



Protective Activity Against Oxidative Stress of Plants Indigenous to Korea

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ABSTRACT. We have screened the cytoprotective effect against H₂O₂ and γ -ray radiation induced oxidative stress from 32 Korean plants. *Betula ermani* var. *saitoana* (caulis, leaves), *Rosa wichuriana* (caulis), *Sorbus commixta* (caulis), *Weigela florida* (leaves), *Cirsium rhinoceros* (whole plant), and *Viburnum erosum* (caulis) were found to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and intracellular reactive oxygen species (ROS). As a result, extracts of six plants reduced cell death of Chinese hamster lung fibroblast (V79-4) cells induced by H₂O₂ treatment. In addition, these extracts protected cell death of V79-4 cells damaged by γ -ray radiation. In addition, these extracts scavenged ROS generated by radiation. Taken together, the results suggest that *Betula ermani* var. *saitoana*, *Rosa wichuriana*, *Sorbus commixta*, *Weigela florida*, *Cirsium rhinoceros*, and *Viburnum erosum* protect V79-4 cells against oxidative damage by radiation through scavenging ROS.

Keywords: γ -ray radiation, Oxidative stress, Reactive oxygen species.

INTRODUCTION

The potential application of radioprotectors in the event of planned exposure or radiation accidents has been investigated from the beginning of the nuclear era (Weiss and Simic, 1988). Exposure to ionizing radiation is believed to cause cell damage via the production of reactive oxygen species to induce oxidative stress (Lin *et al.*, 2003), and apoptotic signaling via mitochondrial pathway involving caspase-9 and -3 activation (Verheij and Bartelink, 2000; Fei and El-Deiry, 2003). The role of reactive oxygen species in radiation injury and the potential of antioxidants to reduce these deleterious effects have been studied in animal models for more than 50 years (Weiss and Landauer, 2000). And radioprotective agents minimize this damage by reducing

generation of free radicals (Jonathan *et al.*, 1999). It has also been considered possible that radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissue. Early investigators attempted to use radioprotectors to help elucidate the mechanism of interaction of radiation and molecules of biological importance. It is suggested that both radiation injury and oxygen poisoning occur through the formation of ROS (Gerschman *et al.*, 1954). Sulfhydryl agents such as cysteine, glutathione, β -mercaptoethylamine (cysteamine), and other antioxidants shown to protect mice against the lethal effects of radiation could also increase survival of mice exposed to high oxygen tension. Although many synthetic compounds for this purpose showed good radioprotection in *in vitro* studies, most of them failed *in vivo* application because of their acute toxicity to the mammalian system (Gandhi and Nair, 2004). The herbal drugs can be offered as alternative to the synthetic chemicals (Jagetia and Baliga, 2003). Because plants have been used by mankind for the treatment of various ailments since the time

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immemorial and there is strong evidence that co-administration of herbal medicines with chemotherapy or radiation therapy can reduce the side effects of these treatments, improving the condition of patients (Song *et al.*, 2003). Therefore, screening of herbal drugs offers a major avenue for new radioprotector discovery. Increased understandings of the interrelationship between oxygen effects and the radiation exposure lead to a rational application of naturally occurring antioxidants (Weiss and Landauer, 2000).

In present study, we screened the antioxidative effect of plant extracts and in addition, from the selected antioxidative plant extracts, it was investigated whether it may show protective effect against γ -ray radiation.

MATERIALS AND METHODS

Plant material and its extract

The plant materials were purchased or obtained from Korea Institute of Bioscience and Biotechnology (KRIBB) and Jeju-do Agricultural Research & Extension Service, respectively. Voucher specimens were deposited in the KRIBB and Jeju-do Agricultural Research & Extension Service.

Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical and 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) were purchased from Sigma Chemical Company, St. Louis, MO, USA. The other chemicals and reagents were of analytical grade.

