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Anti-herpetic Activity of Various Medicinal Plant Extracts

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In order to find antiviral compounds against *Herpes simplex* virus type I (HSV-1) and II (HSV-2) from natural products, a convenient virus-induced cytopathic effect (CPE) inhibition assay was introduced. More than 300 fractions were prepared by solvent fractionation from sixty collected plants or purchased herbal medicines, and their anti-herpetic activities were evaluated. Among them, several medicinal plants showed potent anti-herpetic activity. Selective indexes (SI) of the EtOAc extract of Caraganae Radix (*Caragana sinica*) against HSV-1 and HSV-2 were more than 8.06 and 24.79, SI of the MeOH extract of *Acer okamotoanum* leaves were 3.92 and 3.51, SI of the CH_2Cl_2 extract of Veratri Rhizoma et Radix (*Veratrum patulum*) were 5.49 and 1.31 and SI of the MeOH extract of aerial part of Osmundae Rhizoma (*Osmunda japonica*) were more than 3.45 and 1.25, respectively.

Key word : Antivirals, Natural products, Screening, *Herpes simplex* virus type I, II, Selective index (SI)

INTRODUCTION

Although the average life span has increased very dramatically by virtue of the progress of modern medicine, many people suffer from viral diseases including AIDS, hepatitis B and herpetic diseases. Even though, nucleoside analogs (Mitsuya *et al.*, 1986) such as AZT (Moore *et al.*, 1991, Pizzo *et al.*, 1988, Yarchoan *et al.*, 1986, Fischl *et al.*, 1987), ddI (Yarchoan *et al.*, 1989) and ddC (Yarchoan *et al.*, 1988) were used for the treatment of HIV infection, and Acyclovir was approved for the treatment of herpetic disease, undesired side effects (Richman *et al.*, 1987) and the appearance of resistant strains (Larder *et al.*, 1989) make the development of new antiviral agents an urgent task. In spite of many efforts to discover new antiviral agents, progress has been poor. Development of new drugs from herbal medicines may avoid the side effects or toxicity of synthetic drugs. Two main approaches are used to obtain lead compounds from natural products. The first method involves research based on findings from a thorough literature search of the gold mine of medicine, folk medicine and traditional medicine. The second method uses blind screening of natural extracts against a suitable bioassay. During our search for antiviral agents from natural products, we carried out anti-herpetic assay on about 300 species of plants. A convenient virus-induced cytopathic effect (CPE) inhibition assay was carried out to evaluate *in vitro* anti-herpetic activity of various medicinal plants. The present paper describes partial results of the evaluation of anti-herpetic activity against HSV-1, 2 for various medicinal plants.

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MATERIALS AND METHODS

Plant material and reagents

Plant materials were collected in South Korea or purchased from local Korean herb drug markets and identified taxonomically by an expert of Korea Institute of Science & Technology (KIST). All voucher specimens were deposited in our laboratory. Dulbecco's modified eagle (DME), Fetal bovine serum (FBS) and Trypsin were purchased from Gibco. Gentamycin and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Sigma. Vero cell (African green monkey kidney cell, ATCC CCL 81), *Herpes simplex* virus type I (HSV-1) strain F (ATCC VR-733), *Herpes simplex* virus type II (HSV-2) strain MS (ATCC VR-734) were purchased from American Type Culture Collection (ATCC).

Preparation of plant test samples

Air-dried plants or purchased materials were extracted twice with MeOH at room temperature. The extracts were filtered and the filtrates were concentrated under reduced pressure to dryness. The resulting MeOH extracts were then partitioned consecutively with H_2O, CH_2Cl_2, EtOAc, and n-BuOH. Each organic layer was evaporated under reduced pressure and the resulting organic extracts were used as test sample fractions. Weighed samples of 40 mg each were dissolved in 1 mL of 100% dimethyl sulfoxide (DMSO) then diluted with culture medium to the appropriate concentration. They were filtered again with a microfilter (Sterivex 0.22 μm) prior to testing.

