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Screening of Antiviral Activities of Korean Medicinal Herbs and Traditional Prescriptions Against Herpes Simplex Virus Type-1

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=국문초록=

한약단미제 및 탕제의 항 Herpes Simplex Virus Type-1 활성탐색

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강봉주 · 양기상 · 김명희[1] · 박갑주

향약집성방 및 동의보감등 한의학 서적을 기초로 하여 선택한 탕제 45종과 단미제 80종의 메탄올과 열수추출물을 가지고 MTT assay를 실시하여 항Herpes simplex virus-1 (HSV-1)에 대한 활성을 조사하였다. 열수추출 탕제 45종 중 백사전방, 현람방I, 현람방II, 반천청방 4종에서 항 HSV-1 활성을 나타냈고, 이들의 SI (selective index)값은 2.1±0.5에서 11.8±2.2범위의 값을 가졌으며 메탄올추출 탕제 45종 중에서는 단치시호탕III, 반천청방, 정향울금방, 대황오배자고, 홍인락삼등방, 호장해독탕에서 활성을 보였고 이들의 SI값은 1.7±0.2에서 10.5±3.1범위의 값을 나타냈다. 열수추출 단미제 중 계지, 관중, 구인분, 대황, 자화지정, 포공영, 호장근, 황백등 8종에서 항 HSV-1 활성이 나타났고, 이들의 SI값은 1.6±0.1에서 10.2±0.7범위의 값을 가졌으며 메탄올추출 탕제 중에서는 계지, 목방기, 상지, 호장근에서 활성을 보였고 이들의 SI값은 2.9±1.5에서 9.3±0.5범위의 값을 나타내었다. 처방제 중 반천청방은 열수추출물과 메탄올 추출물 모두에서 항바이러스활성을 나타냈고, 단미제 중 계지와 호장근도 열수와 메탄올 추출물 모두에서 항바이러스 활성을 보였다. 이들 탕제 및 단미제는 분획 및 분석실험을 실시하여 활성성분을 추적하고 있다.

Key Words: Herpes simplex virus-1 (HSV-1), MTT assay, SI (selective index)

INTRODUCTION

Herpes simplex viruses (HSV) are among the most common infectious agents of man. A high prevalence rate of HSV infection has been found in virological and serological studies of healthy subjects. Herpes simplex virus type 1 (HSV-1) infection occur most frequently during childhood and affect most often the mouth, lips, and skin site above the waist. HSV-1 is a neurotropic virus which is capable of establishing a lifelong latent state in the nervous system [1~4].

The herpetic infection is common in humans and is a major cause of morbidity especially in immunosuppressed patients with the acquired immunodeficiency syndrome or in transplant recipients. Though chemotherapies have been developed in many countries, the anti-HSV agents are not satisfactory to human kind because of their toxic reactions and side effects [5,6]. Consequently, efficient HSV treatments have not been developed. Acycloguanosine (Zovirax) and vidarabin have been licensed for use in HSV patients in the United States and other countries [7]. However, acycloguanosine is not a complete treatment agent, and in addition, it

raises adverse side effects and can lead to the recovery of multiple strains of acycloguanosine-resistant HSV from treated patients [8]. Generally, natural products are less toxic to normal cells, but more toxic to virus-infected cells than the chemotherapeutic agents [9,10].

In Korea, many Korean medicinal herbs and Korean traditional prescriptions are expected to be safe anti-HSV agents without major adverse effects and we have therefore focused on these as anti-HSV agents [11]. In this study, medicinal herbs and traditional prescriptions extracted by methanol and boiling water were screened to detect anti-HSV-1 activity, by means of the MTT (3-4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay.

MATERIALS AND METHODS

Viruses, cells and Media

Herpes simplex virus type-1 (HSV-1) F strain (ATCC VR-733, Rockville, MD) was obtained from Korean AIDS (Acquired Immune Deficiency Syndrome) Center, and the Vero cell line (African green monkey kidney cell, KCLB10081) was obtained from Korean Cell Line Bank (KCLB). The virus was purified by plaque assay. The cells were grown as monolayer cell culture in 75 cm^2 plastic tissue culture flasks (Nunc, Roskilde, Denmark) in minimum essential medium (MEM, GibcoBRL, Gaithersburg, MD) with 10% fetal bovine serum (FBS, GibcoBRL), 0.22% sodium bicarbonate (Sigma, St. Louis, MO), 50 μg/ml gentamicin (GibcoBRL) and routinely maintained in MEM with 2% fetal bovine serum [12].