Cell culture

It is reported that lung is an organ sensitive to oxidative stress (Pryor *et al.*, 1998; Murray *et al.*, 2004). To study the effect of plant extracts on oxidative stress, we used Chinese hamster lung fibroblasts (V79-4 cells). The V79-4 cells from the American type culture collection, were maintained at 37°C in an incubator with a humidified atmosphere of 5% CO₂ and cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 µg/ml) and penicillin (100 units/ml).

Irradiation

Cells were exposed to γ -ray from a ⁶⁰Co γ -ray source (MDS Nordion C-188 standard source, located in Cheju National University, Jeju, Korea).

DPPH radical scavenging activity

Various concentrations of plant extracts were added to a 1 × 10⁻⁴ M solution of DPPH in methanol, and the

reaction mixture was shaken vigorously. After 1 h, the amount of residual DPPH was determined at 520 nm using a spectrophotometer (Lo *et al.*, 2004).

Intracellular reactive oxygen species measurement

The DCF-DA method was used to detect the intracellular ROS level (Rosenkranz *et al.*, 1992). DCF-DA diffuses into cells, where it is hydrolyzed by intracellular esterase to polar 2',7'-dichlorodihydrofluorescein. This non-fluorescent fluorescein analog gets trapped inside the cells and is oxidized by intracellular oxidants to a highly fluorescent, 2',7'-dichlorofluorescein. The V79-4 cells were seeded in a 96 well plate. Sixteen hours after plating, the cells were treated with plant extracts and

Table 1. The list of species used in these experiments

Scientific name	Family	Used part
<i>Tetragonia tetragonoides</i> O. Kuntze	Aizoaceae	Whole plant
<i>Lepiota procera</i>	Agaricaceae	Whole plant
<i>Melia azedarah</i> var. <i>japonica</i>	Meliaceae	Cortex
<i>Smilax china</i> L.	Liliaceae	Roots
<i>Smilax china</i> L.	Liliaceae	Fruits
<i>Lespedeza cuneata</i>	Leguminosae	Whole plant
<i>Spiraea prunifolia</i> var. <i>simpliciflora</i>	Rosaceae	Caulis
<i>Elaeocarpus sylvestris</i>	Elaeocarpaceae	Fruits
<i>Elaeocarpus sylvestris</i>	Elaeocarpaceae	Bark
<i>Elaeocarpus sylvestris</i>	Elaeocarpaceae	Leaves, twig
<i>Boehmeria pannonia</i>	Urticaceae	Whole plant
<i>Euphorbia jolkini</i>	Euphorbiaceae	Whole plant
<i>Peucedanum japonicum</i>	Umbelliferae	Roots
<i>Platycarya strobilacea</i>	Juglandaceae	Twig
<i>Mallotus japonicus</i>	Euphorbiaceae	Leaves
<i>Morus bombycis</i>	Moraceae	Caulis
<i>Morus bombycis</i>	Moraceae	Leaves
<i>Morus bombycis</i>	Moraceae	Bark
<i>Morus bombycis</i>	Moraceae	Pith
<i>Ligustrum lucidum</i> <i>Rubus</i>	Oleaceae	Caulis
<i>Gynostemma pentaphyllum</i>	Cucurbitaceae	Whole plant
<i>Empetrum nigrum</i>	Empetraceae	Herba
<i>Cornus controversa</i>	Cornaceae	Leaves
<i>Cimicifuga acerina</i>	Ranunculaceae	Whole plant
<i>Kalopanax pictus</i>	Araliaceae	Leaves
<i>Robinia pseudoacacia</i>	Leguminosae	Leaves, Twig
<i>Hemistepta lyrata</i>	Compositae	Whole plant
<i>Oenothera laciniata</i> Hill.	Onagraceae	Whole plant
<i>Stephanandra incisa</i>	Rosaceae	Caulis
<i>Maackia fauriei</i>	Leguminosae	Caulis
<i>Betula ermani</i> var. <i>saitoana</i>	Betulaceae	Caulis
<i>Betula ermani</i> var. <i>saitoana</i>	Betulaceae	Leaves
<i>Rosa wichuraiana</i>	Rosaceae	Caulis
<i>Sorbus commixta</i>	Rosaceae	Leaves
<i>Sorbus commixta</i>	Rosaceae	Caulis
<i>Weigela florida</i>	Caprifoliaceae	Leaves
<i>Aster hayatae</i>	Compositae	Whole plant
<i>Cirsium rhinoceros</i>	Compositae	Whole plant
<i>Hepatica insularis</i>	Ranunculaceae	Whole plant
<i>Viburnum erosum</i>	Caprifoliaceae	Caulis