Cells and viruses

Vero cell was cultured with dulbecco's modified eagle (DME) medium supplemented with 5% (v/v) heat-inactivated fetal bovine serum (FBS) and 4 μg/mL gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. Vero cell was subcultured twice a week. The stock of *Herpes simplex* virus type I (HSV-1) and type II (HSV-2) were prepared from culture supernatant of HSV-1 and HSV-2 infected vero cells. The virus titer of the supernatant was determined using a MTT assay. The virus stock was stored as aliquots at -70°C until used.

Quantitation of the titer of HSV-1, HSV-2

In order to determine the titer of the virus, a MTT assay was carried out as follows (Pauwels *et al.*, 1988, Françcois *et al.*, 1986) : Vero cells (3×10^3 cells/well) were seeded into a 96-well plate. After three to four days of incubation, a confluent monolayer was generally obtained. After washing the cells, 100 μL of various tenfold-diluted concentrations of the virus solution was added to each well and incubated for 60 min at 37°C. After absorption of the virus, 100 μL of culture medium was added, then incubated for three days. After removing the culture medium, 50 μL of 0.3% MTT soln. (3 mg MTT was dissolved in 1 mL DME medium supplemented with 2% FBS) was added then incubated for 120 min at 37°C in a humidified atmosphere with 5% CO_2. The acidified isopropanol/6% triton X-100 solution, 100 μL, was added to each well. The plate was then vigorously shaken in order to ensure solubilization of the blue formazan. The optical density was measured using a microplate reader (Vmax, Molecular Devices) with a 540 nm test wavelength and a 690 nm reference wavelength.

The evaluation of anti-herpetic activity by CPE (cytopathic effect) inhibition assay

Vero cells (3×10^3 cells/well) were seeded into a 96-well plate. After three to four days of incubation, a confluent monolayer was generally obtained. After washing the cells, 100 μL of the virus solution, diluted with DME medium supplemented with 2% FBS, which was equivalent to 50% cell culture inhibitory dose ($CCID_{50}$) was added to each well and incubated for 60 min at 37°C. After absorption of the virus, the culture medium was removed and 100 μL of culture medium including various concentrations of sample was added to each well in duplicate, then incubated at 37°C in a humidified atmosphere with 5% CO_2 for three days. After removing the culture medium, MTT assay was carried out as described above. The antiviral effective concentration was expressed as an EC_{50}, the concentration of the sample required to inhibit virus-induced CPE by 50%. In order to make clear the cytotoxicity of sample, mock-infected cells were also prepared simultaneously. After addition of various concentrations of sample to each confluent monolayer in duplicate, further incubation was followed for three days. After removing the culture medium, MTT assay was carried out. CC_{50} (50% cytotoxic concentration) was determined by comparing the relative cell number of the sample treated well with the cell number of the non-treated well. Antiviral activity and cytotoxicity were calculated as follows:

$$\frac{(A_T)_{HSV}-(A_C)_{HSV}}{(A_C)_{mock}-(A_C)_{HSV}}\times100$$

$$\frac{1-(A_T)_{mock}}{(A_C)_{mock}}\times100$$

$(A_T)_{HSV}$ is the Optical density (OD) of the cell, treated with the virus and samples

$(A_C)_{HSV}$ is the OD of the cell, treated with the virus (virus control).

$(A_T)_{mock}$ is the OD of the mock-infected cell, treated with the samples

$(A_C)_{mock}$ is the OD of the mock-infected cell only (cell control).

