the Arctic and mountainous regions. For centuries, *R. rosea* has been used for food and as medicine because it invigorates and detoxifies like ginseng and acanthopanax. *R. rosea* produces various compounds, such as flavonoids [Zapesochnaya and Kurkin, 1983; Kurkin *et al*., 1986; Petsalo *et al*., 2006], terpenoids [Kurkin *et al*., 1986; Ma *et al*., 2006], phenylpropanoids [Tolonen *et al*., 2003; Kurkin *et al*., 2003; Akgul *et al*., 2004], and phenylethanol derivatives [Akgul *et al*., 2004], some of which showed hepatoprotective activities, antiallergic activities, glycosidase inhibitory effects, and antioxidant activities [Ryu *et al*., 1998; Ohsugi *et al*., 1999; Lee *et al*., 2000]. However studies on these compounds have been relatively restricted. For example, although Lee *et al*. [2000] reported the antioxidant activities of phenolic compounds and extracts from *R. rosea*, the scope was limited, focusing only on the scavenging effects against DPPH (1,1-diphenyl-2-picryl hydrazyl) radical.

Here we report the antioxidant activity-guided fractionation of an extract of the root of this plant resulted in the isolation of two flavonol glycosides (**1** and **2**). These compounds were evaluated for antioxidant activities using DPPH, superoxide, hydroxyl radical, and

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Abbreviations: HPLC, high pressure liquid chromatography; MDA, malondialdehyde; NMR, nuclear magnetic resornance; UWLA, ultraweak chemiluminescence

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lipid peroxidation inhibition (MDA) assays in mice after γ-ray irradiation exposure. We also evaluated quantitative analysis of compounds **1** and **2** by HPLC.

Materials and Methods

Materials and chemicals. Commercial herb samples of *R. rosea* L. dried root were purchased from the Korea *Rhodiola rosea* Association, Korea. Herbacetin (**3**) was purchased from Extrasynthese (Genay, France) for comparing biological activities. RPMI 1640, fetal bovine serum (FBS), and streptomycin mixture were purchased from GIBCO BRL (Grand Island, NY). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), phorbol 12-myistate 13-acetate (PMA), 2,7-Dichlorofluorescin diacetate (DCFH-DA), ascorbic acid, dimethylsulfoxide (DMSO), and thiobarbituric acid were obtained from Sigma Chemical Co. Cell viability assay kit (WST-1) was purchased from Dojindo Laboratories (Kumanoto, Japan). All other chemicals were of analytical grade.

Instruments. The following instruments were used: Spectrophotometer (UV-1600PC, Shimadzu, Co., Tokyo, Japan), Spectrofluorometer (FCA, Baxter, Mundelein, IL), and BJL ultraweak chemiluminescence analyzer with a high-sensitivity detector from Jye Horn Co. (Taipei, Taiwan). NMR spectra were recorded on Jeol JNM ECA 500 instrument (^{1}H–NMR, 500 MHz, ^{13}C, 125 MHz).

Extraction and isolation. The dried and powdered root of *R. rosea* (1.0 kg) was extracted two times with ethanol for 10 days at room temperature. The non-soluble precipitate was filtered and removed and the solution was concentrated to dryness under reduced pressure. The residue (380 g) was suspended in H_2O, and then successfully partitioned with *n*-hexane, $CHCl_3$, EtOAc and BuOH. The EtOAc fraction (59.8 g) was subjected to silica gel (230-400 mesh, Merck, 5×40 cm) column chromatograph with a MeOH/$CHCl_3$ solvent system (5:95→100:0) and elutes were separated into three fractions (Fr.1-3) based on the comparison of TLC profile using MeOH/$CHCl_3$ (1:3). Fraction 2 (25.1 g) was chromatographed over silica gel with a MeOH/$CHCl_3$ (5:95→100:0) to yield five fractions (Fr. 2.1-2.5). Fraction 2.2 (0.21 g) was purified by silica gel column chromatography eluted with MeOH/$CHCl_3$ (1:6) to give compound **1** (92 mg). Fraction 2.3 (2.6 g) was separated by a combination of silica gel column chromatography using MeOH/$CHCl_3$ (1:10→1:1) and C_{18} column chromatography (MeOH/H_2O, 4:6) to give compound **2** (89 mg).

