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Antihepatotoxic effect of Heat-treated *Allium victorialis* var. *platyphyllum* in CCl_4-induced Rats and the Gas Chromatographic Analysis of Volatile Sulfur Substances

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Abstract – The ethanolic extracts of the leaves and bulbs of *Allium victorialis* var. *platyphyllum* (Liliaceae) collected from Daegwallyoung (D) and Ullung Island (U) in Korea were obtained using three different extracting methods. The first extracts, DL-1 DB-1, UL-1 and UB-1, were obtained from leaves (L) and bulbs (B) dried at 90°C, respectively, and the second extracts, DL-2, DB-2, UL-2 and UB-2, were obtained by extracting the leaves and bulbs of fresh plant parts. The third extracts DL-3, DB-3, UL-3 and UB-3 were obtained by incubating leaves and bulbs at 36°C. The six extracts obtained from *A. victorialis* var. *platyphyllum* at Daegwanllyoung (cultivated site) were orally administered to examine for a possible antihepatotoxic effect in CCl_4-induced rats. DL-1 exhibited the most pronounced effect. The extracts inhibited serum ALT, AST, SDH, γ-GT, ALP and LDH activities elevated by CCl_4 injection and attenuated decreased glutathione S-transferase, glutatione reductase and γ-glutamylcysteine synthetase activities and a decreased hepatic glutathione. However, the extracts obtained from Ullung Is. (native site) were less active than the extracts from Daegwallyoung, suggesting that *A. victorialis* var. *platyphyllum* from the cultivated site is more useful for functional food than of native site. These results also suggest that the antihepatotoxic effect is due to a higher content of hepatic glutathione. Gas chromatography of the twelve extracts showed significantly different sulfides, disulfides or trisulfides contents belonging to volatile sulfur substances (VSS). Nine components were identified on the basis of their mass spectra, namely, dimethyl disulfide, dimethyl trisulfide, diallyl disulfide, dipropyl disulfide, allyl methyl sulfide, allyl methyl trisulfide, 2-vinyl-4H-1,3-dithiin, 3,4-dihydro-3-vinyl-1,2-dithiin, and allithiamine. Extract DL-1 had the highest VSS content. Dried plant materials contained larger amounts of the VSSs than other extracts, and the leaves contained larger amount than the bulbs. These results suggest that heat treatment increases the antiheaptotoxic ability of A. victorialis var. platyphyllum by increasing the proportion of VSSs.
Keywords – *Allium victorialis* var. *platyphyllum*, antihepatotoxic, glutathione, Liliaceae, volatile sulfur substance

Introduction

Allium victorialis var. *platyphyllum* (Liliaceae) are natively grown in Ullung Island, Korea and also cultivated in Daegwanllyoung area, Korea and this plant is common on Ullung Is. as a wild plant species but is uncommon in other regions of Korea. Recently, a number of Korean farmers cultivate this plant as a food crop.

This edible plant *A. victorialis* var. *platyphyllum* is used in traditional medicine to treat gastritis and heart disease (Moon *et al.*, 1984). It has been reported that the leaves of this plant contain 2-3% carbohydrates and ascorbic acid and that the bulbs have sulfur-containing substances (Moon *et al.*, 1984). S-alkenyl- and S-alkyl-L-cysteines are native constituents of this plant and are decomposed to a variety of disulfides by alliinase, which is activated by tissue injury. These sulfuric substances are reported to have an inhibitory effect on platelet aggregation (Lawson *et al.*, 1991). We previously performed the taxonomical investigation (Yoo *et al.*, 1998) have also reported on the isolation of gitogenin 3-O-lycotetroside, astragalin,

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kaempferol 3,4'-di-O-β-D-glucoside. (Lee *et al.*, 2001) Moreover, extract of *Allium victorialis* var. *platyphyllum* exhibited antihepatotoxic, antihyperlipemic activity in the rat (Choi *et al.*, 2003).

On the other hand, the production of volatile sulfur substances (VSS) in this plant during storage causes food spoilage. In addition, wild and cultivated *A. victorialis* var. *platyphyllum* should be compared in terms of their pharmacological activity because the cultivated plant has not been pharmacologically examnined. In this study, cultivated plant was grown in Daegwanllyoung and wild plant was obtained from Ullung Is.. The VSS contents and the antihepatotoxic effects in CCl_4-induced rat were investigated. Because the leaves are easier to obtain than the bulbs, the two parts of the whole plant were pharmacologically compared.

