Antioxidative Activity of *Allium victorialis* L. Extracts

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Abstract. *Allium victorialis L. (Liliaceae, "Hon-Gyoujya Nin-Niku" in Japanese) was successively extracted with hexane, acetone, methanol and 70% methanol and the extracts were further separated into a total of twenty-five fractions by silica gel and ODS column chromatographies. The biological activities of these four extracts and 25 column fractions were compared. The cytotoxic activity of all extracts and fractions against two oral tumor cell lines was significantly higher than that against normal human gingival fibroblasts, suggesting their tumor-specific action. Three methanol column fractions* ***[M2, M3, M6]*** *and a 70% methanol column fraction* ***[70M6]*** *most effectively reversed the multidrug resistance (MDR) against L5178 mouse T cell lymphoma. The electron spin resonance (ESR) spectroscopy showed that methanol column fractions and 70% methanol extracts produced the highest amount of radical(s) and most efficiently scavenging* $O_2^{\bullet -}$*, generated by the hypoxanthine-xanthine reaction system, suggesting that the same substances in these fractions display both prooxidant and antioxidant properties. They showed no anti-human immunodeficiency virus (HIV) or anti-Helicobacter pylori activity. These data suggest the medicinal efficacy of Allium victorialis extract.*

Allium victorialis L. (Liliaceae, "Hon-Gyoujya Nin-Niku" in Japanese) has been used as a folk medicine with garlic (*Allium sativum*) and onions (*Allium cepa*) especially in Japan, Korea and China. Although many chemical compounds of garlic and onions have been previously studied (1-3), the biological activity of *Allium victorialis* L. has not yet been investigated.

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The *Allium* genus, such as garlic and onions, are rich in sulfur-containing components, sulfides, disulfides and trisulfides. In particular, garlic oils contain 66% diallyl disulfide and 14% diallyl sulfide (4). Garlic oil and its allyl methyl trisulfide or diallyl sulfide significantly inhibited the incidence of 7,12-dimethylbenzanthracene-induced, phorbol-myristate-acetate-promoted skin papillomas (5), benzo[*a*]pyrene-induced neoplasia in the fore-stomach and lung (6) and 1,2-dimethylhydrazine-induced colorectal adenocarcinoma in mice (7). This protective effect of organosulfur compounds may result from activation of gluthathione *S*-transferase (GST), which conjugates electrophile and protects the host from carcinogenesis and mutagenesis (8-13).

We investigated here various biological activities of *Allium victorialis* L. extracts such as cytotoxic activity, reversal of multidrug resistance of tumor cells, anti-HIV activity, antibacterial effect on *H. pylori,* radical generation and superoxide anion radical ($O_2^{\bullet -}$) scavenging activity, using electron spin resonance (ESR) spectroscopy.

Materials and Methods

Materials. Dried total herbs of *Allium victorialis* L. in China was kindly supplied by the Itagaki Trading Co. LTD. (Chiyoda-ku, Tokyo, Japan) (14) (Figure 1). The following chemicals and reagents were obtained from the indicated companies: RPN91640 medium, Dulbecco's modified Eagle medium (DMEM) (Gibco BRL, Grand Island, NY, USA); McCoy's 5A medium (Gibco, BRL, Grand Island, NY, USA); fetal bovine serum (FBS) for RPMI 1640 medium (JRH Bioscience, Lenexa, KS, USA); horse serum for McCoy's 5A medium; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Wako Pure Chem Ind., Ltd., Osaka, Japan); 3-azido-2',3'-dideoxythymidine (AZT) (Sigma Chem. Co., St. Louis, MO, USA); dideoxycytidine (ddC) (Sigma Chem. Co., St. Louis, MO, USA); dextran sulfate (8kD) (Kowa Chem. Co., Tokyo, Japan); diethylenetriaminepentaacetic acid (DETAPAC(Sigma Chem. Co., St. Louis, MO, USA); 5,5-dimethyl-l-pyrroline-N-oxide (DMPO) (a spin trap agent) (Dojin, Kumamoto, Japan); superoxide dismutase (SOD) from bovine erythrocytes. A strain of *Helicobacter pylori* (ATCC43504) was purchased from the American Type Culture Collection (Rockville, MD, USA).