Plaque assay and isolation of virus clone

Vero cell monolayers in 75 cm^2 tissue culture flask were inoculated with HSV-1 at a multiplicity of infection (MOI) of approximately 0.1 pfu (plaque forming unit) per cell. The virus adsorbed to the

Table 1. Korean traditional prescriptions

No.	Prescriptions	No.	Prescriptions
1	Sajŏnyobang	23	Chŏngsŏnch'ŏn-gamyobang's prescription 1
2	Paeksajŏnbang	24	Chŏngsŏnch'ŏn-gamyobang's prescription 2
3 – 1	Hyŏllambang I	25	Folk medicinal prescription 1
3 – 2	Hyŏllambang II	26	Folk medicinal prescription 2
4	Hyŏllambang 1	27	Folk medicinal prescription 3
5	Mach'ihyŏnhaedokt'ang	28	Folk medicinal prescription 4
6	Mach'ihyŏnhaedokt'ang 1	29	Folk medicinal prescription 5
7	Mach'ihyŏnhaedokt'ang 2	30	Folk medicinal prescription 6
8	Mach'ihyŏnhaedokt'ang 3	31	Folk medicinal prescription 7
9	Tanch'ishihot'ang	32	Folk medicinal prescription 8
10	Tanch'ishihot'ang 1	33	Folk medicinal prescription 9
11	Tanch'ishihot'ang 2	34	Folk medicinal prescription 10
12	Tanch'ishihot'ang 3	35	Folk medicinal prescription 11
13	Tanch'ishihot'ang 4	36	Folk medicinal prescription 12
14	Tanch'ishihot'ang 5	37	Folk medicinal prescription 13
15	Tanch'ishihot'ang 6	38	Myŏngbanhaedokt'ang
16	Tanch'ishihot'ang 7	39	Hojanghaedokt'ang
17	Tanch'ishihot'ang 8	40	Tansamyongch'ot'ang
18	Panch'ŏnch'ŏngbang	41	P'ojinhapche
19	Chŏnghyang-ulgŭmbang	42	Paengnyŏnsan
20	Ch'oramgwihobang	43	Paekpujasan
21	Taehwang-obaejago	44	Ch'isajŏnch'ang-ilbang
22	Hong-illaksamdŭngbang	45	Kyuhwasan

Table 2. Korean medicinal herbs

No	Scientific name	Plant part
1	*Glycyrrhiza uralensis* Fisch.	radix
2	*Angelica koreana* Max.	radix
3	*Cinnamomum cassia* Blume	ramulus
4	*Trichosanthes kirilowii* Maxim.	semen
5	*Dryopteris crassirhizoma* Nakai	rhizoma
6	*Pheretima communissima* (Goto et Hatai)	
7	*Lonicera japonica* Thunb.	plos
8	*Trachelospermum asiaticum* var. *intermedium* NAKAI	caulis
9	*Salvia miltiorrhiza* Bunge	radix
10	*Angelica gigas* Nakai	radix
11	*Clerodendron cyrtophyllum* Turcz.	folium
12	*Rheum palmatum* L.	rhizoma
13	*Prunus persica* (L.) Batsch	semen
14	*Portulaca oleracea* L.	herba
15	Mirabilite ($Na_2SO_4.10H_2O$)	
16	Alunite ($AlK(SO_4)_2.12H_2O$)	
17	*Paeonia suffruticosa* Andr.	cortex
18	*Cocculus trilobus* DC.	radix
19	*Akebia quinata* Decne.	caulis
20	*Lobeliae chinensis* Lour.	herba
21	*Ledebouriella seseloides* (Hoflm.) Wolff	radix
22	*Bletilla striata* (Thunb.)Reichb. f.	rhizoma
23	*Imperata cylindrica* Beavu. var. *major* (Nees) C. E. Hubb.	rhizoma
24	*Angelica dahurica* Benth. et Hook. f.	radix
25	*Oldenlandia dffusa* (Willd.) Roxb.	herba
26	*Poria cocos* Wolf	Poria
27	*Pueraria thunbergiana* Benth.	radix
28	*Zizyphus jujuba* Mill.	semen
29	*Morus alba* L.	ramulus
30	*Rehmannia glutinosa* var. *purpurea* Makino	radix et rhizoma
31	Gypsum	
32	*Cryptotympana pustulata* Fabricius	
33	*Asarum sieboldii* Miq.	radix
34	*Bupleurum falcatum* L.	radix
35	*Forsythia viridissima* Lindl.	pructus
36	*Scolopendra subspinipes* mutilans L. Koch	
37	*Rhus chinensis* Mill.	galla
38	*Vaccaria segetalis* (Neck.) Garcke	semen
39	*Stegodon orientalis* Dwen.	
40	*Gentiana scabra* var. *buergeri* (Miq.) Max.	radix
41	*Achyranthes japonica* Nakai	radix
42	*Curcuma longa* L.	rhizoma