Rhodionin (1): green amorphous powder; mp 234-237°C; HREIMS *m/z* 448.1003 (calculated for $C_{21}H_{20}O_{11}$ $[M]^+$, 448.1006); ^{1}H NMR (500 MHz, CD_3OD) δ 8.20 (s, *J*=8.3 Hz, 2H, H-2', 6'), 6.90 (d, *J*=8.6 Hz, 2H, H-3', 5'), 6.64 (s, 1H, H-6), 5.52 (s, 1H, H-1''), 4.14 (s, 1H, H-2''), 3.98 (dd, *J*=3.15Hz, 9.45 Hz, 1H, H-5''), 3.69 (m, 1H, H-3''), 3.49 (t, *J*=9.6 Hz, 1H, H-4''), 1.26 (d, *J*=6.0 Hz, 3H, -CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 177.7 (C-4), 160.7 (C-4), 153.8 (C-5), 151.5 (C-7), 148.7 (C-2), 146.1 (C-9), 137.3 (C-3), 131.0 (C-2', 6'), 128.4 (C-8), 123.8 (C-1'), 116.2 (C-3', 5'), 106.0 (C-10), 100.9 (C-1''), 99.2 (C-6), 73.7 (C-3''), 72.1 (C-4''), 71.7 (C-2''), 71.1 (C-5''), 18.0 (C-6'').

Rhodiosin (2): green amorphous powder; mp 195-199°C; HREIMS *m/z* 610.1518 (calculated for $C_{27}H_{30}O_{16}$ $[M]^+$, 610.1534); ^{1}H NMR (500 MHz, CD_3OD) δ 8.20 (d, *J*=8.85 Hz, 2H, H-2', 6'), 6.91 (d, *J*=9.15 Hz, 2H, H-3', 5'), 6.64 (s, 1H, H-6), 5.56 (s, 1H, H-1''), 4.67 (d, *J*=7.45 Hz, 1H, H-1'''), 4.42 (d, *J*=1.15 Hz, 1H), 4.16 (dd, *J*=3.30 Hz, 9.30 Hz, 1H), 3.90 (d, *J*=11.45 Hz, 1H), 3.75 (m, 2H), 3.67 (t, *J*=9.45 Hz, 1H), 3.42 (m, 1H), 3.37 (m, 2H), 3.35 (m, 1H), 1.28 (d, *J*=6.0 Hz, 3H, -CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 177.7 (C-4), 160.7 (C-4), 153.6 (C-5), 150.9 (C-7), 148.7 (C-2), 145.9 (C-9), 137.2 (C-3), 131.0 (C-2', 6'), 128.5 (C-8), 123.8 (C-1'), 116.3 (C-3', 5'), 106.1 (C-1'''), 105.7 (C-10), 100.5 (C-6), 99.1 (C-1''), 82.3 (C-3''), 77.8 (C-5'''), 77.7 (C-2'''), 75.4 (C-3'''), 72.4 (C-4''), 71.2 (C-4'''), 71.0 (C-2''), 70.8 (C-5''), 62.4 (C-6'''), 18.1 (C-6''').

HPLC apparatus and measurements. The roots of *R. rosea* (1.0 g) were extracted with 10 mL of 95% MeOH for 24 h at room temperature. The extracts used for HPLC analysis were filtered through a 0.45-mm syringe filter (Milipore, MSI, Westboro, MA) and 10 μL of the filtrate was injected to the HPLC system. The HPLC was a Agilent Technology 1200 instrument equipped with a Zorbax SB-Aq C_{18} Column (150×4.6 mm, 5 mm). HPLC conditions were as follows: eluent A, water; eluent B; MeOH; gradient, 0-5 min (20-35% B), 0-25 min (35-50% B), 25-50 min (50-70% B), and then equilibrated with 10% B for 10 min at a flow of 0.7 mL/min with the column temperature at 27°C. The peak was monitored at 280 nm.

Superoxide and hydroxyl radical scavenging activity. A 2.2 mL superoxide radical formation reaction mixture consisted of the following components: 1.0 mL of 2.0 mM lucigenin; 1.0 mL of phosphate buffer saline, pH 7.4; 0.05 mL of 1.0 M arginine; 0.05 mL of 1.4 mM methlglyoxal [Tsai *et al*., 2003]. A hydroxyl radical formation reaction mixture consisted of the following components: 1 mL of 3 mM IBG [dissolved in phosphate buffered saline, pH 7.4]; 0.1 mL of 1.0 mM $FeSO_4$; 1.6 mL of 3% H_2O_2; and 0.05 mL of 10 mM EDTA [Tsai *et al*., 2001]. After gently mixing the components, the each reaction mixture was added to a quartz, round-bottom cuvette in the black-box unit of the ultraweak

chemiluminescence analyzer. The reproducibility of the ^{14}C-light source was <1.0%. After 12 min, the initiation of the radical formation reaction, 100 μM of test compounds was added to the reaction mixture. Varying degrees of sudden drops of counts were observed that represented degrees of radical scavenging abilities.