1 hour later, 1 mM H₂O₂ or γ -ray radiation at 10 Gy was added to the plate. The cells were incubated for an additional 30 min at 37°C. After addition of 25 μ M of DCF-DA solution, the fluorescence of 2',7'-dichlorofluorescein was detected at 485 nm excitation and at 535 nm emission using a PerkinElmer LS-5B spectrofluorometer.

Cell viability

The effect of plant extracts on the viability of the V79-4 cells was determined using the [3-(4,5-dimethylthiazol-

2-yl)-2,5-diphenyltetrazolium] bromide (MTT) assay, which is based on the reduction of a tetrazolium salt by mitochondrial dehydrogenase in the viable cells (Carmichael *et al.*, 1987). To determine the effect of plant extracts on the viability of V79-4 cells on H₂O₂ or γ -ray radiation, cells were seeded in a 96 well plate at 1×10^5 cells/ml. Sixteen hours after plating, cells were treated with 10 μ g/ml of plant extracts for 1 h. Plates were treated 1 mM H₂O₂ or irradiated at 10 Gy and the plate was incubated at 37°C for 24 h and the cell viability was measured using MTT test. Fifty μ l of the MTT stock

Table 2. Effect of Korean plant extracts on scavenging DPPH

Scientific name	Concentration (μ g/ml)		
	0.1	1	10
<i>Tetragonia tetragonoides</i> O. Kuntze	0	0	0
<i>Lepiota procera</i>	0	0	0
<i>Melia azedarah</i> var. <i>japonica</i>	0	4.2 \pm 1.1	35.3 \pm 1.3
<i>Smilax china</i> L. (Roots)	0	0	36.9 \pm 2.2
<i>Smilax china</i> L. (Fruits)	0	0	20.1 \pm 1.6
<i>Lespedeza cuneata</i>	0	0	11.3 \pm 2.7
<i>Spiraea prunifolia</i> var. <i>simpliciflora</i>	0	0	27.3 \pm 2.3
<i>Elaeocarpus sylvestris</i> (Fruits)	0	0	2.1 \pm 0.4
<i>Elaeocarpus sylvestris</i> (Bark)	0	0	38.0 \pm 2.3
<i>Elaeocarpus sylvestris</i> (Leaves, twig)	0	2.7 \pm 0.2	39.7 \pm 3.5
<i>Boehmeria pannosa</i>	0	3.3 \pm 0.8	48.1 \pm 2.6
<i>Euphorbia jolkini</i>	2.6 \pm 0.5	5.2 \pm 1.4	41.7 \pm 2.4
<i>Peucedanum japonicum</i>	0	0	0
<i>Platycarya strobilacea</i>	0	3.8 \pm 1.7	32.5 \pm 3.3
<i>Mallotus japonicus</i>	4.7 \pm 1.3	8.1 \pm 1.8	37.4 \pm 3.4
<i>Morus bombycis</i> (Caulis)	0	2.7 \pm 0.2	18.6 \pm 1.2
<i>Morus bombycis</i> (Leaves)	0	1.7 \pm 1.2	15.0 \pm 1.7
<i>Morus bombycis</i> (Bark)	0	0	14.7 \pm 1.2
<i>Morus bombycis</i> (Pith)	1.6 \pm 0.8	5.5 \pm 1.2	45.4 \pm 2.3
<i>Ligustrum lucidum</i> Rubus	0	0	16.9 \pm 1.4
<i>Gynostemma pentaphyllum</i>	0	0	3.7 \pm 0.4