Table 2. Continued

No	Scientific name	Plant part
43	Realgar	
44	*Lonicera japonica* Thunb.	caulis
45	*Glycyrrhiza uralensis* Fisch.[a]	radix
46	*Lithospermum erythrorhizon* Sieb. et Zucc.	radix
47	*Viola yedoensis* Mak.	herba
48	*Polyporus umbellatus* (Pers.) Fries	
49	*Poria cocos* Wolf	
50	*Phaseolus angularis* W. F. Wight	semen
51	*Paeonia lactiflora* var. *hortensis* Makino	radix
52	*Eugenia caryophyllata* Thunb.	plos
53	*Uncaria sinensis* (Oliv.) Havil	Ramulus et Uncus
54	*Citrus aurantum* L.	fructus
55	*Anemarrhena asphodeloides* Bunge.	rhizoma
56	*Citrus unshiu* Markovich	Pericarpium
57	Plantago asiatica L.	semen
58	*Atractylodes japonica* Koidz.	rhizoma
59	*Cnidium officinale* Makino	rhizoma
60	*Melia azedarach* var. *japonica* Makino	fructus
61	*Zanthoxylum bungeanum* Maxim.	Pericarpium
62	*Polygonum tinctoria* H. Gross	naturalis
63	*Atractylodes japonica* Koidz.	rhizoma
64	*Thuja orientalis* L.	Folium et Ramulus
65	*Gardenia jasminoides* Ellis.	fructus
66	*Alisma orientale* (Sam.) Juz.	rhizoma
67	*Isatis indigotica* Fort.	radix
68	*Patrinia scabiosaefolia* Fisch.	herba
69	*Taraxacum platycarpum* H. Dahlst.	herba
70	*Chrysanthemum morifolium* Ramat.	flos
71	*Cyperus rotundus* L.	rhizoma
72	*Corydalis ternata* Nakai	rhizoma
73	*Polygonum cuspidatum* Sieb. et Zucc.	radix
74	*Carthamus tinctorius* L.	plos
75	Talc	
76	*Scutellaria baicalensis* Georgi	radix
77	*Astragalus membranaceus* Bunge	radix
78	*Coptis japonica* Makino	rhizoma
79	*Phellodendron amurense* Rupr.	cortex
80	*Magnolia obovata* Thunb.	cortex

Note [a] It was roasted.

cells for 1 hour at 37℃ in 5% CO_2 incubator, and then 15 ml MEM with 2% serum were added to the flask. The culture was incubated at 37℃ until advanced cytopathic effect was observed. The medium was centrifuged at 25,000 × g for 30 min at 4℃. Cell debris was removed and supernatant was used for plaque assay [13].