Experimental

Chemicals – NADPH and bovine serum albumin were from Sigma Co. (USA). Malondialdehyde and 2-pyridone from Aldrich (USA); reduced- and oxidized glutathione from Fluka (USA); *p*-nitrophenol, 2,4-dinitrochlorobenzene, and thiobarbituric acid from Katayama (Japan); amino-transferase and γ-glutamyltrnsferase from Youngdong Pharm. Co. (Korea); and sorbitol dehydrogenase and lactate dehydro-genase from Sigma (USA).

Plant material and extraction – The cultivated *Allium victorialis* var. *platyphyllum* was collected at Daegwallyoung, Pyongchang-gun, Gangwon province, Korea, and the wild plant was collected at Sungin Mountain, Ullung Island, Korea. Collected plant materials were preserved in a refrigerator before the experiment. After dividing plant material into leaves and bulbs, both portions were dried at 90°C for 24 h in a drying oven. The extract was prepared by extracting 1 kg of heat treated plant material in EtOH under reflux for 5 h three times, evaporated on a rotary evaporator in vacuo, and further dried using a freeze dryer. From the Daegwallyoung-plant material (1 kg), the weight of leaf extract (DL-1) was 185 g and of the bulb extract (DB-1) was 58 g. From Ullung Is.-plant material, the leaf extract (UL-1) was 134 g and the bulb extract (UB-1) was 276 g. For the 2nd extract group, fresh plant materials (each, 1 kg) were extracted in EtOH three times under reflux for 5 h, dried in vacuo, and then dried further by freeze drying. By this extracting process, Daegwallyoung -leaf extract (DL-2, 100 g) and bulb extract (DB-2, 74 g) were obtained from 1 kg starting material. For the 2nd extract group from Ullung Is.-plant material (1 kg), the leaf extract (UL-2) and the bulb extract (UB-2) quantities were 59 g and 41 g, respectively. For the 3rd extract group, fresh leaves and bulbs (1 kg of each) were incubated in H_2O at 36°C for 24 h. The extracts obtained were dried on a rotatory evaporator, and freeze dried. Using this incubation process, the Daegwallyoung-leaf extract (DL-3, 55 g) and Daegwallyoung-bulb extract (DB-3, 71 g), and the Ullung Is.-leaf extract (UL-3, 59 g) and bulb extracts (UB-3, 40 g) were obtained. The abbreviated names of these extracts are interoperated as follows: e.g., in case of DL-1, D represents Daegwallyoung, L leaves, and the number represent the extracting method. Extract yields are shown in Table 1 together with the plant samples used.

Animals and treatment – Extracts were dissolved in 10% Tween 80 and diluted with saline for the animal experiments. Four week-old Sprague-Dawley male rats were purchased from the Dae-Han Bio Link Co.. Animals were adapted to constant conditions (temperature: 20 ± 2°C,

Table 1. Extraction yield of *A. victorialis* var. *platyphyllum* leaves using the different work-up protocols

Sample name	Collected Site	Plant part	Plant drying	Extraction	Yield (%)
DL-1	Daegwallyoung	Leaves	Dried in oven	Under reflux	18.5
DB-1	Daegwallyoung	Bulbs	Dried in oven	Under reflux	5.8
DL-2	Daegwallyoung	Leaves	Fresh tissue	Under reflux	10.0
DB-2	Daegwallyoung	Bulbs	Fresh tissue	Under reflux	7.4
DL-3	Daegwallyoung	Leaves	Fresh tissue	Incubation	5.5
DB-3	Daegwallyoung	Bulbs	Fresh tissue	Incubation	7.1
UL-1	Ullung Is.	Leaves	Dried in oven	Under reflux	13.4
UB-1	Ullung Is.	Bulbs	Dried in oven	Under reflux	27.6
UL-2	Ullung Is.	Leaves	Fresh tissue	Under reflux	5.9
UB-2	Ullung Is.	Bulbs	Fresh tissue	Under reflux	4.1
UL-3	Ullung Is.	Leaves	Fresh tissue	Incubation	1.5
UB-3	Ullung Is.	Bulbs	Fresh tissue	Incubation	4.0