<i>Empetrum nigrum</i>	0	0	20.4 \pm 2.8
<i>Cornus controversa</i>	0	2.4 \pm 0.3	41.6 \pm 3.3
<i>Cimicifuga acerina</i>	0	0	11.1 \pm 3.6
<i>Kalopanax pictus</i>	0	0	37.7 \pm 3.9
<i>Robinia pseudoacacia</i>	0	3.1 \pm 0.8	24.7 \pm 1.8
<i>Hemistepta lyrata</i>	2.9 \pm 0.8	2.7 \pm 0.8	12.9 \pm 2.5
<i>Oenothera laciniata</i> Hill.	1.8 \pm 1.2	2.8 \pm 1.2	27.4 \pm 2.3
<i>Stephanandra incisa</i>	0	3.6 \pm 1.2	46.4 \pm 2.3
<i>Maackia fauriei</i>	0	0	4.7 \pm 0.3
<i>Betula ermani</i> var. <i>saitoana</i> (Caulis)	1.7 \pm 0.8	7.3 \pm 1.3	62.4 \pm 1.9*
<i>Betula ermani</i> var. <i>saitoana</i> (Leaves)	0	13.7 \pm 1.1	65.3 \pm 2.2*
<i>Rosa wichuraiana</i>	0	3.1 \pm 1.1	52.1 \pm 0.8*
<i>Sorbus commixta</i> (Leaves)	0	1.2 \pm 0.2	22.9 \pm 1.2
<i>Sorbus commixta</i> (Caulis)	1.1 \pm 0.2	7.9 \pm 0.7	63.4 \pm 0.5*
<i>Weigela florida</i>	0	6.8 \pm 1.4	62.1 \pm 1.7*
<i>Aster hayatae</i>	0	0	10.5 \pm 0.3
<i>Cirsium rhinoceros</i>	0	1.7 \pm 0.3	53.3 \pm 1.2*
<i>Hepatica insularis</i>	0	0	7.1 \pm 0.6
<i>Viburnum erosum</i>	0	5.9 \pm 1.1	53.8 \pm 1.1*

The amount of DPPH radicals was determined spectrophotometrically. The measurements were made in triplicate and values are expressed as mean \pm S.E. *significantly different from H₂O₂ treated cells (p < 0.05).

solution (2 mg/ml) was then added to each well to attain a total reaction volume of 200 μ l. After incubating for 4 h, the plate was centrifuged at 800 \times g for 5 min and the supernatants were aspirated. The formazan crystals in each well were dissolved in 150 μ l dimethylsulfoxide (DMSO) and the A_{540} was read on a scanning multi-well spectrophotometer.

Statistical analysis

All the measurements were made in triplicate and all values were represented as mean \pm S.E. The results

were subjected to an analysis of the variance (ANOVA) using the Tukey test to analyze the difference. $p < 0.05$ were considered significantly.

RESULTS AND DISCUSSION

A large number of plants contain antioxidant phytochemicals reported to be radioprotective in various model systems. Antioxidants interfere with the initial stage of apoptosis by ROS (Salganik, 2001), as well as later membrane lipid peroxidation, which is characteris-

Table 3. Effect of Korean plant extracts on scavenging intracellular ROS induced by H_2O_2