Approximately 3 × 10^6 Vero cells, in MEM supplemented with 10% serum, were transferred to petri dishes (10 × 15 mm, Nunc) and allowed to at-

Table 3. Korean traditional prescriptions

No.	Prescription name	Scientific name	Plant part
2	Paeksajŏnbang	*Bletilla striata* (Thunb.) Reichb. f. Elephas spp.	rhizoma
3 – 1	Hyŏllambang I	*Clerodendron cyrtophyllum* Turcz. *Taraxacum platycarpum* H. Dahlst. *Portulaca oleracea* L.	folium herba herba
3 – 2	Hyŏllambang II	*Isatis indigotica* Fort. *Taraxacum platycarpum* H. Dahlst. *Portulaca oleracea* L.	radix herba herba
11	Tanch'ishihot'ang	*Paeonia suffruticosa* Andr. *Gardenia jasminoides* Ellis. *Bupleurum falcatum* L. *Angelica gigas* Nakai *Paeonia lactiflora* var. *hortensis* Makino *Cnidium officinale* Makino *Curcuma longa* L. *Corydalis ternata* Nakai	cortex fructus radix radix radix rhizoma rhizoma rhizoma
18	Panch'ŏnch'ŏngbang	*Lobelia chinensis* Lour. *Polygonum tinctoria* H. Gross	herba folium
19	Chŏnghyangulgŭmbang	*Eugenia caryophyllata* Thunb. *Curcuma longa* L. *Bupleurum falcatum* L. *Poncirus trifoliata* (Linne) Rofinesque *Cnidium officinale* Makino *Paeonia lactiflora* var. *hortensis* Makino *Isatis indigotica* Fort. *Glycyrrhiza uralensis* Fisch.	plos rhizoma radix fructus rhizoma radix radix radix
21	Taehwang-obaejago	*Rheum palmatum* L. *Phellodendron amurense* Rupr. *Rhus chinensis* Mill. Mirabilite ($Na_2SO_4.10H_2O$)	rhizoma cortex galla
22	Hong-illaksamdŭngbang	*Uncaria sinensis* (Oliv.) Havil *Lonicera japonica* Thunb. *Viola mandshurica* W. Becker *Oldenlandia diffusa* (Willd.) Roxb. *Trachelospermum asiaticum* var. *intermedium* Nakai *Rehmannia glutinosa* var. *purpurea* Makino *Polygonum cuspidatum* Sieb. et Zucc. *Forsythia viridissima* Lindl. *Paeonia suffruticosa* Andr. *Dryopteris crassirhizoma* Nakai	ramulus et uncus caulis herba herba caulis radix et rhizoma radix fructus cortex rhizoma
39	Hojanghaedokt'ang	*Polygonum cuspidatum* Sieb. *et* Zucc. *Isatis indigotica* Fort. *Paeonia suffruticosa* Andr. *Paeonia lactiflora* var. *hortensis* Makino *Cryptotympana pustulata* Fabricius *Glycyrrhiza uralensis* Fisch.	rhizoma radix cortex radix periostracum radix

Table 4. Anti-HSV-1 activities of Korean traditional prescriptions extracted by boiling water

Prescriptions	ED_{50} (μg/ml)[a]	CD_{50} (μg/ml)[b]	SI [c]
Paeksajŏnbang	2,141.2±158.1	12,437±1311.3	5.8±0.9
Hyŏllambang I	89.5±6.3	519.8±50.6	5.8±0.8
Hyŏllambang II	156.3±42.4	324.2±50.2	2.1±0.5
Panch'ŏnch'ŏngbang	21.2±6.8	242.1±43.1	11.8±2.2
Acycloguanosine (control)	0.11±0.02	49.0±4.4	448.2±94.9

Each value represents mean±S.D., n=5.
Note. [a] 50% cytotoxic dose means dose required to reduce the number of vialble uninfected cells by 50%.
[b] 50% effective dose means dose required to achieve 50% protection of the cells against the cytopathic effect of HSV-1.
[c] Selective index means the ratio of CD_{50} to ED_{50}.