Cell culture and animals. The human promyelocytic leukemia cell line (HL-60) was cultured routinely in RPMI 1640 medium (Invitrogen-Gibco, Breda, The Netherlands) supplemented with 10% FBS and 10 U/mL penicillin, 10 mg/mL streptomycin, and kept in a humidified atmosphere containing 5% CO_2 at 37°C. C57BL/6 mice were purchased from the Orient Inc (Charles River Technology, Seoul, Korea). The mice were housed in polycarbonate cages and were fed with a standard animal diet and water. The animals were kept at 22-24°C with a 12 h light/dark cycle. Research was conducted according to principles enunciated in the 'Animal Care Act', prepared by the Ministry of Agriculture and Forestry, Republic of Korea.

Cell viability assay. The HL-60 cell viability assays were followed in the manufacturer' manual used the WST-1 reagents (Dojindo, Kumamoto, Japan). The cells were seeded in a 96 well flat-bottom plate at 2×10^5 cells/100 μL/well (n=3). After incubation with test compounds at 50, 100, 150, and 300 mM for 24 h, WST-1 reagent was added to each well and incubation continued at 37°C in a humidified 5% CO_2 air atmosphere for 4 h. The absorbance was measured at 450 nm, with reference absorbance 650 nm on a microplate spectrophotometer (UV-1600PC; Shimadzu Corp., Kyoto, Japan).

Measurement of H_2O_2 and O_2 scavenging activity. ROS production inhibitory activity tests of PMA-induced H_2O_2 and O_2 generation in DMSO-differentiated HL-60 cells were performed with some modification of the published protocol [Bass *et al.*, 1983; Markert *et al.*, 1984; Rosenkranz *et al.*, 1992]. HL-60 cells were suspended in RPMI 1640 medium supplemented with 10% FBS, 100 U/mL penicillin, and 10 μg/mL streptomycin and kept at 37°C in a humidified atmosphere of 5% CO_2. The cells were seeded in 96-well flat-bottom plates at 2×10^5 cells/85 mL/well (n=3). After treatment with test compounds at 6.25, 12.5, 25, 50 and 100 mM for 1h, the cells were stimulated with 100 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma, St. Louis, USA) for 30 min. The cells were then incubated for 15 min after the addition of 5 μg/mL DCFH-DA (Molecular Probes, Eugene, USA) or the addition 5 μL of SOD kit-WST (Dojindo, Kumamoto, Japan) working solution to each well and incubation continued at 37°C in a humidified 5% CO_2 air atmosphere for 30 min. The absorbance was measured at 450 nm, with a reference absorbance of 650 nm on a microplate spectrophotometer (680, Bio-Rad Laboratories, Hercules, CA). The levels of DCF were measured using a fluorescence measurement system (FCA, Baxter, Mundelein, IL) with an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

Animal treatment. A total of 3 to 4-week-old female C57BL/6 mice were used for the experiment. The mice were fed with a standard animal diet and water before the experiment. The animals were kept at 22-24°C with a 12 h light/dark cycle. Mice were divided into three groups of ten mice each and each group was housed in a separate cage. The group I was the control which treated no compound with irradiation. The group II was irradiated only and group III was administered compound **2** (100 mg/kg) orally once daily for 10 consecutive days. All the animals in groups II and III were exposed to an acute dose of gamma radiation (6 Gy/mice) on the whole body. One day after irradiation, all animals in the three groups were anesthetized with CO_2. Compound **2** was dissolved in PBS and this stock solution was diluted further with PBS to obtain the desired concentration. Each mouse was administered compound **2** orally at a daily dose of 100 mg/kg body weight for 10 consecutive days. The II and III groups were exposed to an acute dose of gamma radiation (6 Gy/mice) at the dose rate of 1.12 Gy/min on the 10th day.