RESULTS AND DISCUSSION

In the present study, we carried out a convenient and rapid CPE (cytopathic effect) inhibition assay to evaluate anti-herpetic activity against HSV-1, 2 for various medicinal plants. The feasibility of *in vitro* mass screening holds the key to the success of new drug development. It is especially important in the case of trying to find lead compounds from natural products through activity-guided fractionation, since accuracy and rapidity of bio-assay are the determining factors. Because of its rapidity and accuracy, CPE inhibition assay was used more frequently for mass screening than plaque assay. The virus titer of HSV-1, 2 was determined by a MTT assay. A diluted

Table I. Results on various plant extracts for the anti-herpetic activity[a]

Plants	Parts of plant used	Frac.[b]	Toxicity CC_{50}[c] (μg/ml)	Antiviral activity EC_{50} (μg/ml)[d]		Selectivity index (SI)[e]	
				HSV-1	HSV-2	HSV-1	HSV-2
Acer okamotoanum	leaf	M	297.8	76	84.9	3.92	3.51
		D	59.3	>59.3	>59.3	<1	<1
		E	25.4	>25.4	>25.4	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Acer okamotoanum	stem	M	219	>219	>219	<1	<1
		D	150	69.5	>150	2.16	<1
		E	69.6	>69.6	>69.6	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Allium victorialis var. *platyphyllum*	whole plant	M	>300	>300	>300	NC	NC
		D	156	>156	>156	<1	<1
		E	>300	251	>300	>1.2	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Ambrosia artemisiifolia var. *elatior*	whole plant	M	>300	>300	240	NC	>1.25
		D	106	>106	>106	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Angelica tenuissima	root	M	>300	>300	>300	NC	NC
		D	95.9	>95.9	>95.9	<1	<1
		E	135.1	>135.1	>135.1	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Cacalia auriculata var. *matsumurana*	aerial part	M	>300	285	>300	>1.05	NC
		D	116.4	>116.4	>116.4	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Cacalia auriculata var. *matsumurana*	underground part	M	>300	>300	>300	NC	NC
		D	>300	300	>300	>1	NC
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Callicarpa japonica	aerial part	M	>300	>300	>300	NC	NC
		D	119	>119	>119	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Caulophyllum robustum	aerial part	M	>50.7	>50.7	>50.7	<1	<1
		D	30.9	>30.9	>30.9	<1	<1
		E	30.6	>30.6	>30.6	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Caulophyllum robustum	underground part	M	31	>31	>31	<1	<1
		D	126	>126	>126	<1	<1
		E	19.6	>19.6	>19.6	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC

Table I. Continued

Plants	Parts of plant used	Frac.[b]	Toxicity CC_{50}[c] (μg/ml)	Antiviral activity EC_{50} (μg/ml)[d]		Selectivity index (SI)[e]	
				HSV-1	HSV-2	HSV-1	HSV-2
Caragana sinica	root	M	>300	>300	>300	NC	NC
		D	188	>188	>188	<1	<1
		E	>300	37.2	>12.1	>8.06	>24.79
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Cayratia japonica	aerial part	M	>300	171	>300	1.75	NC
		D	>300	92.7	237	>3.24	>1.27
		E	>300	241	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Celastrus orbiculatus	stem	M	263	>263	>263	<1	<1
		D	61.6	>61.6	>61.6	<1	<1
		E	166	>166	>166	<1	<1
		B	274	>274	>274	<1	<1
		H	>300	>300	>300	NC	NC
Centella asiatica	aerial part	M	>300	180	>300	>1.67	NC
		D	108	>108	>108	<1	<1
		E	56.9	32.5	>56.9	1.75	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Cimicifuga heracleifolia	root	M	158.1	59.9	97.9	2.64	1.61
		D	63.4	27.4	>63.4	2.31	<1
		E	164.2	62.3	>164.2	2.64	<1
		B	266.8	84.8	>266.8	3.15	<1
		H	>300	>300	>300	NC	NC
Cirsium pendulum	aerial part	M	>300	>300	>300	NC	NC
		D	>300	>300	>300	NC	NC
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Clematis apiifolia	aerial part	M	>300	>300	>300	NC	NC
		D	230	>230	>230	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Clinopodium chinense var. *parviflorum*	whole plant	M	>300	>300	>300	NC	NC
		D	148	<148	<148	<1	<1
		E	>300	>300	236	NC	>1.27
		B	160	>160	>160	<1	<1
		H	>300	>300	>300	NC	NC
Condonopsis lanceloata	root	M	>300	>300	>300	NC	NC
		D	>300	>300	>300	NC	NC
		E	>300	>300	>300	NC	NC
		B	25.1	>25.1	>25.1	<1	<1
		H	>300	>300	>300	NC	NC
Dystaenia takeshimana	aerial part	M	>300	>300	>300	NC	NC
		D	150	>150	>150	<1	<1
		E	68.7	>68.7	>68.7	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC

Table I. Continued

Plants	Parts of plant used	Frac.[b]	Toxicity CC_{50}[c] (μg/ml)	Antiviral activity EC_{50} (μg/ml)[d]		Selectivity index (SI)[e]	
				HSV-1	HSV-2	HSV-1	HSV-2
Dystaenia takeshimana	underground part	M	>300	>300	>300	NC	NC
		D	155	>155	>155	<1	<1
		E	25.4	>25.4	>25.4	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Elsholtzia ciliata	aerial part	M	>300	224	249	>1.34	>1.2
		D	165	>165	>165	<1	<1
		E	56.1	>56.1	>56.1	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Epilobium pyrricholophum	whole plant	M	>300	>300	>300	NC	NC
		D	242	>242	>242	<1	<1
		E	120	>120	>120	<1	<1
		B	95	>95	>95	<1	<1
		H	>300	>300	>300	NC	NC
Euonymus oxyphyllus	stem	M	>300	>300	>300	NC	NC
		D	119	>119	>119	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Eupatorium lindleyanum	whole plant	M	57.5	>57.5	>57.5	<1	<1
		D	114.9	>114.9	>114.9	<1	<1
		E	140.4	78.09	>140.4	1.8	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Fagus crenata var. *multinervis*	stem	M	154	>154	>154	<1	<1
		D	53.5	>53.5	>53.5	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Hepatica maxima	aerial part	M	>300	>300	>300	NC	NC
		D	>300	>300	>300	NC	NC
		E	>300	141	>300	2.1	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Hepatica maxima	unerground part	M	>300	>300	>300	NC	NC
		D	107	>107	>107	<1	<1
		E	50.5	>50.5	>50.5	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Heracleum moellendorffii	aerial part	M	>300	>300	>300	NC	NC
		D	>300	>300	>300	NC	NC
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Hydrangea serrata for. *acuminata*	leaf	M	137	>137	>137	<1	<1
		D	210	>210	>210	<1	<1
		E	>300	>300	>300	NC	NC
		B	24.2	>24.2	>24.2	<1	<1
		H	>300	>300	>300	NC	NC

