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Screening for Various Herb Medicines Extracts against Human immunodeficiency virus & Herpes simplex virus type Ⅰ and Herpes simplex virus type Ⅱ

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국문초록

바이러스 질환 필요처방의 선정을 위한 수종 한방 처방의 효능연구

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면역 결핍 바이러스와 허피즈 바이러스 -1,2에 대한 한약의 항바이러스 작용을 관찰하기 위하여, 세포 바이러스 검색에 초기 독성과 감염 바이러스의 생존 세포 수를 비교적 단기간내 볼 수 있는 96-well plate를 이용한 MTT assay로 측정하였다. 본 실험은 예비 실험 단계에서 사용된 총 85가지의 한약중 단미제 6가지와 복합처방 44가지를 선정하였으며 복합처방은 유사한 치법으로 분류하였고 한약의 시료 추출은 순수하게 물로 전탕하여 여과하였다. 실험 결과 파두와 맥아가 면역 결핍 바이러스에 대해 감염초기 유의성이 있었으며 호장근, 갈근우방자탕과 형방패독산이 허피즈 바이러스-1,2에 대해 감염초기 유의성이 있었으나 실험의 시간 경과에 따른 지속적인 약효 안정성을 보이지는 못하였다. 이 실험을 통하여 한약을 이용한 우수한 바이러스 치료를 개발하기 위해서는 단미제나 복합 처방에서 항바이러스 작용이 큰 유효성분의 대량 분리와 세포 실험에 있어서 오차를 줄일 수 있는 세포면역학적 실험의 도입 등이 필요할 것으로 사료된다.

중심 낱말 : 항바이러스, 한약, MTT assay, 면역 결핍 바이러스, 허피즈 바이러스 1, 허피즈 바이러스 2.

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Introduction

The basic principles of treatment to viral disease in oriental medicines are in invigorating qi-recuperate yin(補氣養陰), replenishing qi-removing heat from the blood (益氣凉血), restored spleen-recuperate stomach(健脾養胃), removing fever and toxic material from the body(淸熱解毒), removing fever and heat from the blood(淸熱凉血), promoting blood circulation to remove blood stasis(活血化瘀), and regulating the flow of qi to alleviate mental depression(理氣解鬱), which inhibits the strength of infection and encourages the split and energy of the patients(扶正祛邪)[1,2,3]

Recently several studies reported the anti-viral effects herb remedies of oriental medicine. The extract of *Herba Urinariae*(珍珠草) reported to be effective in the treatment of hepatitis B by antagonizing the DNA replication of duck hepatitis B virus(DHBV)[4], *Radix Scutellariae*(黃芩) and *Rhizoma Pinellia*(半夏) are reported to be effective in constrain virus activity and DNA replication[5]. *Rhizoma Polygoni Cuspidatis*(虎杖根) and *Herba Prunellae*(夏枯草) are reported to have antiviral effect in screening test[6], and a refined extract of *Fructus Arctii* (牛蒡子) and *Radix Asragali*(黃芪) was effective for anti-viral action through screening test aganist HIV infection[7]. *Descalzo AM and Coto C* [8] reported that the extract of *Melia toosendan Sieb* leave inhibited the multiplication of pseudorabies, a variant of herpes virus and *Sakagami et al.*[9] found the antiviral effect against herpes virus of pine cone extract. Also the anti-viral effects of combined prescription were studied[10,11]. *Guo el al.*[5] reported that *soshihotang* has therapeutic effect on viral hepatitis by antagonizing DNA replication of DHBV and *Tang et al.*[6] reported the results of screening tests for anti-viral effect of *soshihotang* against HIV. In Korea, *Woo et al.*[12] reported the outcome of screening tests for anti herpes virus effect of several extracts of natural substance and *Kang et al.*[13] published the study of anti-HSV activity of Korean traditional prescriptions, and *Park et al.*[14] reported the study on the anti-HSV activity of natural complex products. However, all the studies on complex treatment of virus disease are less significant than studies on single herbal treatment. We have chosen 50 recipes of Herbal medicines as possible candidates for development of anti-viral agents and tested cytotoxicity and anti-viral activity against *Human immunodeficiency virus*(HIV), *Herpes simplex virus I*(HSV-1), and *Herpes simplex virus II*(HSV-2)[15,16,17,18,19,20]. We tried to point out the problems associated with the development of effective anti-viral agents with less side effect herb remedies and to show the guidelines in screening in selecting appropriate remedies for the development process.