humidity: 40-60%, light/dark cycle: 12 hr) for at least two weeks; rats of 200 ± 1 g body weights were used for the experiments. The plant extracts were administered orally to the rats once a day for two weeks at 100 mg/kg. Thirty minutes after the last treatment, 0.2 ml of CCl_4-olive oil (1:1, v/v) per 100 g body weight of the rat was intraperitoneally injected. Animals were sacrificed 48 h after CCl_4-injection. After being fasted for 8 h water was supplied *ad libitum*. Untreated animals were administered at the same volume of 5% Tween 80 and olive oil. Sera obtained by centrifuging blood were tested for ALT (alanine transaminase), AST (aspartate transaminase), γ-GT (γ-glutamyltransferase), SDH (sorbitol dehydrogenase) and LDH (lactate dehydrogenase) enzyme activity.

Measurement of AST, ALT, SDH, γ-GT, ALP and LDH – Alanine transaminase (ALT) and aspartate transaminase activities (AST) were measured using a kit (Asan Pharm. Co., Korea), based upon Reitman and Frankel's method (Reitman *et al.*, 1957). Enzyme activities were calculated using a standard calibration curve and shown by Karmen unit/ml. Sorbitol dehydrogenase (SDH) activity was measured using the method described by Weisner *et al.* (Weisner *et al.*, 1965). This enzyme activity was calculated as the reduction in NADH due to oxidation to NAD^+ in 3 min and is presented as mU/ml. γ-Glutamyltransferase (γ-GT) activity was measured using the method described by Szasa (Szasa *et al.*, 1969). Data is shown as the *p*-nitroaniline formation rate (unit: mU/ml). Alkaline phosphatase (ALP) activity was measured using a kit reagent (Asan Pharm. Co., Korea) based on the method of Roos (Roos, K.A., 1996). Enzyme activity was calculated using a standard calibration curve, and is quoted in Karmen units. Lactate dehydrogenase (LDH) activity was measured as described by Berga and Boida (Berga *et al.*, 1960), its activity (in Wrobleski units) was also calculated using a standard calibration curve.

Measurement of TBARS – Thiobarbituric acid reactive substances (TBARS) in the liver were measured as a marker of lipid peroxidation using a modification of the method described by Ohkawa *et al.* (1979). Briefly, an aliquot (0.4 ml) of 10% liver homogenate in 0.9% NaCl was added to 1.5 ml of 8.1% SDS, 1.5 ml of 20% acetate buffer (pH 3.5), and 1.5 ml of 0.8% TBA solution. The mixture was then heated at 95°C for 1 h, cooled, and extracted with 5.0 ml of n-butanol-pyridine (15:1), and the absorbance of the n-butanol-pyridine layer was measured at 532 nm to determine the level of TBARS.

Measurement of hepatic glutathione content, γ-glutamylcysteine synthetase (γ-GCS) activity and glutathione reductase activity – 1) Measurement of glutathione content: Cysteine was determined using a modification of Gaitonde *et al.*'s method (Gaitonde *et al.*, 1967). Briefly, glutathione content was determined by subtracting cysteine-SH from nonprotein bound-SH. 10% Trichloroacetic acid was added to the homogenate, which was then centrifuged. And then 0.5 ml acetic acid and 0.5 ml ninhydrin reagent were added to the supernatant, which was heated for 10 min and cooled. Immediately after adding 3 ml ethanol absorbance was measured.

2) γ-GCS was determined based on the method described by Richman and Meister (Richman *et al.*,1975), 3.5 ml reaction mixture was prepared by adding 0.1 M tris HCl buffer (pH 8.0), 8.9 mM L-glutamic acid, 0.94 mM EDTA, 3.2 mM $MgCl_2$, 1.35 mM ATP and enzyme mixture (100-300 ml protein) and then incubated at 37°C for 10 min. The absorbance of this reaction mixture was measured at 600 nm using a UV spectrophotometer.