Tissue and plasma preparation. Animals were examined at the end of the feeding period and were not given food for 16 h before sacrifice. Under ether anesthesia, blood was drawn by cardiac puncture and plasma was collected by vacutainer tube into EDTA. After centrifugation, plasma was immediately frozen on dry ice and stored at −70°C. Liver tissues were washed in 0.9% NaCl, then dried on filter paper and stored at −60°C. One gram tissue was homogenized in a homogenizer in a volume of 1.15% KCl. The hemolysate was stored −70°C until analysis. The livers were used to obtain 10% homogenates in 0.15 M KCl-10 mM potassium phosphate buffer, pH 7.4. These were centrifuged at 600 g for 10 min and recentrifuged at 13,000×g for 20 min to obtain postnuclear homogenate and postmitochondrial supernatant fractions. MDA standard was prepared by dissolving 1,1,3,3tetraethoxypropane (TEP) in 50 mL of water to give a 0.5 mM stock solution. The resulting MDA standard of 100 nmol/mL was further diluted with 1% sulfuric acid to yield final concentrations of 12.5, 25, 50, and 100 nmol/mL to get the standard curve for the estimation of total MDA.

Gamma-ray irradiation. Gamma irradiation was performed using a ^{137}Cs γ-rays gamma cell (Gammacell 40 exactor, MDS Nordion international Inc., Ontario) in the Advanced Radiation Technology Institute, the Korea