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Preparation and fractionation of Allium victorialis L. extracts. Dried herbs of *Allium victorialis* L. (500 g) were successively extracted with hexane, acetone, MeOH and 70% MeOH at room temperature. After evaporation of the solvent *in vacuo,* the hexane extract **[H0]** (1.65 g), acetone extract **[A0]** (24.31 g), MeOH extract **[M0]** (33.73 g) and 70% MeOH extract **[70M0]** (14.34 g) were obtained, respectively (Figure 2). First, the aliquot of hexane extract **[H0]** (1.5 g) was applied to a silica gel column chromatography, which was then eluted with a hexane-acetone gradient. The hexane fraction [H1] (0.03 g), hexane-acetone (9:1) fraction **[H2]** (0.46 g), hexane-acetone (4:1) fraction **[H3]** (0.08 g), hexane-acetone (3:2) fraction **[H4]** (0.13 g) and hexane-acetone (2:3) fraction **[H5]** (0.18 g) were step-wisely eluted. Second, the acetone extract **[A0]** (20 g) was applied to a silica gel column chromatography, which was then eluted with a benzene-EtOAc gradient. The benzene fraction **[A1]** (0.06 g), benzene-EtOAc (9:1) fraction **[A2]** (0.35 g), benzene-EtOAc (4:1) fraction **[A3]** (0.13 g), benzene-EtOAc (3:2) fraction **[A4]** (0.10 g) and benzene-EtOAc (2:3) fraction **[A5]** (0.14 g), were step-wisely eluted. The acetone fraction **[A6]** (0.77 g) and MeOH fraction **[A7]** (10.98 g) were then eluted, respectively. Third, the MeOH extract **[M0]** (20 g) was applied to silica gel column chromatography, which was then eluted with a $CHCl_3$-MeOH gradient. The $CHCl_3$ fraction **[M1]** (0.19 g), $CHCl_3$-MeOH (49:1) fraction **[M2]** (0.96 g), $CHCl_3$-MeOH (24:1) fraction **[M3]** (0.14 g), $CHCl_3$-MeOH (9:1) fraction **[M4]** (0.62 g), [M5] (0.83 g) and $CHCl_3$-MeOH (4:1) fraction **[M6]** (1.25 g) were step-wisely eluted. The MeOH fraction **[M7]** (9.31 g) was then eluted. Finally, the 70% MeOH extract **[70M0]** (10 g) was applied to ODS column chromatography, which was then eluted with a H_2O-MeOH gradient. The H_2O-MeOH (3:1) fractions **[70M1]** (5.74 g), **[70M2]** (2.44 g), **[70M3]** (0.18 g), H_2O-MeOH (2:1) fraction **[70M4]** (0.04 g) and H_2O-MeOH (1:1) fraction **[70M5]** (0.12 g) were step-wisely eluted. The MeOH fraction **[70M6]** (0.09 g) was then eluted (Figure 2).

Assay for cytotoxic activity. Human squamous cell carcinoma (HSC-2), human salivary gland tumor (HSG) and human gingival fibroblasts (HGF) (5-7 population doubling levels) were cultured in DMEM medium supplemented with 10% heat-inactivated FBS. These cells were incubated for 24 hours with the indicated concentrations of test samples. The relative viable cell number (absorbance at 540 nm (A_{540}) was then determined by the MTT assay. The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve (Table I) (15).

Assay for anti-human immunodeficiency virus (HIV) activity (16). Human T-cell leukemia virus 1 (HTLV1)-bearing CD4-positive human T-cell lines, MT-4 cells, were infected with HIV-1_{IIIB} at a multiplicity of infection (m.o.i.) of 0.01. HIV- or MOCK-infected MT-4 cells (1.5 x 10^5/mL, 200 μL) were placed into 96-well microtiter plates and incubated in the presence of varying concentrations of the fractions in RPMI1640 medium supplemented with 10% FBS. After incubation for 5 days at 37°C in a 5% CO_2 incubator, cell viability was quantified by a colorimetric assay (at 540 nm and 690 nm), monitoring the ability of viable cells to reduce MTT to a blue formazan product. All data represent the mean values of triplicate measurements. CC_{50} was determined with MOCK-infected cells, whereas EC_{50} was determined with HIV-infected cells. Selectivity index (SI) was defined as follows: $SI=CC_{50}/EC_{50}$ (Table I) (16).

Anti-Helicobacter pylori activity. A strain of *Helicobacter pylori* (ATCC43504) was purchased from the American Type Culture Collection (Rockville, MD, USA). Mueller-Hilton broth containing 5% FBS was used as the medium and was cultured in a jar conditioned with Campylo Pack (Dia Iatron) for 48 hours. Briefly, *H. pylori* strains were inoculated onto a Brucella agar plate containing 10% horse serum and cultured at 37°C for 48 hours. The bacterial colonies collected were diluted to 10^7 colony forming unit (CFU)/mL with 0.9% saline. The extracts were dissolved in DMSO and then diluted with Mueller-Hilton broth. To the solution of the extracts, each bacterial suspension was added to a density of 10^6 colony forming units (CFU)/100 mL/well. The mixture was incubated at 37°C for 48 hours. The minimum inhibitory concentration (MIC) of each fraction was calculated from the dose-response curve (17, 18) (Table I).