Material and Methods

The lists of tested herbs

Single taste medicine of six herbs (g)

Semen Crotnis ——40

Semen Armeniacae Amarum ——40

Radix Achyranthis Bidentae ——40
Fructus Hordei Germanitus ——40
Squama Manitus ——40
Rhizoma Polygoni Cuspidati ——40

Compound prescriptions of fifty five herbal medicines (g)

Tanglisodogum 金銀花 陳皮 12 黃芪鹽水炒 天花粉 8 防風 當歸 川芎 白芷 桔梗 厚朴 穿山甲炒 皂角刺 4

Jungdokbusaetang 金銀花 12 地骨皮 牡蠣粉 皂角刺 乳香 沒藥 牛蒡子 連翹 梔子 8 木通 天花粉 6

Naetagsan 金銀花 12 黃芪 牡蠣粉 皂角刺 10

Wanpeitang 金銀花 20 蒲公英 12 天花粉 甘草 桔梗 6 人蔘 黃芩 4

Sunbangwalmyungeum 金銀花 8 當歸 陳皮 甘草 天花粉 貝母 白芷 4 防風 3 皂角刺 赤芍藥 乳香 沒藥 2 穿山甲 2片

Naetagoksultang 大黃酒蒸 8 當歸 白芍藥 甘草 黃芪 射干 連翹 白芷 貝母 陳皮 皂角刺 天花粉 木香 乳香 沒藥 4

Sunbangwalmyungeum plus daewhang 大黃 20 金銀花 12 當歸 皂角刺 陳皮 乳香 貝母 天花粉 赤芍藥 甘草 穿山甲 白芷 4 防風 3 沒藥 2

Yongdamsagantang plus daewhang 大黃 20 金銀花 16 草龍膽 當歸 乾地黃 柴胡 澤瀉 木通 車前子 赤茯苓 6 梔子 黃芩 甘草 3 牧丹皮 玄胡索 4

Euinbujapaejangsan 薏苡仁 8 附子 2 敗醬 4

Euiintang 薏苡仁 防己 赤小豆炒 甘草炙 6

Sanpoongkosamwhan 苦蔘 15 大黃酒炒 防風 枳角 玄蔘 獨活 黃連 8 黃芩 梔子 菊花 4

Homasan 胡麻子 苦蔘 荊芥 何首烏 威靈仙炒 防風 石菖蒲 枳實 甘菊 蔓荊子 白蒺藜 甘草 3

Chungyolyanghyulhaedoktang 茵陳 16 連翹 虎杖根 生地黃 牧丹皮 8 大黃 黃連 黃柏 梔子 6 甘草 3

Injinhotang 茵陳 40 大黃 20 梔子 8

Whadoktang 蒲公英 12 大黃 金銀花 6 當歸 4 赤芍藥 黃芪 3 升麻 甘草 2

Sodokeum 蒲公英 玄蔘 12 升麻 8 麥門冬 桔梗 甘草 4

Gilkyungsakantang 山豆根 12 牛蒡子 6 連翹 竹瀝 荊芥 防風 玄蔘 3 桔梗 射干 甘草 3

Gikyungtang 桔梗 20 甘草 40

Dohongsamultang plus geumjacgeun 金雀根 20 當歸 川芎 生地黃 白芍藥 12 桃仁 紅花 8

Yongdamsagantang 龍膽草炒 柴胡 澤瀉 木通 4 車前子 赤茯苓 生地黃 當歸 梔子 黃芩 甘草 2

Chesupwilyungtang 蒼朮炒 厚朴炒 陳皮 猪苓 澤瀉 赤茯苓 白朮炒 滑石 防風 梔子 木通 4 肉桂 甘草 6 燈心炒 6 生薑 3片 大棗 2個