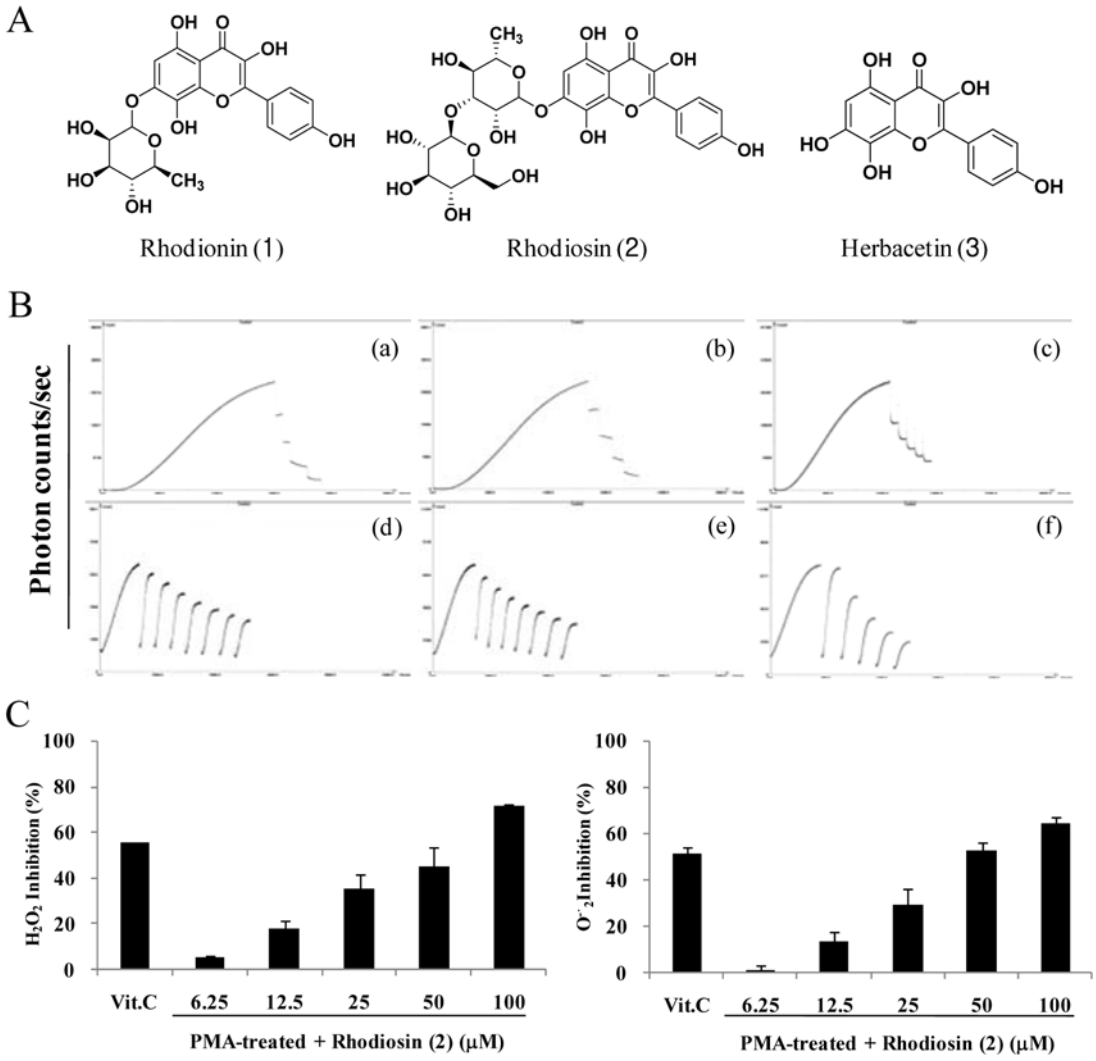

Fig. 1. (A) The chemical structures of compounds 1-3. (B) UWLA spectrum of superoxide and hydroxyl radicals scavenging activities of compounds 1-3: (a-c) Spectra of the superoxide radical scavenging effects of rhodionin (a), rhodiosin (b), and herbacetin (c). (d-f) Spectra of the hydroxyl radicals scavenging effects of rhodionin (d), rhodiosin (e), and herbacetin (f). (C) Effect of rhodiosin (**2**) on the generation of H_2O_2 and O^-_2 in HL-60 cells.

Atomic Energy Research Institute (Jeongeup, Korea). Mice were irradiated at room temperature with at a dose rate of 6 Gy (1.12 Gy/min). In these experiments, the mice were put under whole-body irradiation.

MDA content in tissues. Malondialdehyde (MDA) levels were determined according to the method of Ohkawa *et al*. [1979]. Samples of less than 0.2 mL of 10% (w/v) tissue homogenate were added to 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid solution, and 1.5 mL of 0.8% aqueous solution of TBA. The mixture was brought to 4.0 mL with distilled water and heated at 95°C for 60 min. After cooling, 1.0 mL of distilled water and 5.0 mL of butanol:pyridine (15:1, v/v) were added and shaken vigorously. After centrifugation at 3,500×g for 10 min, the organic layer was removed and its absorbance at 532 nm was measured. Total TBA-reactive materials were expressed as MDA, using a molar extinction coefficient for MDA of 1.56×10^5/cm/M. MDA level was expressed as nmol/mg/protein.

MDA content in plasma. Plasma MDA levels were measured by a spectrophotometric method [Beuge and Aust, 1978; Cynamon *et al.,* 1985]. Briefly, 0.5 mL plasma was shaken with five volumes of 20% (w/v) trichloroacetic acid (TCA), 0.375% (w/v) thiobarbituric acid (TBA) and 0.25 N hydrochloric acid. The mixture was heated for 30 min in a boiling water bath, followed by rapid cooling. It was then extracted into 0.5 mL of n-butanol in a separation tube. MDA content in plasma was determined by the absorbance at 535 and at 520 nm. Total thiobarbituric acid (TBA) reactive materials were expressed as MDA, using a molar extinction coefficient for MDA of 1.56×10^5/cm/M. Plasma MDA levels were

Table 1. Radical scavenging effects of compounds 1-3

Compounds	UWLA		ROS inhibition in HL-60 cell	
	IC_{50} (μM)		IC_{50} (μM)	
	•OH	$O^{\cdot-}_2$	H_2O_2	$O^{\cdot-}_2$
Rhodionin (**1**)	0.22	0.24	-	-
Rhodiosin (**2**)	0.21	0.15	59.5	47.3
Herbacetin (**3**)	0.48	0.5	-	-

Table 2. Effect of rhodiosin (2) on liver and plasma MDA levels in irradiated mice

Groups	Rhodiosin (**2**) 100 mg/kg body weight	
	Liver MDA level (μmol/mL)	Plasma MDA level (μmol/mL)
Normal	5.5±1.4	1.35±0.6
Irradiation	19.8±2.9	2.89±0.2
Irradiation+Rhodiosin (**2**)	12.5±2.3*	±0.5*

n=10, results were presented as means±SD. *p<0.05 versus irradiation.

expressed as nmol/mg/protein.

Statistical analysis. All experiments were repeated at least three times. Results are expressed as mean±SD (standard deviation). The statistical significance of differences between groups was evaluated for two parallel experiments using the student's t-test.

Results and Discussion

Initially, the dried roots of *R. rosea* were extracted with 80% ethanol. Among the extracts, the EtOAc-soluble extract showed the most scavenging effect on DPPH-radicals (95% electron donating ability at 50 μg/mL). The EtOAc-soluble fraction of the ethanolic extract yielded two flavonol-glycosides by chromatography. Two compounds (**1** and **2**) are known compounds and were identified by their spectroscopic data as rhodionin (**1**) and rhodiosin (**2**). All spectroscopic data were compared with and matched published values [Yu *et al*., 1993; Lee *et al*., 2002] (Fig. 1A).