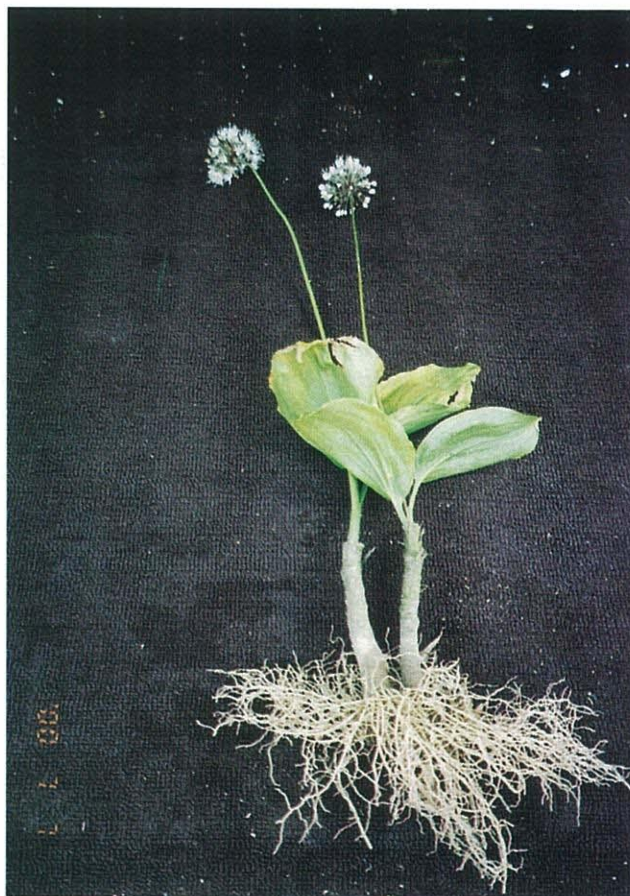

Figure 1. *Total herb of Allium victorialis L.*

Assay for radical intensity. Radical intensity was determined at 25°C (RT) using ESR spectroscopy (JEOL JES RE1X, X-band, 100 kHz modulation frequency). Instrument settings: center field, 335.6 ± 5.0 mT; microwave power, 8 mW; modulation amplitude, 0.1 mT; gain, 630 time constant, 0.1 second; scanning time, 2 minutes. Radical intensity was determined in 0.1M $NaHCO_3/Na_2CO_3$ buffer (pH 10.5), or 0.1M KOH (pH 12.5) containing 50% DMSO. The final concentration of each extract or column fraction was 3 mg/mL and the radical intensity was defined as the ratio of peak heights of these radicals to that of MnO (Figure 3) (19).

Superoxide anion ($O_2\bullet^-$) scavenging activity. A superoxide anion radical ($O_2\bullet^-$) was generated by the hypoxanthine (HX) and xanthine oxidase (XOD) reaction system (200 μL) [2 mM HX (in 0.1 M phosphate-buffer, pH 7.8) (PB) 50 μL, 0.5 mM DETAPAC 20 μL, DMPO 10 μL, sample (in DMSO) 50 μL, H_2O or SOD 30 μL, SOD (0.5 U/mL in 0.1M PB) 40 μL]. After 1 minute, the measurement was initiated. The gain was changed to 250. $O_2\bullet^-$ scavenging activity was expressed as SOD unit/mg sample (Table II) (20).

Reversal of multidrug resistance of tumor cells (Cell and fluororescence uptake). The L5178 mouse T-cell lymphoma cell line was transfected with a *MDR1/A* containing retrovirus, as previously described (21). MDR1-expressing cell lines were selected by culturing the infected cells with 60 ng/mL colchicine to maintain expression of the MDR phenotype. The L5178 MDR cell line and the L5178Y parent cell line were grown in McCoy's 5A medium with 10% heat-inactivated horse serum, L-glutamine and antibiotics. The cells were adjusted to a concentration of 2 x 10^6/mL and resuspended in serum-free McCoy's 5A medium and 0.5 mL aliquots of the cells were distributed into Eppendorf centrifuge tubes. Next, 2.0 μL of 10 mg/mL of tested fractions were added and incubated for 10 minutes at room temperature. Then 10 μL rhodamine 123 (R123) indicator (5.2 μM final concentration) was added and incubated for a further 20 minutes at 37°C. After washing twice and resuspension in 0.5 mL phosphate-buffered saline (PBS), the fluorescence of the cell population was measured by flow cytometry, using Beckton Dickinson FACScan instrument (cell sorter). (±)-Verapamil was used as a positive control in the R123 accumulation experiments (22). The R123 accumulation was calculated from the fluorescence intensity of the samples. The percentage of control of untreated mean fluorescence intensity was calculated for parental and MDR cell lines and compared to the fluorescence intensity of treated cells. An activity ratio was calculated by the equation in Table III (22, 23) (Tables III and IV).