Chongyolchesuptang 蛇床子 12 苦蔘 鷄內金 6 黃連 白礬 3

Chesuptang 黃柏 蟬退 苦蔘 土茯苓 白鮮皮 地膚子 8 荊芥 防風 赤芍藥 甘草 6

Whangyonhaedoktang 黃連 黃柏 黃芩 梔子 4

Daewhangmokdnapitang 大黃 亡草 6 牧丹皮 桃仁 瓜樓仁 8

Galkunwoobangjatang 牛蒡子 升麻 葛根 麻黃 連翹 玄蔘 桔梗 甘草 4 生薑 2片

Chongkihaedoktang 牛蒡子 犀角 荊芥 甘草 黃芩 葛根 梔子 連翹 黃柏 知母 天花粉 赤芍藥 4

Galkunhaegitang 葛根 8 麻黃 桂枝 芍藥 甘草 炙 4 黃芩 3 生薑 3片 大棗 2個

Yikoyunkyosankintang 當歸酒洗 連翹 蓬朮酒

炒 三棱酒炒 10 土毛根 龍膽草酒洗 8 柴胡 6 黃芩酒洗 甘草炙 3 黃連酒炒 2 蒼朮 12 赤芍藥 2

Sammyosan 黃柏 蒼朮 牛膝 6

Insamyangyoungtang 白芍藥 8 當歸 人蔘 白朮 黃芪蜜炙 4 肉桂 2 甘草炙 4 熟知黃 五味子 防風 3 遠志 2 生薑 3片 大棗 2個

Naetakwhanggisan 黃芪 6 金銀花 4 牡蠣粉 4 甘草 3

Gamibaenongtang 桔梗 12 甘草 6 杏仁 4 五味子 4 麥門冬 4 天門冬 4 當歸 4 生薑 3片 大棗 2個

Paljintang 人蔘 白朮 白茯苓 甘草 熟知黃 白芍藥 川芎 當歸 4

Gamroum 熟知黃 12 生地黃 12 天門冬 8 麥門冬 8 黃芩 黃連 枳角 石斛 枇杷葉 6 甘草 4

Samginaetaksan 人蔘 黃芪炒 當歸酒洗 白朮炒 陳皮 甘草 升麻 川芎 生地黃 姜活 4

Toonongtang 黃芪 16 川芎 6 當歸 4 皂角刺 8 穿山甲 8

Hyungbangpaedoksan 人蔘 柴胡 前胡 姜活 獨活 枳角 桔梗 川芎 4 赤茯苓 甘草 荊芥 4 防風 6 生薑 3片

Soshihitang 柴胡 12 黃芩 8 人蔘 半夏 4 甘草 2 生薑 3片

Soshihitang plus hagocho 柴胡 12 黃芩 8 人蔘 半夏 4 甘草 2 生薑 3片 夏古草 10

Jungsihoum 柴胡 12 白芍藥 8 陳皮 防風 甘草 4

Sogonjunwonsan 白朮 神麴 香附子 枳實 玄胡索 海粉 4 赤茯苓 陳皮 靑皮 砂仁 麥芽 山査 甘草 3 生薑 3片

Chungwhajiyangtang I 黃柏 黃芩 8 梔子 地膚子 蒼朮 車前子 6

Chungwhajiyangtang II 黃柏 黃芩 黃連 川椒 8 白礬 12

Preparation of Extracts :

Since oriental herbal medicines have been clinically taken as water extracts each sample was extracted with complete submerged boiling water for 2 hours, in an open vessel. The hot extract was filtered and lyophilized and the resulting extract was tested. Samples of 5㎎ each were dissolved in water, then diluted to appropriate concentrations(500㎍/㎖, 166.67㎍/㎖, 55.56㎍/㎖, 18.52㎍/㎖, 6.17㎍/㎖) and filtered with microfilter prior to testing.

Preparation of Reagents :

Dulbecco's modified eagle (DME), Fetal bovine serum (FBS) and Trypsin were purchased from Gilbo. Gentamycin and MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterrazolium bromide} were purchased from Sigma. Vero cell(African green monkey kidney cell, ATCC CCL 81), *Human immunodifficiency virus* (HIV) strain M (HTLV-1), *Herpes simplex virus* type I (HSV-1) strain F (ATCC VR-733), *Herpes simplex virus* II (HSV-2) strain MS (ATCC VR-734) were purchased from American Type Culture Collection (ATCC).

Cell and Virus :

Vero cell was cultured with dulbecco's modified eagle (DME) medium supplemented with 5% (v/v) heatinactivated fetal bovine serum (FBS) and 4㎍/㎖ 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 *Human immunodifficiency virus* (HIV), *Herpes simplex virus* type I (HSV-1) and

type II (HSV-2) prepared from culture supernatant of HIV, 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.