In this study, three flavonols, two isolated flavonol glycosides (**1** and **2**) and a commercial available flavonol, herbacetin (**3**), were evaluated for their scavenging effects in various antioxidant assays. As shown in Fig. 1B and Table 1, all flavonols (**1-3**) scavenged the hydroxyl radical with IC_{50} values of 0.22, 0.21, and 0.48 μM, respectively, as determined by UWLA (ultraweak chemiluminescence) analyzer which is monitors the production of a specific oxygen-derived reactive species. Generally, hydroxyl radicals were generated by the Fe(III)-catalyzed Fenton reaction. Therefore, hydroxyl radical scavenging activities may be influenced by the electron-donating and metal-chelating abilities of possessing hydroxyl groups. These compounds did not change the spectral shift on UV/Vis spectrum (data not shown). These compounds (**1-3**) also showed antioxidant effects on the superoxide radical, which was generated by lucigenin-based chemiluminescens system. Diglycoside flavonol **2** exhibited three fold more effective scavenging activity with an IC_{50} value of 0.15 μM, compared to herbacetin (IC_{50}=0.5 μM) (Fig. 1B and Table 1). Rhodionin (**1**) showed two fold more radical scavenging activity (IC_{50}=0.24 μM) than herbacetin (**3**).

The inhibition effect of rhodiosin (**2**) on exogenous cytoplasmic ROS-catalyzed oxidation of DCFH-DA in HL-60 cells is shown in Fig. 1C. Rhodiosin (**2**) exhibited moderate activity with IC_{50} values of 59.5 μM against hydrogen peroxide and 47.3 μM against superoxide anion and has a low cytotoxicity (CC_{50}=500 μM). In exogenous cytoplasmic antioxidants, the antioxidant efficacy depends on the ability to penetrate the cell membranes.

We also investigated whether rhodiosin (**2**) could reduced the malondialdehyde (MDA) levels in liver and plasma. Some studies report that irradiation increases the formation of malonedialdehyde (MDA), the main product of lipid peroxidation, in the liver [Kergonou *et al*., 1981; Varshiney and Kale, 1990]. In this study, when mice were exposed to a single total body γ-irradiation dose (10.0 Gy), lipid peroxidation significantly increased in the liver. As shown in Table 2, the level of liver MDA in the γ-irradiation-plus 100 mg/kg rhodiosin (**2**) groups (12.5± 2.3 μmol/mL) significantly decreased compared to the γ-irradiation-only groups (19.8±2.9 μmol/mL). The plasma MDA content in the irradiation group (2.89±0.2 μmol/mL) was higher than in the group without irradiation (1.35±0.6 μmol/mL) and in the group fed rhodiosin (**2**)