Results

Cytotoxic activity. We first compared the cytotoxic activity of four extracts of *Allium victorialis* L. : hexane extract [**H0**], acetone extract [**A0**], methanol extract [**M0**] and 70% methanol extract [**70M0**] (Table I). The first three extracts [**H0, A0, M0**] showed higher cytotoxic activity against HSC-2 (CC_{50}=298, 314 and 222 μg/mL, respectively) and HSG cells (CC_{50}=280, 234 and 182 μg/mL, respectively) when compared with **70M0** (CC_{50}>400 and 269 μg/mL, respectively). When these extracts were applied to silica gel column chromatography, major cytotoxic activities remained in the column and were eluted by hexane-acetone (9:1, 4:1, 3:2, 2:3: corresponding to **H2, H3, H4, H5**), by benzene-EtOAc (4:1, 3:2) [**A3, A4**] or by $CHCl_3$-MeOH (49:1, 24:1, 9:1, 4:1) [**M2, M3, M4, M5, M6**]. The cytotoxic activity of all of the 70% methanol extracts was relatively weak, but enhanced by application to ODS column chromatography and elution with 70% methanol [**70M6**] after wash-out of **70M1-70M5** (Table I). Normal cells (human gingival fibroblasts) (HGF) were relatively resistant to all extracts and eluted fractions, as demonstrated by the selectivity index (SI) ratio (Table I).

Anti-human immunodeficiency virus (HIV) activity. All extracts and column fractions showed no apparent anti-HIV activity (selectivity index(SI)<1), in contrast to popular anti-HIV agents, such as dextran sulfate (SI>185), curdlan sulfate (SI>864), AZT (SI=6925) and ddC (SI=2896) (Table I).

Anti-Helicobacter pylori activity. All extracts and column fractions showed no measurable anti-*H. pylori* activity (Table I).

Radical generation and $O_2^{\bullet -}$ scavenging activity. ESR spectroscopy showed that crude extracts of *Allium victorialis* L. [**H0, A0, M0, 70M0**] did not produce any radical(s) under alkaline conditions (Figure 3). However, some column fractions such as **A3, M3, M4, 70M3, 70M4, 70M5, 70M6** produced detectable amounts of radical(s) and their radical intensity was increased when the pH of the assay buffer changed from 10.5 to 12.5 (Figure 3). This indicates that the radical intensity of fractions roughly corresponded to the water-solubility (Table II).

Hexane extract [**H0**] very weakly scavenged $O_2^{\bullet -}$ which was generated from the hypoxanthine-xanthine oxidase reaction system, when compared with acetone, methanol and 70% methanol extracts [**A0, M0, 70M0**] (Table II). Column fractions of 70% methanol extracts [**70M3, 70M4, 70M5, 70M6**] showed the highest $O_2^{\bullet -}$ scavenging activity (7.57-14.75 SOD unit/mg), followed by those of methanol extracts [**M2, M3, M4, M5, M6**] (5.06-9.46 SOD unit/mg) and those of acetone extracts [**A3, A4, A5, A6, A7**] (2.82-5.69 SOD unit/mg) (Table II). Combined together, the $O_2^{\bullet -}$ scavenging activity of these fractions roughly paralelled their radical intensity.

Reversal of multidrug resistance (MDR). Enhancement (enforcement) of the expression of *MDR1/A* in the L15178 mouse T-cell lymphoma cell line resulted in MDR, as reflected by the reduced intracellular accumulation of R123 and addition of a known resistance modifier, (±)-verapamil, reversed the MDR, as reflected by the increase in R123 accumulation (7.88-fold increase) (Table III). We unexpectedly found that column fractions (40 μg/mL) of *Allium victorialis* L. hexane extracts [**H3, H4, H5**] (41.36-, 85.54-and 65.99-fold increase), acetone extracts [**A3, A4, A5, A6**] (33.37-, 19.66-, 26.26- and 25.98-fold increase), methanol extracts [**M0, M2, M3, M4, M5, M6**](74.16-, 100.82-, 111.06-, 82.79-, 78.12- and 120.86-fold increase) and 70% methanol extract [**70M6**] (106.82-fold increase) increased the R123 accumulation more efficiently than (±)-verapamil (Table III). When the accumulation of these fractions was reduced to 4 μg/mL, the MDR reversal effects were slightly reduced, but those of most fractions [**H3, H4, H5, A3, A4, M0, M2, M3, M4, M5, M6**] still exceeded that of (±)-verapamil (Table IV).