3) Glutathione reductase was determined based on the method described by Mize and Langdon (1962) (Mize *et al.*, 1962). Briefly, a 3.5 ml reaction mixture was prepared by adding 0.1 M potassium phosphate buffer (pH 7.5), 0.94 mM EDTA, 4.6 mM oxidized glutathione, 0.16 mM NADPH and the enzyme mixture and then incubated at 37°C for 10 min. Absorbance was measured at 340 nm to determine NADPH level.

GC-Analysis – Gas chromatography was performed using a Varian 3400 instrument as follows: Column-DB-1 capillary (length 30 meters, i.d. 0.25 mm, film thickness 0.25 mm, J&W scientific, USA), initial temp. 50°C (rate 10°C), final temp. 250°C (for 11 min); injection size-2 μl, temperature-injector 250°C and transfer line 250°C, carrier gas {He (99.99%), flow rate (1.5 ml/min)}.

Mass spectroscopy – MS was performed using a Finnigan-Mat TSQ 700, Q1-single stage unit, ionization by electron impact (EI) at a 70 eV, Scan range: low mass range (LMR) 35-1000 amu, 0.56 scan/sec, temperature of ion source 150°C, dynode: 15 kV.

Statistics – Statistical significance between experimental groups was analyzed using Duncan's new multiple range test.

Results and Discussion

Antihepatotoxic activity – As shown in Table 2, the intraperitoneal administration of CCl_4 increased serum ALT, AST, SDH, γ-GT, ALP, LDH as hepatotoxicity indices. And the oral administration of the six extracts of *A. victorialis* var. *platyphyllum* at 100 mg/kg significantly reduced these enzyme activities. DL-1 exhibited the most potent antihepatotoxic effect and others did much less

Table 2. Effect of *A. victorialis* var. *platyphyllum* extracts on aminotransferases (ALT, AST), sorbitol dehydrogenase (SDH), γ-glutamyltransferase (γ-GT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities in CCl_4-induced hepatitis rats (100 mg/kg, *p.o.*)

Treatment	ALT (IU/L)	AST (IU/L)	SDH (mU/ml)	γ-GT	ALP (K-A)	LDH (Wrobl.)
Vehicle	32.4±4.56^{d}	61.7±9.23^{d}	19.4±11.8^{c}	25.6±3.06^{e}	39.2±3.11^{e}	25.3±3.11^{e}
CCl_4	120.8±9.47^{a}	200.6±20.3^{a}	80.6±7.43^{a}	79.6±4.90^{a}	89.8±4.21^{a}	51.6±3.27^{a}
DL-1	80.2±3.46^{b}	154.3±9.3^{c}	65.0±3.47^{b}	54.1±3.21^{d}	53.6±3.47^{d}	33.9±3.37^{d}
DB-1	83.6±6.43^{b}	174.5±7.29^{bc}	81.3±8.25^{a}	64.2±2.93^{c}	69.5±3.22^{c}	40.6±3.59^{c}
DL-2	110.5±7.57^{ab}	190.8±13.5^{ab}	78.5±5.39^{a}	75.5±3.47^{ab}	81.3±3.49^{b}	48.7±3.25^{ab}
DB-2	120.0±8.56^{ab}	201.7±13.5^{a}	81.3±8.25^{a}	78.6±3.47^{a}	85.6±5.12^{ab}	50.6±2.35^{ab}
DL-3	108.6±6.25^{b}	181.9±15.0^{ab}	75.6±3.27^{ab}	70.0±2.53^{b}	79.5±2.43^{b}	45.6±2.09^{bc}
DB-3	111.8±76.23^{ab}	198.4±10.7^{a}	79.6±3.57^{a}	76.8±2.43^{a}	82.9±4.20^{b}	48.5±2.25^{ab}

Values are the means ± S.D. of eight experiments. Data followed by different superscript are significantly different ($p < 0.05$) by Duncan's new multiple range test.