Scientific name	Concentration (μ g/ml)		
	0.1	1	10
<i>Tetragonia tetragonoides</i> O. Kuntze	16.2 \pm 1.5	25.3 \pm 2.1	39.9 \pm 1.6
<i>Lepiota procera</i>	38.1 \pm 2.3	38.0 \pm 2.5	60.7 \pm 1.3
<i>Melia azedarah</i> var. <i>japonica</i>	0	0	66.8 \pm 1.5
<i>Smilax china</i> L. (Roots)	0	0	67.7 \pm 2.2
<i>Smilax china</i> L. (Fruit)	22.1 \pm 1.3	21.6 \pm 1.6	51.3 \pm 1.6
<i>Lespedeza cuneata</i>	11.5 \pm 0.8	22.3 \pm 1.3	50.1 \pm 2.7
<i>Spiraea prunifolia</i> var. <i>simpliciflora</i>	12.5 \pm 0.6	19.6 \pm 1.1	81.0 \pm 2.3
<i>Elaeocarpus sylvestris</i> (Fruits)	0	0	12.2 \pm 0.4
<i>Elaeocarpus sylvestris</i> (Bark)	0	0	74.5 \pm 2.3
<i>Elaeocarpus sylvestris</i> (Leaves, twig)	20.8 \pm 1.4	33.4 \pm 0.2	91.0 \pm 3.5
<i>Boehmeria pannosa</i>	0	3.1 \pm 0.8	72.4 \pm 2.6
<i>Euphorbia jolkini</i>	1.4 \pm 0.5	29.1 \pm 1.4	87.6 \pm 2.4
<i>Peucedanum japonicum</i>	0	0	2.0 \pm 1.3
<i>Platycarya strobilacea</i>	3.5 \pm 0.5	26.4 \pm 1.7	84.1 \pm 3.3
<i>Mallotus japonicus</i>	10.0 \pm 1.1	13.3 \pm 1.8	80.0 \pm 3.4
<i>Morus bombycis</i> (Caulis)	12.9 \pm 1.3	16.7 \pm 0.2	74.4 \pm 1.2
<i>Morus bombycis</i> (Leaves)	17.5 \pm 1.3	17.1 \pm 1.2	57.4 \pm 1.7
<i>Morus bombycis</i> (Bark)	0	0	6.1 \pm 1.2
<i>Morus bombycis</i> (Pith)	0	0	82.7 \pm 2.3
<i>Ligustrum lucidum</i> Rubus	0	0	70.7 \pm 1.4
<i>Gynostemma pentaphyllum</i>	5.3 \pm 1.3	16.7 \pm 0.8	16.7 \pm 0.4
<i>Empetrum nigrum</i>	16.1 \pm 1.2	18.6 \pm 1.3	68.8 \pm 2.8
<i>Cornus controversa</i>	22.2 \pm 1.6	26.0 \pm 0.3	83.0 \pm 3.3
<i>Cimicifuga acerina</i>	27.6 \pm 1.1	33.2 \pm 1.3	54.1 \pm 3.6
<i>Kalopanax pictus</i>	39.1 \pm 0.3	41.0 \pm 1.5	88.9 \pm 3.9
<i>Robinia pseudoacacia</i>	35.8 \pm 1.3	36.7 \pm 0.8	54.0 \pm 1.8
<i>Hemistepta lyrata</i>	40.9 \pm 0.8	46.1 \pm 0.8	70.7 \pm 2.5
<i>Oenothera laciniata</i> Hill.	8.8 \pm 1.2	10.0 \pm 1.2	59.3 \pm 2.3
<i>Stephanandra incisa</i>	3.8 \pm 0.3	22.3 \pm 1.2	72.1 \pm 2.3
<i>Maackia fauriei</i>	0	0	16.7 \pm 1.5
<i>Betula ermani</i> var. <i>saitoana</i> (Caulis)	36.0 \pm 1.8	60.3 \pm 3.3	76.9 \pm 1.1*
<i>Betula ermani</i> var. <i>saitoana</i> (Leaves)	30.5 \pm 1.3	56.1 \pm 1.1	74.3 \pm 1.9*
<i>Rosa wichuraiana</i>	30.0 \pm 1.4	57.7 \pm 1.1	87.6 \pm 1.2*
<i>Sorbus commixta</i> (Leaves)	13.0 \pm 1.6	25.4 \pm 0.2	68.8 \pm 1.2
<i>Sorbus commixta</i> (Caulis)	23.1 \pm 0.2	42.2 \pm 0.7	66.7 \pm 1.6*
<i>Weigela florida</i>	17.1 \pm 2.3	55.4 \pm 1.4	74.3 \pm 1.3*
<i>Aster hayatae</i>	54.3 \pm 1.7	56.1 \pm 0.2	70.7 \pm 0.3
<i>Cirsium rhinoceros</i>	34.2 \pm 2.3	55.6 \pm 0.3	74.7 \pm 1.5*
<i>Hepatica insularis</i>	10.4 \pm 1.3	17.8 \pm 1.3	49.7 \pm 0.4
<i>Viburnum erosum</i>	19.6 \pm 1.8	42.1 \pm 1.1	62.0 \pm 1.6*