Discussion

We compared four crude extracts and their column fractions for their diverse biological activities. Hexane extract [**H0**] showed moderate cytotoxic activity, while separation by silica gel chromatography yielded fractions [**H3, H4**] with enhanced cytotoxic activity. These fractions did not produce any detectable amount of radical, showed the weakest $O_2^{\bullet -}$ scavenging activity and some MDR reversing activity.

Acetone extract [**A0**] showed comparable cytotoxic activity, while silica gel chromatography fractions [**A3, A4**]

Table I. *Cytotoxic, anti-HIV and anti-H. pylori activity of Allium victorialis L. extracts and fractions.*

Extract or fraction	Cytotoxic activity [1)] (CC_{50}: μg/mL)				Anti-HIV activity			Anti-*H.pylori* activity [2)]
	Human oral tumor cell lines		Human gingival fibroblast (HGF)	SI (HGF/ HSC-2)	CC_{50} (μg/mL)	EC_{50} (μg/mL)	SI (CC_{50} EC_{50})	MIC_{50} (μg/mL)
	HSC-2	HSG						
H0	298	280	> 400	>1.34	> 200	> 200	>< 1	–
H1	357	187	> 400	>1.12	> 200	> 200	>< 1	–
H2	173	177	> 400	>2.31	= 76	> 200	< 1	–
H3	183	124	327	>1.79	= 122	> 200	< 1	–
H4	125	127	340	2.72	= 104	> 200	< 1	–
H5	179	224	> 400	>2.23	= 102	> 200	< 1	–
A0	314	234	> 400	>1.27	> 200	> 200	>< 1	–
Al	370	197	> 400	>1.08	> 200	> 200	>< 1	–
A2	218	88	359	1.65	> 200	> 200	>< 1	–
A3	150	40	315	2.10	= 109	> 200	< 1	–
A4	234	68	345	1.47	= 100	> 200	< 1	–
A5	346	167	> 400	>1.16	> 200	> 200	><1	–
A6	340	50	> 400	>1.18	> 200	> 200	><1	–
A7	193	166	252	1.31	= 168	> 200	< 1	–
M0	222	182	>400	>1.80	> 200	> 200	>< 1	–
Ml	347	190	>400	>1.15	> 200	> 200	>< 1	–
M2	145	64	289	1.99	= 111	> 200	< 1	–
M3	195	95	382	1.96	= 100	> 200	< 1	–
M4	171	81	>400	>2.34	= 95	> 200	< 1	–
M5	158	89	365	2.31	= 84	> 200	< 1	–
M6	126	118	> 400	>3.17	= 136	> 200	< 1	–
M7	214	148	> 400	>1.87	> 200	> 200	>< 1	–
70M0	>400	269	> 400	>1.00	> 200	> 200	>< 1	–
70M1	368	240	> 400	>1.09	> 200	> 200	>< 1	–
70M2	364	326	> 400	>1.10	> 200	> 200	>< 1	–
70M3	337	363	> 400	>1.19	> 200	> 200	>< 1	–
70M4	>400	348	> 400	>1.00	> 200	> 200	>< 1	–
70M5	370	320	> 400	>1.08	> 200	> 200	>< 1	–
70M6	49	68	120	2.45	= 90	> 200	< 1	–
Gallic acid					= 12	> 180	< 1	–
Dexatran sulfate					>1000	5.4	> 185	–
Curdlan sulfate					>1000	1 2	>864	–
AZT(μM)					277	0.040	6925	–
ddC(μM)					753	0.26	2896	–

1) Near confluent HSC-2, HSG and HGF cells were incubated for 24 hours with various concentrations of *Allium victorialis* L. extracts and fractions, and the relative viable cell number (A_{540}) was determined by MTT method. 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve. Each value represents the mean from duplicate determinations. Control A_{540} values of HSC-2, HSG and HGF cells were 1.154, 0.701 and 0.262, respectively. 2) -: inactive (>100μg/mL).