tach and form a monolayer overnight. The medium was removed and the virus inoculum, diluted with PBS, was inoculated onto the cell monolayer. The virus was allowed to adsorb to the cells at 37℃ for 1 hour with intermittent tilting for uniform virus distribution. The cells were then overlaid with 5 ml of MEM supplemented with 5% serum and containing 0.9% agarose. 72 to 96 hours later, another 3 ml of agarose-containing overlay medium was added for feeding purpose. The overlay medium was prepared by autoclaving 9g of low melting temperature agarose in 91 ml of distilled water, cooling the agarose solution to 45℃ and dilution it (1:10) in MEM (with 2% serum) preheated to 40℃. The cells were incubated at 37℃ in a saturated humidity for 3 to 4 days. The plaques were visualized by staining with a 0.01% solution of neutral red (GibcoBRL) in MEM (with 2% serum) for 2 hours. Plaques were picked from a petri dish infected with 10^{-4} diluted virus. A plaque was transferred into 0.5 ml of PBS, pipetted gently to release virus from the agarose and used for inoculation of cell monolayers to develop a virus stock for second plaque assay purification step. The supernatant was stored as virus stock in a -70℃ freezer.

Extracts from Korean Medicinal Herbs and Korean Traditional Prescriptions

Korean medicinal herbs and Korean traditional prescriptions were obtained on the basis of a review of Korean traditional medicine books [14,15] and on the basis of recommendations of Korean traditional medical doctors. Scientific names of herbs and common names of traditional prescriptions are shown Table 1, Table 2 and Table 3.

80 medicinal herbs and 45 traditional prescriptions were screened to detect anti-HSV-1 activities. Both methanol extracts and boiling water extracts were prepared by the following methods. A sample of each herbs or prescriptions was cut to a size of 10 mesh using a cutting mill machine. Thirty grams of each specimen were measured and added to 500 ml of methanol and incubated at 60℃ in a water bath for 18 hours. The aqueous extracts from the samples were lyophilized. The lyophilized extracts were dissolved in mixture of DMSO and distilled H_2O (1:9). The dissolved samples were filtered through membranes of 0.2 μm pore size [16].

Boiling-water extracts were prepared from dried plants. Each plant specimen was added to 1,100 ml of sterilized water, boiled for 150 min. Following procedure was the same method as above for methanol.

The following terminology was used for the extract: the first letter M or C represents mono-herb or complex (prescription) respectively, while the second letter M or W represents methanol or water extract.

Estimation of the Cytotoxicity effect

Extracts were diluted sequentially five fold in six steps: original solution, 5^{-1}, 5^{-2}, 5^{-3}, 5^{-4}, 5^{-5} and added to replicate 96-well culture plates (Falcon, Franklin Lakes, NJ) containing 1.0×10^3 of Vero

Table 5. Anti-HSV-1 activities of Korean traditional prescriptions extracted by methanol

Prescriptions	ED_{50} (μg/ml)	CD_{50} (μg/ml)	SI
Tanch'ishihot'ang	125.8±5.6	319.0±12.9	2.5±0.1
Panch'ŏnch'ŏngbang	153.2±19.9	263.8±19.8	1.7±0.2
Chŏnghyangulgŭmbang	9.0±3.6	86.4±6.9	10.5±3.1
Taehwang-obaejago	18.9±0.4	171.7±9.1	9.0±0.5
Hong-illaksamdŭngbang	91.1±1.8	395.5±16.3	4.3±0.1
Hojanghaedokt'ang	117.1±13.3	409.9±19.0	3.5±0.2
Acycloguanosine (control)	0.11±0.02	49.0±4.4	448.2±94.9

Each value represents mean±S.D., n=5.

cells per well. The culture plates were then incubated at 37℃, in 5% CO_2 incubator for 3 days and used in estimation of cytotoxic dose (CD_{50}), by means of MTT (tetrazolium-based colorimetric) assay [17].

Anti-viral assay

1×10^3 of Vero cells per well were infected with HSV-1 at 0.1 MOI. Each extract was diluted sequentially five fold in six steps: original solution, 5^{-1}, 5^{-2}, 5^{-3}, 5^{-4}, 5^{-5}, dispensed within replicate 96-well culture plates in 100 μl volume using a multichannel pipette and incubated at 37℃, in 5% CO_2 incubator for 3 days. Antiviral activity of each extract, and acycloguanosine (Sigma) as control drug, were estimated by means of MTT (tetrazolium-based colorimetric) assay.