Table 3. Effect of *A. victorialis* var. *platyphyllum* extracts on hepatic glutathione *S*-transferase (GST), glutathione content, glutathione reductase (GR), γ-glutamylcysteine synthetase (γ-GCS) in CCl_4-induced rats (100 mg/kg, *p.o.*)

Treatment	Lipid peroxide	GST	Glutathione	GR	γ-GCS
	nmol MDA/g of tissue	CDNB nmol/mg protein/min	mol/g of tissue	Gltut. nmol/mg protein/min	Pi nmol/mg potein/min
Vehicle	23.4 ± 2.49^{e}	283.6±31.1^{a}	5.38±0.70^{a}	27.8±2.19^{a}	15.9±1.37^{a}
CCl_4	98.7 ± 3.32^{a}	126.5±18.8^{d}	2.46±0.18^{c}	11.8±3.10^{c}	6.47±0.85^{e}
DL-1	54.6 ± 3.87^{d}	210.4±21.5^{b}	4.17±0.50^{b}	17.7±3.14^{b}	12.6±0.98^{b}
DB-1	70.4 ± 5.33^{c}	170.3±17.60^{c}	2.97±0.15^{c}	15.4±2.16^{bc}	8.54±0.7^{c}
DL-2	90.5 ± 2.47^{b}	147.8±19.0^{cd}	2.59±0.22^{c}	13.6±2.11^{bc}	7.21±0.69^{cde}
DB-2	91.8 ± 2.56^{b}	150.2±23.6^{cd}	2.58±0.27^{c}	14.6±4.17^{bc}	6.53±0.54^{e}
DL-3	85.9 ± 3.25^{b}	158.9±20.0^{cd}	2.74±0.30^{c}	11.5±3.13^{c}	8.43±0.90^{cd}
DB-3	86.4 ± 3.49^{b}	149.3±15.2^{cd}	2.97±0.1^{c}	13.9±3.20^{bc}	6.98±0.47^{de}

Values are the means ± S.D. of eight experiments. Data followed by different superscript are significantly different ($p < 0.05$) by Duncan's new multiple range test.

Table 4. Effect of pretreated *A. victorialis* var. *platyphyllum* extracts on the hepatic lipid peroxide, glutathione content and related enzyme activities in CCl_4-induced hepatitis rats (100 mg/kg, p.o.)

Treatment	lipid peroxide	GST	Glutathione	γ-GCS
	nmol/mg MDA/g of tissue	CDNB nmol/mg Protein/min	mol/g of tissue	Pi nmol/mg potein/min
Vehicle	24.6±3.11^{e}	279.8±29.7^{a}	5.42±0.98^{a}	16.4±2.11^{a}
CCl_4	97.3±2.96^{a}	124.5±20.1^{c}	2.50±0.23^{c}	6.33±0.90^{c}
UL-1	73.6±2.43^{d}	170.9±19.4^{b}	3.17±0.23^{b}	9.54±0.86^{b}
UB-1	85.2±3.38^{c}	150.4±18.6^{bc}	2.85±0.19^{c}	7.53±0.47^{c}
UL-2	96.8±3.49^{a}	130.6±18.5^{c}	2.81±0.31^{c}	6.49±0.58^{c}
UB-2	95.2±3.49^{ab}	129.5±20.8^{c}	2.53±0.27^{c}	6.39±0.54^{c}
UL-3	89.6±2.48^{bc}	141.7±20.80^{bc}	2.77±0.28^{c}	6.84±0.33^{c}
UB-3	93.8±4.18^{ab}	134.6±19.9^{bc}	2.66±0.20^{c}	6.62±0.40^{c}

Values are the means ± S.D. of eight experiments. Data followed by different superscript are significantly different ($p < 0.05$) by Duncan's new multiple range test.

effect; DB-1 was also potent but less so than DL-1. The data observed by treatment with Ullung Is. samples were much less active than with Daegwanllyoung samples (data of Ullung samples not shown).

Inhibitory effect on lipid peroxidation and glutathione metabolism – As shown in Table 3, treatment with CCl_4 increased the lipid peroxide content and hepatic aniline hydroxylase and aminopyrine N-demethylase activities versus the vehicle only-treated group. Table 3 shows that treatment with *A. victorialis* var. *platyphyllum* of Daegwanllyoung site attenuated lipid peroxide formation, and the most of test samples had no significant effect of activities of these two hepatic enzymes and the second most potent was DB-1. No significant activities of aniline ahydroxylase- and aminopyrine N-demethylase activities were observed (data not shown).