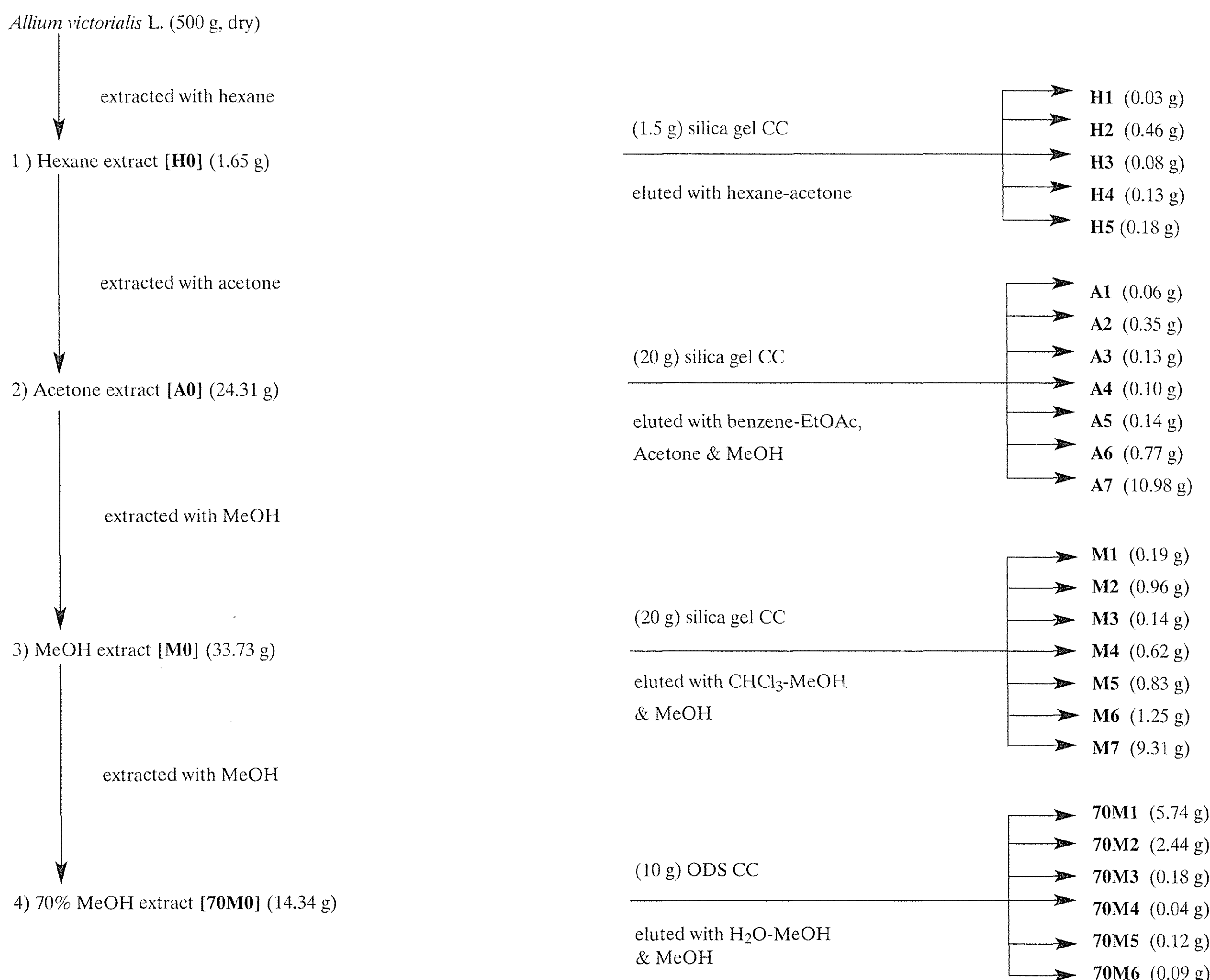

Figure 2. *Fractional separation of Allium victorials L. extracts. CC: column chromatography.*

showed higher cytotoxic activity and produced weak radical signal and $O_2\bullet^-$ scavenging activity. The MDR reversing activity was slightly lower than that of **H0, H3** or **H4**.

Methanol extract [**M0**] was obtained at the highest yield from the starting material (Figure 2) and showed the highest cytotoxic activity among the four extracts. Silica gel column chromatography yielded higher cytotoxic fractions, which produced higher radical intensity and $O_2\bullet^-$ scavenging activity. These fractions showed the highest MDR reversing activity.

The 70% extract [**70M0**] showed the lowest cytotoxic activity. ODS column chromatography yielded fractions with slightly higher cytotoxic activity. These fractions showed the highest radical intensity and $O_2\bullet^-$ scavenging activity, but their MDR reversing activity was much less than that of **M0** fractions.