MTT (tetrazolium-based colorimetric) assay method

A modification of the MTT [18] assay was used, by means of the cell proliferation kit I (MTT, Boehringer Mannheim, Mannheim). 96-well culture plates containing HSV-1 and extracts from each specimen were cultured for 4 days. 100 μg MTT (50 μl of 2 mg/ml solution) was added to each well. After further 4 hours incubation, 100 μl of solubilization solution (SDS, 10% in HCl, 0.01 mol/l) was added to each well. The absorbance (test wavelength 540 nm, reference wavelength 690 nm) of each well was measured using an 96 well plate reader (Spectra MAX 340, Molecular devices, Sunnyvale, CA). Subsequently, data were stored and ploted through the use of software (SigmaPlot 3.0, Sigma). Antiviral activities of extracts against HSV-1 was determined [19,20].

RESULTS AND DISCUSSIONS

Anti-HSV-1 activity of acycloguanosine

Acycloguanosine was used to estimate anti-HSV-1 activity as the control drug. The ED_{50} value of acycloguanosine was 0.11±0.02 μg/ml and CD_{50} value of acycloguanosine was 49.0±4.4 μg/ml (Tables 4~7).

Anti-HSV-1 activities of extracts from Korean Traditional prescriptions

In an attempt to find new compounds with anti-HSV-1 activities, we screened 45 specimens of Korean traditional prescriptions which were extracted by methanol and boiling-water. Anti-HSV-1 activities of extracts were determined by means of the MTT assay. Vero cells were infected with HSV-1 and various concentration of extracts were added. Among Korean traditional prescriptions tested, 9 specimens showed anti-HSV-1 activities. The SI values calculated from ED_{50} and CD_{50} are shown in Table 4 and Table 5.

$$\text{Selective index (SI)} = \frac{\text{50 \% cytotoxic dose in Vero cells } (CD_{50})}{\text{50 \% effective dose of formazan formation } (ED_{50})}$$

Korean traditional prescriptions showing anti-HSV activities as boiling water extracts were Paeksajŏnbang, Hyŏllambang I, Hyŏllambang II, and

Table 6. Anti-HSV-1 activities of Korean medicinal herbes extracted by boiling water

No.	Medicinal herbs	ED_{50} (μg/ml)	CD_{50} (μg/ml)	SI
3	*Cinnamomum cassia* Blume	18.9±1.7	94.2±16.1	4.9±0.7
5	*Dryopteris crassirhizoma* Nakai	61.3±10.8	555.0±27.2	9.2±1.2
6	*Pheretima communissima* Goto et Hatai	1586.5±46.2	6534.6±41.6	4.1±0.1
12	*Rheum palmatum* L.	106.2±8.0	338.4±7.3	3.1±0.1
47	*Viola yedoensis* Mak.	57.2±6.5	473.0±26.6	8.3±0.9
69	*Taraxacum platycarpum* H. Dahlst.	49.6±3.0	508.1±18.7	10.2±0.7
73	*Polygonum cuspidatum* Sieb. *et* Zucc.	77.1±6.1	129.0±5.9	1.6±0.1
79	*Phellodendron amurense* Rupr.	76.2±16.5	395.5±32.5	5.4±1.3
	Acycloguanosine (control)	0.11±0.02	49.0±4.4	448.2±94.9

Each value represents mean±S.D., n=5.

Table 7. Anti-HSV-1 activities of Korean medicinal herbes extracted by methanol

No.	Medicinal herbs	ED_{50} (μg/ml)	CD_{50} (μg/ml)	SI
3	*Cinnamomum cassia* Presl	74.9±8.1	697.7±75.1	9.3±0.5
18	*Cocculus trilobus* DC.	424.8±79.4	1979.8±98.5	4.7±0.8
29	*Morus alba* L.	84.0±14.9	322.3±71.7	3.9±0.4
73	*Polygonum cuspidatum* Sieb. *et* Zucc.	25.2±5.9	70.7±33.1	2.9±1.5
	Acycloguanosine (control)	0.11±0.02	49.0±4.4	448.2±94.9

Each value represents mean±S.D., n=5.