Quantification of the titer of HIV, HSV-1& HSV-2 :

In order to determine the titer of the virus, a MTT assay[21] was carried out as follows (*Pauwel et al.*, 1988, Franccois et al., 1986) : Vero cells (3 x 10^3 cell/well) were seeds into a 96-well palte. After three to four days of incubation, a confluent monolayer was generally obtained. After washing the cells, 100㎕ of various tenfold-diluted concentrations of the virus solution was added to each well and incubated for 60min at 37°C. After absorption of the virus, virus 100㎕ of culture medium was added, then incubated for three days. After removing the culture medium, 50 ㎕ of 0.3% MTT soln.(3㎎ MTT was dissolved in 1㎖ DME medium supplemented with 2% FBS) was added then incubated for 120min at 37°C in a humidified atmosphere with 5% CO2. The acidified isopropanol/6% triton X-100 solution, 100㎕, 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 (3x10^3 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㎕ 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 incuabted for 60 min at 37°C. After absorption of the virus, the culture medium was removed and 100㎕ of culture medium including various concentration of sample was added to each well in delicate, 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 of the sample required to inhibit virus-induced CEP by 50%. In order to make clear the cytotoxicity of sample, mock-infected cells were also prepared simultaneously. 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:

$$\frac{(A_T)_{HIV,HSV}-(A_C)_{HIV,HSV}}{(A_C)mock-(A_C)_{HIV,HSV}} \times 100$$

$$\frac{1-(A_T)mock}{(A_C)mock} \times 100$$

$(A_T)_{HIV,HSV}$ is the Optical density (OD) of the cell, treated with the samples.
$(A_C)_{HIV,HSV}$ 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 of the mock-infected cell only(cell control).

Results AND Discussion

Since the first development of smallpox vaccine in 1796, preventive vaccines against rabies, poliomyelitis, and meales became available and presently, vaccines are being developed utilizing interferons or to attack the specific enzymes which are used for viral multiplication[22]. The basic principles of treatment to viral diseases are invigorating qi-recuperate yin, replenishing qi-removing heat from the blood, restored spleening-recuperate stomach, removing fever and heat from the blood, promoting blood circulation to remove blood stasis, regulating the flow of qi to alleviate mental depression, which inhabits the strength of infection and encourages the spirit and energy of the patients[1,2,3].

In the present study, we carried out a convenient and rapid CPE (cytopathic effect) inhibition assay to evaluated anti-viral activity against HIV, HSV-1 & 2 for various herbal medicines. 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 herbal medicines through activity-guided fractionation, since accuracy and rapidity of bio-assay are 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 HIV, HSV-1 & HSV-2 was determined by a MTT assay. A diluted HIV, HSV-1 & HSV-2 concentration at 100$\mu\ell$ which was equivalent to 50% cell culture inhibitory dose ($CCID_{50}$) was used as a seeding virus throughout the experiment. AZT, which is clinically used for the treatment of HIV disease[23,24], was used as a positive control under this assay system. Acyclovir (ACV), which is clinically used for the treatment of herpetic disease[25,26], was used as a positive control under this assay system. Abstactions of decocting herbs were prepared by solvent fractionation from fifty purchased herbal medicines, and their toxicity of infected cell and anti-viral activities were evaluated. Among them, the major part of herbal medicines showed cell stability compared with the contrast and the minor part of herbal medicines showed cell toxicity about the early infection cell. Cytotoxic concentration (CC) of the H_2O extracts of *Semen Crotonis* against HIV was <4.0, *Fructus Hordei Germinatus* against HIV was <4.0, *Rhizoma Polygoni Cuspidati* against HSV-1 and HSV-2 was 247.49, CC of the *Galkunwoobangjatang* against HSV-1 and HSV-2 was 239.48, CC of the *Hyongbangpaedoksan* against HSV-1 and HSV-2 was 181.12. These are high level cytotoxic concentration compared with the contrast. Therefore, we assumed that the high level cytotoxic concentration of various herbal medicines play a major role in improvement of antiviral activity for the first infective cell. But continuous antiviral effect was unable to figure out for selective index(SI)=CC_{50}/EC_{50}. The other herbal medicines were unable to showed potent anti-HIV and anti-HSV

activity. The antiviral activation using herbs in this thesis have unlimited objects, to select research object will help to show the direction of antiviral drug development that have less side effect and more excellent efficiency.