Administration of CCl_4 (i.p.) reduced hepatic glutathione content and decreased glutathione S-transferase, glutathione reductase and γ-glutamylcysteine synthethase. (Table 3) *A. victorialis* var. *platyphyllum* collected from Daegwall-young increased the hepatic glutathione concentration in CCl_4-intoxicated rats. DL-1 was the most potent; however, the others were not statistically significant. In terms of glutathione S-transferase activity, only DL-1 and DB-1 significantly increased its value versus the vehicle only-treated group. In terms of glutathione reductase and γ-glutamylcysteine synthethase activities, DL-1 was the most potent followed by DB-1.

We also investigated the effect of six kinds of *A. victorialis* var. *platyphyllum* extracts collected from Ullung Is. in CCl_4-treated rats. Table 4 shows the effect of these extracts on the enzyme activities of lipid peroxide, glutathione concentration, and on glutathione S-transferase, and γ-glutamylcysteine synthethase activities. *A. victorialis* var. *platyphyllum* collected from Ullung Is. reduced lipid peroxide concentration as did the plants from Daegwall-young, but the former was less effective than the latter. In terms of glutathione S-transferase and γ-glutamylcysteine synthethase activities, plants from Ullung Is. were less potent than those from Daegwall-young. The extracts, DL-1 and DB-1, obtained from heat-processed herbs were found to higher levels of these enzyme activities.

Gas chromatographic analysis on the volatile sulfur substances – GC-MS investigation of VSS led to the identification of 9 sulfuric compounds, namely, dimethyl disulfides, dimethyl trisulfide, diallyl disulfide, dipropyl