Panch'ŏnch'ŏngbang and their SI values were 5.8±0.9, 5.8±0.8, 2.1±0.5, and 11.8±2.2, respectively (Table 4). Methanol extracts of Tanch'ishihot'ang, Panch'ŏnch'ŏngbang, Chŏnghyangulgŭmbang, Taehwang-obaejago, Hong-illaksamdŭngbang, and Hojanghaedokt'ang showed anti-HSV activities in tissue culture and their SI values were 2.5±0.1, 1.7±0.2, 10.5±3.1, 9.0±0.5, 4.3±0.1, and 3.5±0.2, respectively (Table 5). So water extract of Panch'ŏnch'ŏngbang is considered as useful for anti-HSV-1 agent. The other Korean traditional prescriptions did not show anti-HSV-1 activity at any concentration without cytotoxicity.

Anti-HSV-1 activities of extracts from Korean Medicinal Herbs

80 Korean medicinal herbs were screened to detect anti-HSV-1 activities. Anti-HSV-1 activities of extracts were determined by means of the MTT assay. Vero cells were infected with HSV-1 and various concentration of extracts were added.

Boiling water extracts of *Cinnamomum cassia* Blume, *Dryopteris crassirhizoma* Nakai, *Pheretima communissima* Goto et Hatai, *Rheum palmatum* L., *Viola yedoensis* Mak., *Taraxacum platycarpum* H., *Polygonum cuspidatum* sieb et Zucc., and *Phellodendron amurense* Rupr. showed anti-HSV activities and their SI values were 4.9±0.7, 9.2±1.2, 4.1±0.1, 3.1±0.1, 8.3±0.9, 10.2±0.7, 1.6±0.1, and 5.4±1.3, respectively (Table 6). Methanol extracts of *Cinnamomum cassia* Blume, *Cocculus trilobus* DC., *Morus alba* L., and *Polygonum cuspidatum* sieb et Zucc. showed anti-HSV activities and their SI values were 9.3±0.5, 4.7±0.8, 3.9±0.4, and 2.9±1.5, respectively (Table 7). The other Korean medicinal herbs did not show anti-HSV-1 activity at any concentration without cytotoxicity. *Cinnamomum cassia* Blume and *Polygonum cuspidatum* sieb et Zucc. were interesting because both water and methanol extracts were active. Especially, *Polygonum cuspidatum* sieb et Zucc. is a part of composition of Hong-illaksamdŭngbang, and Hojanghaedokt'ang which have anti-HSV-1 activity (Table 3 and Table 5). The SI value of water extracts from *Taraxacum platycarpum* H. Dahlst. was relative high as 10.2±0.7. So *Cinnamomum cassia*

Table 8. Cytotoxic effects of Korean traditional prescription and medicinal herb which have above ten SI value, and Acycloguanosine

No.	Prescription and medicinal herb	Viability (%)				
		2500	500	100	20	4.0
		concentration (μg/ml)				
CW-18	Panch'ŏnch'ŏngbang	10.2±4.2	24.8±9.1	89.2±26.4	97.1±10.0	101.4±26.0
MW-69	*Taraxacum platycarpum* H. Dahlst.	12.3±3.9	52.4±8.6	78.1±8.3	100.2±11.4	107.3±5.9
	Acycloguanosine (control)	-	3.45±1.9	2.91±0.9	101.2±5.7	101.3±6.3

Each value represents mean±S.D., n=5.

Blume, *Polygonum cuspidatum* sieb et Zucc. and *Taraxacum platycarpum* H. Dahlst. are considered as potentially useful for anti-HSV-1 agent and will be the focus of further research. On the whole these results indicate that boiling water extracts generally show relatively stronger anti-HSV activity than methanol extracts. Some Korean traditional prescriptions show stronger anti-HSV activity than the individual medicinal herbs.

Cytotoxic effects of acycloguanosine for Vero cell line

Acycloguanosine was used to estimate cytotoxic effect for Vero cell line as the control drug and cytotoxic effect for Vero cell line of acycloguanosine was determined by means of the MTT assay. The CD_{50} value of acycloguanosine was 49.0±4.4 μg/ml (Tables 4~7) and the viability rates of Vero cells at the concentration of 500 μg/ml, 100 μg/ml, 20 μg/ml, and 4.0 μg/ml were 3.45±1.9%, 2.91±0.9%, 101.2±5.7%, and 101.3±6.3%, respectively.