The intracellular ROS was detected by DCF-DA method. The measurements were made in triplicate and values are expressed as mean \pm S.E. *significantly different from H_2O_2 treated cells ($p < 0.05$).

tic of radiation induced apoptosis (McClain *et al.*, 1995). From tested 32 Korean plants (Table 1), *Betula ermani* var. *saitoana* (caulis, leaves), *Rosa wichuraiana* (caulis), *Sorbus commixta* (caulis), *Weigela florida* (leaves), *Cirsium rhinoceros* (whole plant), and *Viburnum erosum* (caulis) were found to scavenge DPPH radical, showing at 10 µg/ml 62%, 65%, 52%, 63%, 62%, 53%, and 54%, respectively (Table 2) and intracellular ROS, showing at 10 µg/ml 77%, 74%, 88%, 67%, 74%, 75%, and 62 % respectively (Table 3). As a result, extracts of six plants reduced cell death of V79-4 cells induced by H₂O₂ treatment, showing the cell viability of 88%, 86%, 93%, 89%, 89%, 91%, and 90% respectively, compared to cell viability of 81% in H₂O₂ treated cells (Table 4). These extracts protected cell death of V79-4 cells damaged by γ-ray radiation, showing the cell viability of 90%, 91%, and 95%, 88%, 89%, 91%, and 91% respectively, compared to cell viability of 84% in 10 Gy radiated cells (Table 5) and scavenged ROS generated by radiation, showing the percentage of intracellular ROS generation of 68%, 60%, 80%, 74%, 65%, 56%, and 81% respectively, compared to 100% in 10 Gy radi-

Table 4. Protective effect of Korean plant extracts upon H₂O₂ induced oxidative damage of V79-4 cells

Scientific name	Cell viability (%)
<i>Betula ermani</i> var. <i>saitoana</i> (Caulis)	88.3 ± 1.3*
<i>Betula ermani</i> var. <i>saitoana</i> (Leaves)	85.7 ± 1.5
<i>Rosa wichuraiana</i>	92.7 ± 1.2*
<i>Sorbus commixta</i> (Caulis)	88.7 ± 1.9*
<i>Weigela florida</i>	88.5 ± 2.1*
<i>Cirsium rhinoceros</i>	91.2 ± 1.6*
<i>Viburnum erosum</i>	89.6 ± 0.2*

Protective effect of Korean plant extracts upon H₂O₂ induced oxidative damage of V79-4 cells. The viability of V79-4 cells upon H₂O₂ was determined by MTT assay. The measurements were made in triplicate and values are expressed as mean ± S.E. *significantly different from H₂O₂ treated cells (p < 0.05).