Taken together, the cytotoxic activity cannot be determined only by radical intensity. Hydrophobic substances in **H0** or **A0** might produce cytotoxic activity, possibly *via* radical-independent mechanisms. On the other hand, more hydrophilic substances in **70M0** might produce cytotoxic activity, possibly *via* radical-mediated mechanisms and their cytotoxic activity might be enhanced in combination with radical generating compounds such as ascorbate (24) or vitamin K_3 (25). We also found that **70M0** fractions, which produced radicals, showed the highest $O_2\bullet^-$ scavenging activity. We had previously found that vitamin K_3 not only generated radicals but also scavenged $O_2\bullet^-$ (26). These data suggest that the same substance in the **70M0** fraction showed both prooxidant and antioxidant properties. Further purification of these fractions would be neccessary to confirm this possibility.

Table II. *Radical generation and* $O_2\bullet^-$ *scavenging activity of Allium victorialis L. extracts and fractions.*

Extract or fraction	Radical intensity		$O_2\bullet^-$ scavenging activity (SOD unit/mg)
	pH10.5	pH12.5	
H0	<0.03	<0.03	0.59
H1	<0.03	<0.03	0.35
H2	<0.03	<0.03	0.39
H3	<0.03	<0.03	0.62
H4	<0.03	<0.03	0.39
H5	<0 03	<0.03	0.62
A0	<0.03	<0.03	4.35
A1	<0.03	<0.03	0.34
A2	<0.03	<0.03	0.69
A3	<0.03	0.06	3.70
A4	<0.03	<0.03	2.82
A5	<0.03	<0.03	3.27
A6	<0.03	<0.03	3.81
A7	N.D.	N.D.	5.69
M0	<0.03	<0.03	5.79
M1	<0.03	<0.03	0.61
M2	<0.03	<0.03	5.64
M3	0.10	0.14	9.46
M4	0.10	0.16	7.66
M5	<0.03	<0.03	5.06
M6	<0.03	<0.03	8.47
M7	<0.03	<0.03	3.96
70MO	<0.03	<0.03	4.04
70M1	<0.03	<0.03	1.27
70M2	<0.03	<0.03	3.86
70M3	0.18	0.81	14.75
70M4	0.24	0.91	12.49
70M5	0.19	0.81	14.12
70M6	0.16	0.31	7.57
Gallic acid	0. 78	0.18	6040

N.D.: not determined.

We also found that human gingival fibroblasts (HGF) were relatively resistant to *Allium victorialis* L. extracts and fractions when compared with human oral tumor cell lines. We recently found that HGF cells were relatively resistant to vitamin K_2 (27), antioxidants (28), tannins (15), flavonoids (29) and saponins (30).

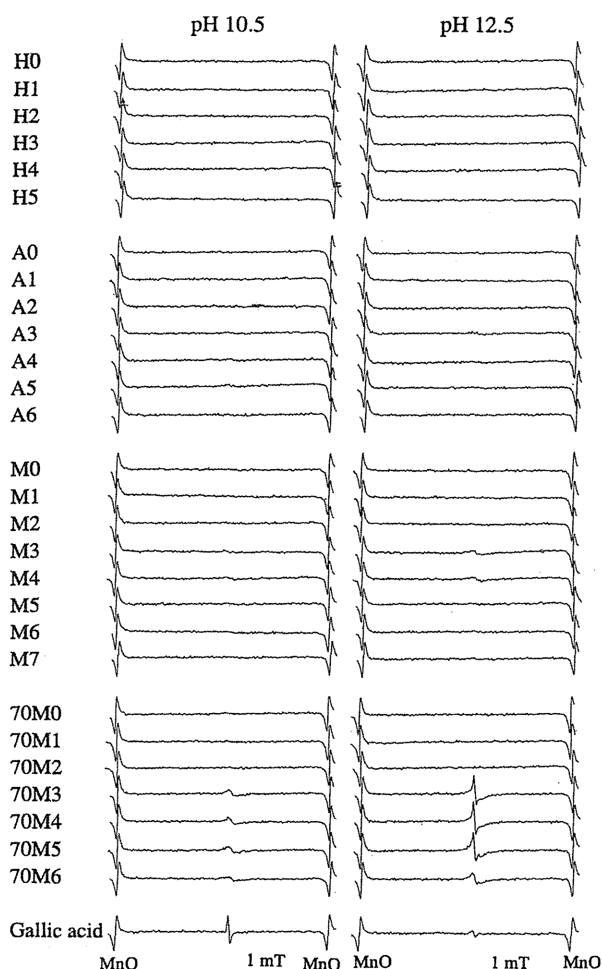

Figure 3. *ESR spectra of Allium victorialis L. extracts and fractions (3 mg/mL) except for gallic acid (0.03 mg/mL) measured 1 minute after dissolving in 0.1M $NaHCO_3/Na_2CO_3$, pH 10.5 or 0.1M KOH (pH 12.5).*