Cytotoxic effect of extracts from Korean Medicinal Herbs and Korean traditional prescriptions which have strong anti-HSV-1 activities for Vero cell line

Water extracts of Panch'ŏnch'ŏngbang (prescription) and *Taraxacum platycarpum* H. (mono herb) which have relatively strong anti-HSV-activities were used to estimate cytotoxic effect for Vero cell line. In order to determine cytotoxicity of the specimens, Vero cells were incubated with the various concentration of water extracts of Panch'ŏnch'ŏngbang and *Taraxacum platycarpum* H., and observed daily by phase-contrast microscope. After 5 days, the formazan produced by living Vero cells was determined by MTT assay. The CD_{50} value of Panch'ŏnch'ŏngbang was 242.1±43.1 μg/ml (Table 4) and the viability rates of Vero cells at the concentration of 2,500 μg/ml, 500 μg/ml, 100 μg/ml, 20 μg/ml, and 4.0 μg/ml of Panch'ŏnch'ŏngbang were 10.2±4.2%, 24.8±9.1%, 89.2±26.4%, 97.1±10.0%, and 101.4±26.0%, respectively (Table 8). The CD_{50} value of *Taraxacum platycarpum* H. Dahlst. was 508.1±18.7 μg/ml (Table 6) and the viability rates of Vero cells at the concentration of 2,500 μg/ml, 500 μg/ml, 100 μg/ml, 20 μg/ml, and 4.0 μg/ml of *Taraxacum platycarpum* H. Dahlst were 12.3±3.9%, 52.4±8.6%, 78.1±8.3%, 100.2±11.4%, and 107.3±5.9%, respectively (Table 8). As shown in Table 8, acycloguanosine showed strong cytotoxic effects on Vero cells at > 100 μg/ml. But water extracts of Panch'ŏnch'ŏngbang (prescription) and *Taraxacum platycarpum* H. showed very weak cytotoxic effects on Vero cells at > 100 μg/ml. This result demonstrated that water extracts of Panch'ŏnch'ŏngbang and *Taraxacum platycarpum* H. Dahlst. proved to have not only weak cytotoxic effect but also relatively strong anti-HSV-activities.

SUMMARY

In order to search for anti-Herpes simplex virus (HSV) type-1 agents from Korean medicinal herbs and Korean traditional prescriptions (herb complexes), we selected 80 medicinal herbs and 45 prescriptions, based on a review of the Korean traditional medicine books. Both methanol extracts and

boiling-water extracts were tested by means of the MTT assay (tetrazolium based colorimetric assay). Ten of the 125 methanol extracts: CM-11, CM-18, CM-19, CM-21, CM-22, CM-39, MM-3, MM-18, MM-29, MM-73 (see explanation of nomenclature below), showed efficacy against HSV-1. Twelve of the water extracts: CW-2, CW-3-I, CW-3-II, CW-18, MW-3, MW-5 MW-6, MW-12, MW-47, MW-69, MW-73 and MW-79 were active. #3 (individual herb) and #73 (individual herb) were interesting because both water and methanol extracts were active. Especially, #3 is a part of composition of Hong-il-laksamdŭngbang and Hojanghaedokt'ang which have anti-HSV-1 activitives. The SI value of MW-69 and CW-18 was relative high as 10.2±0.7 and 11.8±2.2. The cytotoxic effect on Vero cells of Panch'ŏnch'ŏngbang, *Taraxacum platycarpum* H. Dahlst. and acycloguanosine was determined by MTT assay. Water extracts of Panch'ŏnch'ŏngbang (prescription) and *Taraxacum platycarpum* H. Dahlst. showed very weak cytotoxic effects on Vero cells at > 100 μg/ml but acycloguanosine showed strong cytotoxic effects on Vero cells at > 100 μg/ml. As a result, #3, #73, MW-69 and CW-18 are considered as potentially useful for anti-HSV-1 agent and will be the focus of further research.

Abbreviations: CM - methanol extracts of traditional prescriptions; CW - water extracts of traditional prescriptions; MM - methanol extracts of individual herbs; MW - water extracts of individual herbs.

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