Table I. Continued

Plants	Parts of plant used	Frac.[b]	Toxicity CC_{50}[c] (μg/ml)	Antiviral activity EC_{50} (μg/ml)[d]		Selectivity index (SI)[e]	
				HSV-1	HSV-2	HSV-1	HSV-2
Hydrangea serrata for. *acuminata*	stem	M	291	>291	>291	<1	<1
		D	135	>135	99.9	<1	1.35
		E	161	99.4	97.5	1.62	1.65
		B	29.3	>29.3	>29.3	<1	<1
		H	122	>122	>122	<1	<1
Lonicera insularis	aerial part	M	>300	>300	>300	NC	NC
		D	144	>144	>144	<1	<1
		E	>300	225	225	>1.33	>1.35
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Lonicera maackii	stem	M	>300	>300	>300	NC	NC
		D	>300	>300	128	NC	>1.24
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Lythrum anceps	aerial part	M	>300	>300	>300	NC	NC
		D	135	>135	>135	<1	<1
		E	226	>226	>226	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Majanthemum dilatatum	whole plant	M	144	>144	>144	<1	<1
		D	99.2	>99.2	>99.2	<1	<1
		E	227	>227	>227	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Osmunda japonica	underground part	M	>300	204.66	>179.7	>1.47	>1.67
		D	173	>173	>173	<1	<1
		E	156.04	55.77	54.96	2.8	2.84
		B	194	81.37	65.31	2.38	2.96
		H	>300	>300	>300	NC	NC
Osmunda japonica	aerial part	M	>300	86.97	239.6	>3.45	>1.25
		D	>300	249.2	>300	>1.2	NC
		E	153.5	74.18	72.73	2.07	2.11
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Patrinia villosa	whole plant	M	>300	>300	>300	NC	NC
		D	144	>144	>144	<1	<1
		E	86.2	>86.2	>86.2	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Potentilla cryptotaeniae	whole plant	M	>300	>300	>300	NC	NC
		D	>300	>300	>300	NC	NC
		E	204	>204	>204	<1	<1
		B	223	>223	>223	<1	<1
		H	>300	>300	>300	NC	NC
Pyrus calleryana var. *fauriei*	stem	M	>300	184.68	236.5	1.62	1.27
		D	107.23	>107.23	>107.2	<1	<1
		E	>300	283.15	246.2	>1.05	>1.21
		B	93.07	>93.07	>93.07	<1	<1
		H	>300	>300	>300	NC	NC

Table I. Continued

Plants	Parts of plant used	Frac.[b]	Toxicity CC_{50}[c] (μg/ml)	Antiviral activity EC_{50} (μg/ml)[d]		Selectivity index (SI)[e]	
				HSV-1	HSV-2	HSV-1	HSV-2
Quercus dentata	stem	M	107.86	>107.86	107.8	<1	<
		D	28.08	>28.08	>28.08	<1	<1
		E	97.3	>97.3	>97.3	<1	<1
		B	174.35	>174.35	>174.3	<1	<1
		H	229.91	>229.91	>229.9	<1	<1
Rhamnus davurica	leaf	M	>300	>300	>300	NC	NC
		D	123	>123	>123	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Rhamnus davurica	stem	M	>300	>300	>300	NC	NC
		D	181	>181	>181	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Rumohra standishii	aerial part	M	194.5	92.1	>194.5	2.11	<1
		D	81.6	25.2	>81.6	3.24	<1
		E	177	82.2	>177	2.15	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Rumohra standishii	underground part	M	>300	>300	>300	NC	NC
		D	247	>247	>247	<1	<1
		E	274	>96.8	>78.2	2.83	3.5
		B	274	>274	>274	<1	<1
		H	>300	>300	>300	NC	NC
Sambucus sieboldiana var. *pendula*	leaf	M	>300	>300	>300	NC	NC
		D	>300	>300	>300	NC	NC
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Schizophragma hydrangeoides	leaf	M	195	>195	>195	<1	<1
		D	>300	270.3	>300	>1.11	NC
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Schizophragma hydrangeoides	stem	M	>300	>300	>300	NC	NC
		D	174	>174	>174	<1	<1
		E	167	>167	>167	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Scrophularia buergeriana	root	M	123	>123	>123	<1	<1
		D	>300	>300	>300	NC	NC
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Scrophularia koraiensis	aerial part	M	>300	>300	>300	NC	NC
		D	81.6	81.6	>81.6	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC