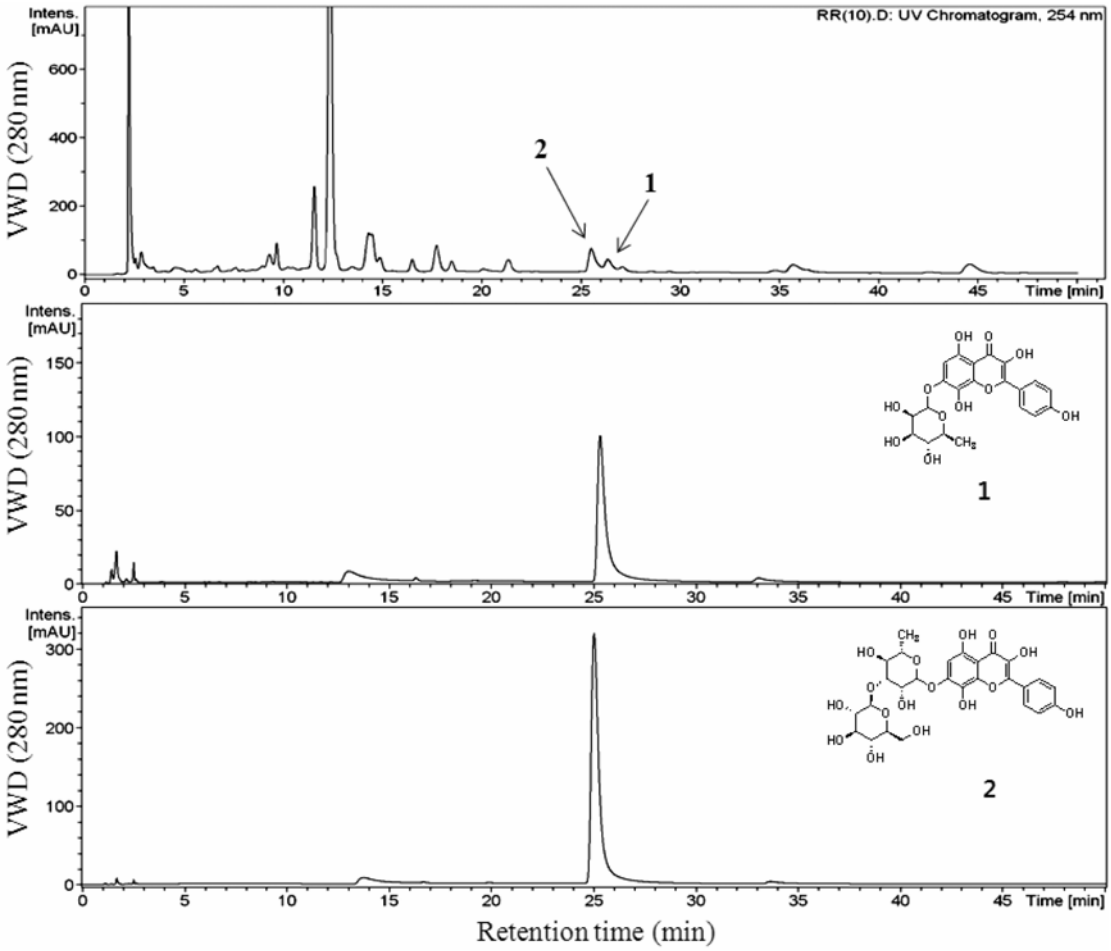

Fig. 2. Representative HPLC chromatograms of ethanol extract of *R. rosea* and compounds. HPLC analysis was performed on a C_{18} column (4.6×150 mm, 5 mm) and an Agilent VWD UV detector at 280 nm. Mobile phase was a 0 min gradient of 20-70% MeOH with water: flow rate 0.7 mL/min: Samples: 10 mg/mL of rhodionin (**1**), rhodiosin (**2**).

Table 3. Quantitative analysis data of compounds 1 and 2 in *R. rosea* ethanol extract

Compound	t_R (min)[a]	Amount (values in μg/10 mg extract)	Calibration curve
Rhodionin (**1**)	26.33	170.12	y=2940x+27.91 (R^2>0.991)
Rhodiosin (**2**)	25.51	189.32	y=9462.5x–618.75 (R^2>0.999)

[a]Elution on the C_{18} column (retention time)

(2.23±0.5 μmol/mL). Rhodiosin (**2**) clearly decreased lipid peroxidation in the liver and plasma induced by total body γ-irradiation.

Furthermore, *R. rosea* contains many secondary metabolites. Of them, isolated compounds (**1** and **2**) were investigated through quantitative analysis using HPLC analysis (C_{18} column), as shown in Fig. 2. The amounts of **1** and **2** were 170.12 μg/10 mg and 189.32 μg/10 mg in the ethanol extract of *R. rosea* roots (Table 3).

In summary, flavonol glycosides **1** and **2** having antioxidant activity were isolated from the EtOAc fraction of *R. rosea* extract. Isolated compounds (**1** and **2**) had stronger antioxidant activities against superoxide radical and hydroxyl radical than herbacetin (**3**). Glycoside groups are a critical functionality for cytoplasm antioxidants. Rhodiosin (**2**) has clear antioxidant properties and is likely to be a valuable drug for protection against γ-irradiation and can be used as an antioxidant against oxidative stress.

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References

Akgul Y, Ferreira D, Abourashed EA, and Khan IA (2004) Lotaustralin from *Rhodiola rosea* roots. *Fitoterapia* **75**, 612-614.

Bass DA, Parce JW, Dechatelet LR, Szejda P, Seeds MC, and Thomas M (1983) Flow cytometric studies of oxidative product formation by neutrophils: A graded response to membrane stimulation. *J Immunol* **130**, 1910-1917.

Beuge JA and Aust SD (1978) Microsomal lipid peroxidation. *Method Enzymol* **12**, 302-310.

Butterfield DA, Castenga A, Pocernich CB, Drake J, Scapagnini G, and Calabrese V (2002) Nutritional approaches to combat oxidative stress in Alzheimer's disease. *J Nurt Biochem* **13**, 444-461.

Cynamon HA, Isenberg JN, and Nguyen CH (1985) Erythrocyte malondialdehyde release *in vitro*: a functional measure of vitamin E status. *Clin Chim Acta* **151**, 169-176.