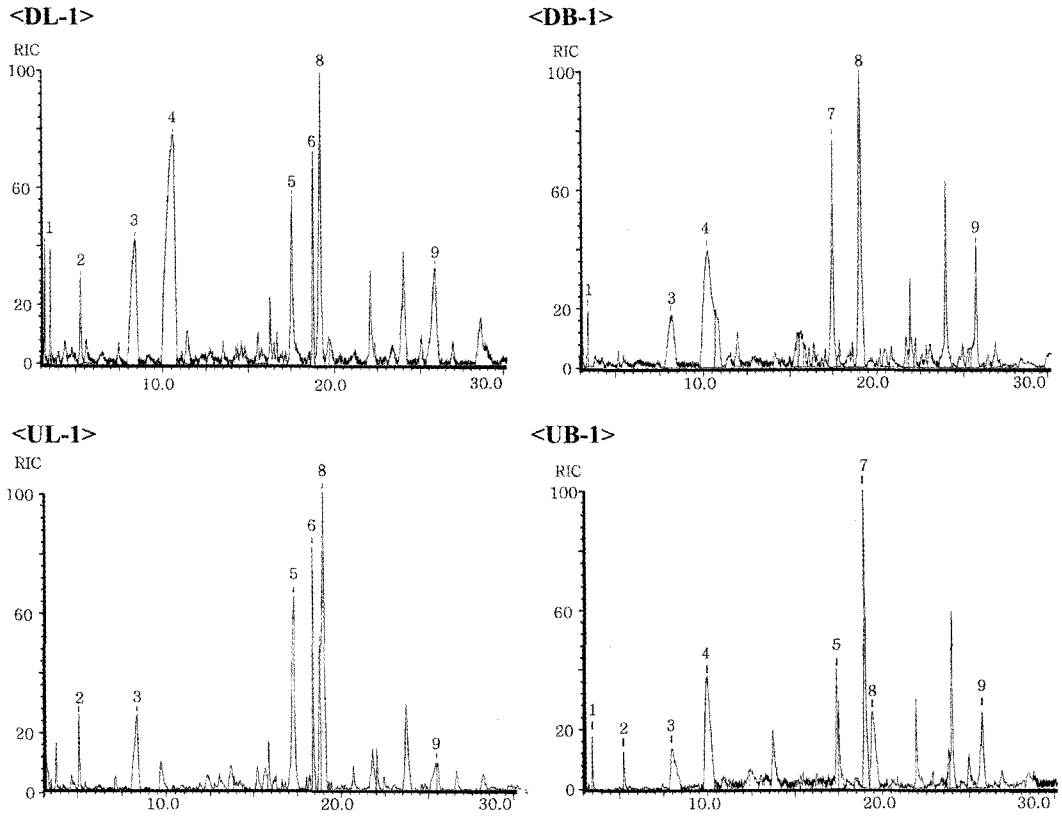

Fig. 1. GC chromatograms of the ethanolic extracts from the leaves (L) and bulbs (B) of *A. victorialis* var. *platyphyllum* collected from Daegwanryung (D) and Ullung Is. (U).
(**1** Dimethyl disulfide, **2** Dimethyl trisulfide, **3** Diallyl disulfide, **4** Dipropyl disulfide, **5** Allyl methyl sulfide, **6** Allyl methyl trisulfide, **7** 2-vinyl-4H-1,3-dithiin, **8** 3,4-dihydr-3-vinyl-1,2-dithiin, **9** Allithiamine)

Table 5. Relative ion count of the volatile sulfuric compounds at GC-MS on several extracts of *A. victorialis* var. *platyphyllum*

Compound	t_R (min)	DL-1	DB-1	DL-2	DB-2	DL-3	DB-3	UL-1	UB-1	UL-2	UB-2	UL-3	UB-3
Dimethyl disulfide (**1**)	3.17	1.667	1.286	1.053	1.013	0.383	1.430	–	0.609	1.061	2.003	–	0.831
Dimethyl trisulfide (**2**)	5.24	2.705	–	1.706	0.983	0.470	–	3.113	0.805	1.274	–	–	0.979
Diallyl disulfide (**3**)	8.31	16.093	8.552	3.600	2.865	1.601	–	10.260	3.969	7.370	–	–	4.486
Dipropyl disulfide (**4**)	10.57	44.647	5.579	6.756	2.819	1.805	–	–	10.050	4.537	–	–	1.461
Allyl methyl sulfide (**5**)	17.49	9.972	12.785	1.249	1.112	0.912	0.721	19.157	3.780	1.600	–	2.421	5.482
Allyl methyl trisulfide (**6**)	18.70	5.758	–	5.128	1.032	5.199	–	8.282	–	1.032	–	-	14.902
2-vinyl-4H-1,3-dithiin (**7**)	19.08	–	–	1.716	1.210	1.961	3.073	4.185	9.561	3.848	0.437	6.586	–
3,4-dihydr-3-vinyl-1,2-dithiin (**8**)	19.12	15.849	22.886	4.982	3.016	2.437	0.222	26.841	6.024	1.188	0.786	0.306	12.527
Allithiamine (**9**)	25.75	11.998	7.170	3.510	1.557	–	2.719	0.358	4.211	-	3.232	0.548	–
Sum		108.689	58.258	29.700	15.607	14.768	8.165	72.196	39.009	21.910	6.458	9.861	40.668

–, trace; Unit: RIC

disulfide, allyl methyl sulfide, allyl methyl trisulfide, 2-vinyl-4H-1,3-dithiin, 3,4-dihydro-3-vinyl-1,2-dithiin, allithiamine. Of these, it is known that allithimine is produced by the reaction between allicin and thiamine (Matsukawa *et al.*, 1953). Four typical chromatograms were shown in Fig. 1.

Peak areas on the gas chromatogram were presented as relative ion counts (RIC), which represent the relative amounts of these compounds. DL-1 with the largest total peak area contained a relatively higher amounts of dipropyl disulfide (4), diallyl disulfide (3), 3,4-dihydro-3-vinyl-1,2-dithiin (8). On examination of the data shown in Table 5, it was found that the extract from the heated plant materials had more VSS than the extracts from fresh or incubated materials. By comparing DL-1 and DB-1 data, it was found that the greater amounts of VSS were produced in the leaves than in the bulbs.

This research was undertaken to determine the antihepatotoxic activity and the relative amounts of VSS in extracts of *A. victorialis* var. *platyphyllum* bulbs and leaves due to various treatment of plant samples. We also compared the biological activities and the amounts of those substances in the plant materials collected from the two sites, Ullung and Daegwallyoung. Plants from Ullung grew wildly whereas those from Daegwallyoung had been cultivated for 2 years.