Table I. Screening for extracts of six herbal medicines against HIV

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			IIIB	CBL20	IIIB	CBL20
1	*Radix Achyranthis Bidentatae*	>300.00	>300.00	>300.00	ND	ND
2	*Semen Crotonis*	<4.0	ND	ND	ND	ND
3	*Squama Manitis*	>300.00	>300.00	>300.00	ND	ND
4	*Semen Armeniaca Amarum*	238.8	>238.82	>238.82	<1	<1
5	*Rhizoma Polygoni Cuspidati*	89.9	>89.91	>89.91	<1	<1
6	*Fructus Hordei Germinatus*	<4.0	ND	ND	ND	ND
7	AZT	1.35	0.003	0.0885	457	15

Table II. Screening for extracts of six herbal medicines against HSV-1,2

No	Herbal Medicine	Toxicity (CC_{50})[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			HSV-1	HSV-2	HSV-1	HSV-2
1	*Radix Achyranthis Bidentatae*	>500.00	>500.00	>500.00	ND	ND
2	*Semen Crotonis*	>500.00	>500.00	>500.00	ND	ND
3	*Squama Manitis*	>500.00	>500.00	>500.00	ND	ND
4	*Semen Armeniaca Amarum*	>500.00	>500.00	>500.00	ND	ND
5	*Rhizoma Polygoni Cuspidati*	247.49	>247.49	>247.49	<1	<1
6	*Fructus Hordei Germinatus*	>500.00	>500.00	>500.00	ND	ND
7	ACV	>250	1.42	4.01	>176	>62.4
8	ARA-C	1.31	1.03	>1.31	1.28	<1

a) 50% Cytotoxic Concentration (CC_{50}) is the concentration of the 50% cytotoxic effect
b) 50% Effective Concentration (EC_{50}) is the concentration of the sample required to inhibit virus-induced CPE 50%
c) Selective index(SI)=CC_{50}/EC_{50}

Table III. Screening for extracts of group-1* herbal medicines against HIV

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			IIIB	CBL20	IIIB	CBL20
1	*Tanglisodogeum*	>100.00	>100.00	>100.00	ND	ND
2	*Jungdokbusaengtang*	112.9	>112.92	>112.92	<1	<1
3	*Naetagsan*	104.9	>104.88	>104.88	<1	<1
4	*Wanvaetang*	>300.00	>300.00	>300.00	ND	ND
5	*Sunbanghwalmyungeum*	>300.00	>300.00	>300.00	ND	ND
6	*Naesooksultang*	>300.00	>300.00	>300.00	ND	ND
7	*Sunbanghwalmyungeumplus daewhang*	182.5	>182.49	>182.49	<1	<1
8	*Yongdamsagantang plus daewhang*	107.7	>107.72	>107.72	<1	<1
9	*Euinbujapaejangsan*	>100.00	>100.00	>100.00	ND	ND
10	*Euiintang*	>300.00	>300.00	>300.00	ND	ND
11	*Sanpoongkosamwhan*	18.5	>18.50	>18.50	<1	<1
12	*Homasan*	>300.00	>300.00	>300.00	ND	ND
13	*Chungyolyanghyulhaed-doktang*	27.5	>27.53	>27.53	<1	<1
14	*Iinjinhotahg*	150.8	>150.82	>150.82	<1	<1
15	*Whadoktang*	101.2	>101.21	>101.21	<1	<1
16	*Sodokeum*	203.5	>203.52	>203.52	<1	<1
17	*Gilkyungsakantang*	63.5	>63.46	>63.46	<1	<1
18	*Gilkyungtang*	130.0	>130.04	>130.04	<1	<1
19	*Dohongsamultang plus geumjacgeun*	352.5	>352.46	>352.46	<1	<1
20	AZT	2.1	0.0028	0.0492	746	43