Table 5. Protective effect of Korean plant extracts upon γ-ray radiation induced oxidative damage of V79-4 cells

Scientific name	Cell viability (%)
<i>Betula ermani</i> var. <i>saitoana</i> (Caulis)	90.0 ± 3.1*
<i>Betula ermani</i> var. <i>saitoana</i> (Leaves)	90.9 ± 1.6*
<i>Rosa wichuraiana</i>	94.7 ± 0.4*
<i>Sorbus commixta</i> (Caulis)	87.9 ± 1.5
<i>Weigela florida</i>	89.3 ± 2.1
<i>Cirsium rhinoceros</i>	90.9 ± 1.1*
<i>Viburnum erosum</i>	90.6 ± 1.7*

The viability of V79-4 cells upon radiation was determined by MTT assay. The measurements were made in triplicate and values are expressed as mean ± S.E. *significantly different from irradiated cells (p < 0.05).

Table 6. Effect of Korean plant extracts on scavenging intracellular ROS generated by radiation

Scientific name	ROS generation (%)
<i>Betula ermani</i> var. <i>saitoana</i> (Caulis)	68.3 ± 2.5*
<i>Betula ermani</i> var. <i>saitoana</i> (Leaves)	59.9 ± 1.6*
<i>Rosa wichuraiana</i>	80.1 ± 3.2*
<i>Sorbus commixta</i> (Caulis)	74.2 ± 1.5*
<i>Weigela florida</i>	65.4 ± 1.2*
<i>Cirsium rhinoceros</i>	55.6 ± 1.4*
<i>Viburnum erosum</i>	80.1 ± 1.1*

The intracellular ROS was detected by DCF-DA method. The measurements were made in triplicate and values are expressed as mean ± S.E. *significantly different from irradiated cells (p < 0.05).

ated cells (Table 6). The antioxidant and anticancer properties of the same genus *Betula* were investigated, and methanol extract showed cytoprotective effects against H₂O₂ in the V79-4 cell line and induced cytotoxicity and apoptosis in human promyelocytic leukemia (HL-60) cells (Ju *et al.*, 2004). There has been a report on antioxidant activity of the extract of the same genus *Rosa*, which was measured by DPPH radical scavenging activity (Moure *et al.*, 2001). The chemical constituents responsible for antioxidant activity of *Rosa wichuraiana* are never been studied thus far, and warrant further study. From the genus *Sorbus*, phenolic compounds isolated (Malterud and Opheim, 1989; Kokubun *et al.*, 1995). These natural polyphenols have an ideal and intrinsic structure of capturing of free radicals and electron delocalization, causing higher antioxidant activity than known antioxidants, such as vitamins A and E (Sokmen *et al.*, 2005). The antioxidant activity of *Sorbus commixta* which belongs to the genus *Sorbus* might be related with these polyphenols. And these polyphenols isolated from the genus *Viburnum* also might be responsible for the antioxidant activity of *Viburnum erosum*. Flavonoids and coumarins have been reported as constituents of the genus *Weigela* (Chang, 1997; Won *et al.*, 2004), and the antioxidative properties of four coumarins, scopoletin, cleomiscosin A, scopolin and fraxin were evaluated (Thuong *et al.*, 2005). Coumarins, which are also benzopyrones (α-pyrone), form a large class of phenolic compounds and are widely distributed in plants (Haraguchi, 2001). Therefore, the antioxidant activity of *Weigela florida* which belongs to genus *Weigela* might be related with the flavonoids and coumarins, and up to present, no studies have been done on the chemical constituents and biological activities of *Weigela florida*. From the genus *Cirsium*, flavone glycosides, hispidulin 7-neohesperidoside, cirsimaritin 4'-glucoside and acacetin 7-rutinoside were isolated (Park *et al.*, 1995). These naturally occurring

flavonoid profiles are widely distributed in plant kingdom and their antioxidant properties are well studied. Therefore, the antioxidant activity of *Cirsium rhinoceros* which belong to Genus *Cirsium* might be related with the flavonoids. Taken together, the results suggest that *Betula ermani* var. *saitoana*, *Rosa wichuraiana*, *Sorbus commixta*, *Weigela florida*, *Cirsium rhinoceros*, and *Viburnum erosum* protect V79-4 cells against oxidative damage by radiation through scavenging ROS.

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