Debashis DD, Bhattacharjee BM, and Banerjee RK (1997) Hydroxyl radicals is the major causative factor in stress-induced gastric ulceration. *Free Radical Bio Med* **23,** 8-18.

Heo SJ, Park EJ, Lee KW, and Jeon YJ (2005) Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Technol* **96**, 1613-1623.

Kergonou JF, Bernard P, Braquet M, and Rocquet G (1981) Effect of whole-body gamma irradiation on lipid peroxidation in rat tissues. *Biochimie* **63**, 555-559.

Kurkin VA (2003) Phenylpropanoids from medicinal plants: distribution, classification, structural analysis and biological activity. *Chem Nat Comp* **39**, 123-153.

Kurkin VA, Zapesochanaya GG, Gorbunov YN, Nukhimovskii EL, Shreter AI, and Shchavlinskii AN (1986) Chemical investigations on some species of Rhodiola L. and Sedum L. genera and problems of their chemotaxonomy. *Rastitelney Resursy* **22**, 310-319.

Kurkin VA, Zapesochanays GG, and Shchavlinskii AN (1984) Flavonoids of the rhizomes of *Rhodiola rosea* III. *Chem Nat Comp* **20**, 367-368.

Lee MW, Lee YA, Park HM, Toh SH, Lee EJ, Jang HD, and Kim YH (2000) Antioxidative phenolic compounds from the roots of *Rhoiola sachalinensis A*. Bor. *Arch Pharm Res* **23**, 455-458.

Ma G, Li W, Dou D, Chang X, Bai H, Satou T, Li J, Sun D, Kang T, Nikaido T, and Koike K (2006) Rhodiolosides A-E, monoterpene glycosides from *Rhodiola rosea*. *Chem Pharm Bull* **54**, 1229-1233.

Markert M, Andrews PC, and Babior BM (1984) Measurement of O_2^- production by human neutrophils. The preparation and assay of NADPH oxidase-containing particles from human neutrophils. *Method Enzymol* **105**, 358-365.

Ohkawa H, Ohishi N, and Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**, 351-358.

Ohsugi M, Fan W, and Hase K (1999) Active-oxygen scavenging activity of traditional nourishing-tonic herbal medicines and active constituents of *Rhodiola sacra*. *J Ethnopharmacol* **67**, 111-119.

Petsalo A, Jalonen J, and Tolonen A (2006) Identification of flavonoids of *Rhodiola rosea* by liquid chromatography-tandem mass spectrometry. *J Chromatogr A* **1112**, 224-231.

Rosenkranz AR, Schmaldienst S, Stuhlmeier KM, Chen W, Knapp W, and Zlabinger GJ (1992) A microplate assay for the detection of oxidative products using 2,7-dichlorofluorescin-diacetate. *J Immunol Methods* **156**, 39-45.

Ryu KY, Kang, WS, and Lee MW (1998) Antioxidant effects of the rhizome *Rhodiola sachalinensis*. *Yakhak Hoeji* **42**, 312-318.

Sussman MS and Bulkley GB (1990) Oxygen derived free radicals in reperfusion. *Method Enzymol* **186**, 711-723.

Tolonen A, Pakonen M, Hohtola A, and Jalonen J (2003) Phenylpropanoid glycosides from *Rhodiola rosea*. *Chem Pharm Bull* **51**, 467-470.

Tsai, H, Chang C, and Chiou F (2001) Rapid and Specific detection of hydroxyl radical using an ultraweak chemiluminescence analyzer and low-level chemiluminescence emitter: application to hydroxyl radical-scavenging ability of aqueous extracts of food constituents. *J Food Drug Anal* **49**, 2137-2141.

Tsai H, Chang C, and Chiou F (2003) Improved superoxide-generating system suitable for the assessment of the superoxide-scavenging ability of aqueous extracts of food constituents using ultraweak chemiluminescence. *J Food Drug Anal* **51**, 58-62.

Vajragupta O, Boonchoong P, and Wongkrajang Y (2000) Comparative quantitative structure-activity study or radical scavengers. *Bioorg Med Chem* **8**, 2617-2628.

Varshiney R and Kale RK (1990) Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int J Radiat Biol* **58**, 733-743.

Yu WS, Chen XM, Li H, and Yang L (1993) Polyphenols from *Rhodiola crenulara*. *Planta Med* **59**, 80-83.

Zapesochnaya GG and Kurkin VA (1983) The flavonoids of the rhizomes *Rhoidiola rosea*. *Khim Prir Soedin* **19**, 23-32.