Table IV. Screening for extracts of group-1* herbal medicines against HSV-1,2

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			HSV-1	HSV-2	HSV-1	HSV-2
1	*Tanglisodogeum*	>300.00	>300.00	>300.00	ND	ND
2	*Jungdokbusaengtang*	85.17	>85.17	>85.17	<1	<1
3	*Naetagsan*	>500.00	>500.00	>500.00	ND	ND
4	*Wanxaetang*	>500.00	>500.00	>500.00	ND	ND
5	*Sunbanghwalmyungeum*	>500.00	>500.00	>500.00	ND	ND
6	*Naesooksultang*	449.81	>449.81	>449.81	<1	<1
7	*daewhang*	>500.00	>500.00	>500.00	ND	ND
8	*Sunbanghwalmyun-geumplus Yongdamsagantang plus daewhang*	>500.00	>500.00	>500.00	ND	ND
9	*Euinbujapaejangsan*	>300.00	>300.00	>300.00	ND	ND
10	*Euiintang*	>500.00	>500.00	>500.00	ND	ND
11	*Sanpoongkosamhwan*	>500.00	>500.00	>500.00	ND	ND
12	*Homasan*	>500.00	>500.00	>500.00	ND	ND
13	*Chongyolyanghyulhae-doktang*	>500.00	>500.00	>500.00	ND	ND
14	*Iijinhotahg*	>500.00	>500.00	>500.00	ND	ND
15	*Whadoktang*	>500.00	>500.00	>500.00	ND	ND
16	*Sodokeum*	>500.00	>500.00	>500.00	ND	ND
17	*Gilkyungsagantang*	>500.00	>500.00	>500.00	ND	ND
18	*Gilkyungtang*	>500.00	>500.00	>500.00	ND	ND
19	*Dohongsamultang plus geumjacgeun*	>500.00	>500.00	>500.00	ND	ND
20	ACV	>10.00	0.2746	0.7823	>36.42	>12.78
21	Ara-C	3.841	>3.84	>3.84	<11	<1

* Treatment methods of herbal medicines are removing fever and heat from the body.

a) 50% Cytotoxic Concentration (CC_{50}) is the concentration of the 50% cytotoxic effect

b) 50% Effective Concentration (EC_{50}) is the concentration of the sample required to inhibit virus-induced CPE 50%

c) Selective index(SI)=CC_{50}/EC_{50}

Table V. Screening for extracts of group-2** herbal medicines against HIV

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			IIIB	CBL20	IIIB	CBL20
1	*Yongdamsagantang*	>100.00	>100.00	>100.00	ND	ND
2	*Chesupwilyungtang*	>500.00	>500.00	>500.00	ND	ND
3	*Chongyolchesuptang*	13.1	>13.14	>13.14	<1	<1
4	*Chesuptang*	274.1	>271.38	>271.38	<1	<1
5	*Whangyonhaedoktang*	15.3	>15.3	>15.3	<1	<1
6	*Daewhangmokdanpi-tang*	>300.00	>300.00	>300.00	ND	ND
7	*Galkunwoobangjatang*	323.3	>323.34	>323.34	<1	<1
8	*Chongkihaedoktang*	45.0	>45.03	>45.03	<1	<1
9	*Galkunhaegitang*	316.4	>316.43	>316.43	<1	<1
10	*Yikoyunkyosankintang*	183.4	>183.42	>183.42	<1	<1
11	*Sammyosan*	165.8	>165.83	>165.83	<1	<1
12	AZT	2.1	0.0028	0.0492	746	43

Table VI. Screening for extracts of group-2** herbal medicines against HSV-1,2

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			HSV-1	HSV-2	HSV-1	HSV-2
1	*Yongdamsagantang*	>300.00	>300.00	>300.00	ND	ND
2	*Chesupwilyungtang*	>500.00	>500.00	>500.00	ND	ND
3	*Chongyolchesuptang*	>500.00	>500.00	>500.00	ND	ND
4	*Chesuptang*	>500.00	>500.00	>500.00	ND	ND
5	*Whangyonhaedoktang*	>300.00	>300.00	>300.00	ND	ND
6	*Daewhangmokdanpi-tang*	>500.00	>500.00	>500.00	ND	ND
7	*Galkunwoobangjatang*	239.48	>239.48	>239.48	<1	<1
8	*Chonggihaedoktang*	>500.00	>500.00	>500.00	ND	ND
9	*Galkunhaegitang*	>500.00	>500.00	>500.00	ND	ND
10	*Yikoyunkyosankintang*	>500.00	>500.00	>500.00	ND	ND
11	*Sammyosan*	>500.00	>500.00	>500.00	ND	ND
12	ACV	>250	1.42	4.01	>176	>62.4
13	ARA-C	1.31	1.03	>1.31	1.28	<1