The antihepatotoxic effects of treatments were assayed in a CCl_4-induced hepatitis rat model and results are shown in Table 2. It was known that the hepatotoxic mechanism of CCl_4 is dependent on the biotransformation of CCl_4 into $\cdot CCl_3$ and the subsequent production of $\cdot OOCCl_3$. These radicals induce lipid peroxidation of cell membranes and then destroy their structure and functions. (Noll *et al.*, 1984; Recknagel *et al.*, 1989).

Heat-treatment increased the antihepatotoxic effect of *A. victorialis* var. *platyphyllum* as compared with the other two treatments, and the bioactivity of the leaf extract was found to be higher than of the bulb. Incubation process was associated with lower levels of VSS than heat treatment. As shown in Table 5, DL-1 had the highest amount of VSS, suggesting that heat-treatment increases VSS. It has been shown that the activation of alliinase causes the biotransformation of S-alkyl-L-cysteine and S-alkenyl-L-cysteine to produce VSS (*2*). Our analytical results supported Nishimura *et al.*'s and Higuchi *et al.*'s reports (Nishimura *et al.*, 2000; Higuchi *et al.*, 2003) that trisulfides and dithiins could be increased at thermochemical reaction in Allium species. Higuchi From these examinations, it appears that the production rate of VSS contributes to the antihepatotoxic activity of *A. victorialis* var. *platyphyllum*. Thus, these results suggest that heat-treatment significantly increases VSS content in *A. victorialis* var. *platyphyllum* and its antihepatotoxic effect.

Based on the above experimental results, it may be that heat-treatment prevents deterioration due to long-term storage without loss of bioactivity. Moreover, our results show that the cultivated species (D-series samples) are probably more beneficial as foods than wild plant (U-series samples) based on its higher VSS content and bioactivity. Moreover, the antihepatotoxic effects associated by the different treatments used were found to be associated with the activities of various enzyme known to be involved in CCl_4-induced hepatotoxicity.

As shown in Table 3, CCl_4-treatment markedly increased lipid peroxide via lipid peroxidation and it is known that cytochrome P-450, a microsomal enzyme (Reddy, C.C., *et al.*, 1981), is increased by CCl_4 treatment in the rat. Aniline hydroxylase- and aminopyrine N-demethylase, which are belonged to cytochrome P-450, are also increased by CCl_4-treatment. Treatment of rats with *A. victorialis* var.

platyphyllum reduced lipid peroxide concentrations without reducing aniline hydroxylase and aminopyrine N-demethylse activities, suggesting that lipid peroxidation had been inhibited without cytochrome P-450 involvement. It is known that lipid peroxidation due to CCl_4-induced hepatotoxicity is closely associated with its hepatptoxicity mechanism. Comparisons of the data in Table 3 and 4 between plant materials from the two sites indicated that plants from Daegwallyoung are more active than those from Ullung Is..

Glutathione S-transferase, a phase 2 enzyme, is responsible for glutathione conjugation (Meister *et al.*, 1983; Gusovsky *et al.*, 1990). Reduced levels of the enzyme activity of this enzyme indicate a loss of detoxifying ability and an increase in the level of xenobiotics (Fried *et al.*, 1975). DL-1 treatment was associated with the highest glutathione S-transferase activity in the present study. The enzyme activities of γ-glutamylcysteine synthetase (γ-GCS), a rate-limiting enzyme of glutathione synthesis, and glutathione reductase (GR) (Klaassen *et al.*, 1974) were determined to investigate effects of *A. victorialis* var. *platyphyllum* extracts on CCl_4-induced hepatotoxicity. Treatment with these extracts variably prevented from the decrease of γ-GCS- and GR activities. In addition, *A. victorialis* var. *platyphyllum* extracts increased hepatic glutathione content, suggesting that this content is closely associated with the antihepatotoxicity. Moreover, treatment with DL-1 most effectively inhibited hepatotoxicity via an antioxidative mechanism.

These experimental results suggest that heat-treatment should be adopted to improve both the preservation of this plant and to improve its antihepatotoxicity. It was also found that *A. victorialis* var. *platyphyllum* cultivated at Daegwallyoung contained more VSS than the native plant from Ullung Is. Taken together, our results suggest that the *A. victorialis* var. *platyphyllum* in cultivation is the better choice in terms of supply, preservation, functional benefit.

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