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Table III. *MDR reversal by Allium victorialis L. extracts and fractions (40 µg/mL) in mouse lymphoma-5178 cells.*

Extract or fraction	Concentration (µg/mL in DMSO)	Forward scatter height (cell size ratio)	Side scatter height (granulation of cell ratio)	Fluorescence one height (FL-1)[a]	Fluorescence activity ratio[b]
Par(control)[c]		473.01	140.75	1063 04	
MDR+R123(mean)[d]		532.47	165.33	**20.98**	**1**
(±)-verapamil (positive control[e])	10	530.02	161.96	165.27	7.88
H0	ND				
Hl	ND				
H2	ND				
H3	40	538.35	153.45	867.67	41.36
H4	40	530.81	170.93	1794.55	85.54
H5	40	506.81	192.21	1384.45	65.99
A0	40	494.24	138.50	92.92	4.43
Al	40	470.94	142.52	20.00	0.95
A2	ND				
A3	40	502.14	149.42	700.14	33.37
A4	40	496.80	142.41	412.38	19.66
A5	40	503.87	149.55	550.92	26.26
A6	40	505.59	149.44	545.00	25.98
A7	40	484.24	132.21	28.98	1.38
Gallic acid	40	480.15	119.76	165.27	7.88
DMSO	40	499.12	147.42	17.16	0.82
Par(control)		466.48	171.20	943.55	
MDR+R123(mean)		561.30	200.57	**9.14**	**1**
(±)-verapamil (positive control)	10	588.04	208.24	21.27	2.33
M0	40	597.01	206.80	677.85	74.16
Ml	40	574.51	205.84	62.27	6.81
M2	40	578.49	201.16	921.50	100.82
M3	40	594.12	211.15	1015.08	111.06
M4	40	594.36	225.20	756.74	82.79
M5	40	600.98	210.35	713.99	78.12
M6	40	592.66	208.93	1104.70	120.86
M7	40	606.19	218.31	14.37	1.57
70MO	ND				
70M 1	ND				
70M2	ND				
70M3	40	593.74	222.90	10.09	1.10
70M4	40	596.82	221.76	10.39	1.14
70M5	40	586.80	216.88	10.58	1.16
70M6	40	570.74	218.26	976.33	106.82
Gallic acid	40	591.50	178.83	16.39	1.79

NB: the *Allium victoriallis* L. extracts of **H0, H1, H2, 70M0, 70M1** and **70M2** were not completely dissolved in DMSO.

a) references 22 and 23.

b) The R123 accumulation was calculated from fluorescence of one height value using the following equation:

$$\log(y)=\log_{10}\frac{\times}{256}$$

then the fluorescence activity ratios were calculated according to the formula given below:

$$\text{MDR reversal activity} = \frac{\text{(MDR-treated/MDR control)}}{\text{(parental-treated/parental control)}}$$

c) Par: a parental cell without *MDR-1* gene. d) MDR: a parental cell with *MDR- 1* gene.

Table IV. *MDR reversal by Allium victorialis L. extracts and fractions(4 μg/mL) in mouse lymphoma-5178 cells.*

Extract or fraction	Concentration (μg/mLin DMSO)	Forward scatter height (cell size ratio)	Side scatter height (granulation of cell ratio)	Fluorescence one height (FL-1)[a]	Fluorescence activity ratio[b]
Par(control)[c]		438.82	114.74	1046.86	
MDR+R123(mean)[d]		580.64	176.41	**14.85**	**1**
(±)-verapamil (positive control)	5	540.94	160.91	38.00	2.56
H0	ND				
H1	ND				
H2	ND				
H3	4	543.70	168.65	172.61	11.62
H4	4	541.33	166.08	384.75	25.91
H5	4	574.80	182.59	109.85	7.40
A0	ND				
A1	ND				
A2	ND				
A3	4	570.10	174.82	98.47	6.63
A4	4	567.15	174.71	69.18	4.66
A5	4	573.75	181.69	17.08	1.15
A6	4	570.30	177.81	14.33	0.96
A7	ND				
M0	4	578.65	176.81	237.07	15.96
M1	4	575.52	181.62	10.61	0.71
M2	4	576.88	175.66	1161.44	78.21
M3	4	576.89	175.10	734.86	49.49
M4	4	588.66	180.87	689.54	46.43
M5	4	590.77	182.61	575.26	38.74
M6	4	580.22	180.21	392.18	26.41
M7	4				
70MO	ND				
70M1	ND				
70M2	ND				
70M3	ND				
70M4	ND				
70M5	ND				
70M6	4	575.37	178.98	22.03	1.48
DMSO	4	585.92	175.21	11.14	0.75

Footnotes: As in Table III.

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