Table I. Continued

Plants	Parts of plant used	Frac.[b]	Toxicity CC_{50}[c] (μg/ml)	Antiviral activity EC_{50} (μg/ml)[d]		Selectivity index (SI)[e]	
				HSV-1	HSV-2	HSV-1	HSV-2
Scrophularia koraiensis	underground part	M	>300	>300	>300	NC	NC
		D	191.6	98	>191.6	>1.96	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Securinega suffruticosa	aerial part	M	>300	>300	>300	NC	NC
		D	>300	>300	>300	NC	NC
		E	130	90.8	>130	1.43	<1
		B	280	>280	>280	<1	<1
		H	>300	>300	>300	NC	NC
Sedum sarmentosum	whole plant	M	>300	>300	>300	NC	NC
		D	129	>129	>129	<1	<1
		E	>300	198	>300	>1.52	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Siegesbeckia glabrescens	whole plant	M	130	>130	>130	<1	<1
		D	59.5	>59.5	>59.5	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Smilax sieboldii var. *inermis*	stem	M	>300	>300	>300	NC	NC
		D	>300	>300	>300	NC	NC
		E	88.7	>88.7	>88.7	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Sophora flavescens	underground part	M	120	64	76	1.88	1.58
		D	170	86	>170	1.98	<1
		E	125	>125	93.3	<1	1.34
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Staphylea bumalda	stem	M	>300	>300	>300	NC	NC
		D	108	>108	>108	<1	<1
		E	124.9	>124.9	>124.9	<1	<1
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Taraxacum coreanum	whole plant	M	>300	>300	>300	NC	NC
		D	120	>120	>120	<1	<1
		E	237	>237	65.0	<1	3.64
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Teucrium veronicoides	aerial part	M	283	>283	>283	<1	<1
		D	22.49	>22.49	>22.49	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC
Veratrum patulum	whole plant	M	>300	168	>300	>1.79	NC
		D	129	23.5	98.2	5.49	1.31
		E	113	76.4	90.8	1.48	1.24
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC

Table I. Continued

Plants	Parts of plant used	Frac.[b]	Toxicity CC_{50}[c] (μg/ml)	Antiviral activity EC_{50} (μg/ml)[d]		Selectivity index (SI)[e]	
				HSV-1	HSV-2	HSV-1	HSV-2
Weigela florida	aerial part	M	>300	>300	>300	NC	NC
		D	87.5	>87.5	>87.5	<1	<1
		E	>300	>300	>300	NC	NC
		B	>300	>300	>300	NC	NC
		H	>300	>300	>300	NC	NC

[a]The anti-herpetic activity was measured by CPE-MTT assay.
[b]M:MeOH ext, D:CH_2Cl_2 ext, E:EtOAc ext, B:BuOH ext, H:H_2O ext
[c]50% Cytotoxic Concentration (CC_{50})is the concentration of the 50% cytotoxic effect.
[d]50% Effective Concentration (EC_{50}) is the concentration of the sample required to inhibit virus-induced CPE 50%.
[e]Selective Index (SI)=CC_{50}/EC_{50}

HSV-1, 2 concentration at 100 μL which was equivalent to 50% cell culture inhibitory dose ($CCID_{50}$) was used as a seeding virus throughout the experiment. Acyclovir (ACV), which is clinically used for the treatment of herpetic desease, was used as a positive control under this assay system. Selective Indexes (SI) of ACV against HSV-1, 2 were more than 333.3 and 156.2 Table 1 shows the inhibitory activity on the cytopathic effect (CPE) of various medicinal plants. Most of the medicinal plants didn't show significant CPE inhibitory activity. Among them, the EtOAc ext. of *Caragana sinica* showed a high level of CPE inhibitory activity. Selective indexes (SI) of the EtOAc extract of Caraganae Radix (*Caragana sinica*) against HSV-1 and HSV-2 were more than 8.06 and 24.79, respectively. SI of the MeOH extract of *Acer okamotoanum* leaves were 3.92 and 3.51, SI of the CH_2Cl_2 extract of Veratri Rhizoma et Radix (*Veratrum patulum*) were 5.49 and 1.31 and SI of the MeOH extract of aerial part of Osmundae Rhizoma (*Osmunda japonica*) were more than 3.45 and 1.25, respectively. Purification of active components is currently under way based on our assay results.

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