** Treatment methods of herbal medicines are removing fever and removing dampness.

a) 50% Cytotoxic Concentration (CC_{50}) is the concentration of the 50% cytotoxic effect

b) 50% Effective Concentration (EC_{50}) is the concentration of the sample required to inhibit virus-induced CPE 50%

c) Selective index(SI)=CC_{50}/EC_{50}

Table Ⅶ. Screening for extracts of group-3*** herbal medicines against HIV

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			ⅢB	CBL20	ⅢB	CBL20
1	*Insamyangyoungtang*	413.7	>413.68	>413.68	<1	<1
2	*Naetakwanggisan*	384.8	>384.81	>384.81	<1	<1
3	*Gamibaenongtang*	>500.00	>500.00	>500.00	ND	ND
4	*Paljintang*	>100.00	>100.00	>100.00	ND	ND
5	*Gamroum*	111.5	>115.0	>115.0	<1	<1
6	*Samginaetaksan*	400.5	>400.48	>400.48	<1	<1
7	*Toonongsan*	>500.00	>500.00	>500.00	ND	ND
8	AZT	2.1	0.0028	0.0028	746	43

Table Ⅷ. Screening for extracts of group-3*** herbal medicines against HSV-1,2

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			HSV-1	HSV-2	HSV-1	HSV-2
1	*Insamyangyoungtang*	>500.00	>500.00	>500.00	ND	ND
2	*Naetakwanggisan*	>500.00	>500.00	>500.00	ND	ND
3	*Gamibaenongtang*	>500.00	>500.00	>500.00	ND	ND
4	*Paljintang*	>300.00	>300.00	>300.00	ND	ND
5	*Gamroum*	>500.00	>500.00	>500.00	ND	ND
6	*Samginaetaksan*	>500.00	>500.00	>500.00	ND	ND
7	*Toonongsan*	>500.00	>500.00	>500.00	ND	ND
8	ACV	>10.00	0.2746	0.7823	>36.42	>12.78
9	Ara-C	3.841	>3.84	>3.84	>3.84	>3.84

*** Treatment merhods of herbal medicines are invigorating qi and requperate yin.

a) 50% Cytotoxic Concentration (CC_{50}) is the concentration of the 50% cytotoxic effect

b) 50% Effective Concentration (EC_{50}) is the concentration of the sample required to inhibit virus-induced CPE 50%

c) Selective index(SI)=CC_{50}/EC_{50}

Table Ⅸ. Screening for extracts of group-4**** herbal medicines against HIV

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			ⅢB	CBL20	ⅢB	CBL20
1	*Hyungbangpaedoksan*	9.3	>9.3	>9.3	<1	<1
2	*Soshihotang*	>100.00	>100.00	>100.00	ND	ND
3	*Soshihotang plus hago -cho*	>300.00	>300.00	>300.00	ND	ND
4	*Jungsihoum*	>100.00	>100.00	>100.00	ND	ND
5	*Sogonjunwonsan*	>300.00	>300.00	>300.00	ND	ND
6	*Chunghuajiyangtan I*	155.8	>155.77	>155.77	<1	<1
7	*Chunghuajiyangtan II*	14.2	>14.20	>14.20	<1	<1
8	AZT	1.25	0.0021	0.00104	595	1202

Table Ⅹ. Screening for extracts of group-4**** herbal medicines against HSV-1,2

No	Herbal Medicine	Toxicity CC_{50}[a]	Antiviral activity(EC_{50})[b]		Selective index[c]	
			HSV-1	HSV-2	HSV-1	HSV-2
1	*Hyungbangpaedoksan*	181.12	>181.12	>181.12	<1	<1
2	*Soshihotang*	>300.00	>300.00	>300.00	ND	ND
3	*Soshihotang plus hago -cho*	>500.00	>500.00	>500.00	ND	ND
4	*Jungshihoum*	>300.00	>300.00	>300.00	ND	ND
5	*Sogonjunwonsan*	>500.00	>500.00	>500.00	ND	ND
6	*Chunghuajiyangtang I*	>500.00	>500.00	>500.00	ND	ND
7	*Chunghuajiyangtang II*	>500.00	>500.00	>500.00	ND	ND
8	ACV	>250	0.77	1.27	>325	>197
9	Ara-C	9.96	0.48	0.88	20.75	11.32

**** Treatment methods of herbal medicines are reducing fever by reconcilation.

a) 50% Cytotoxic Concentration (CC_{50}) is the concentration of the 50% cytotoxic effect

b) 50% Effective Concentration (EC_{50})is the concentration of the sample required to inhibit virus-induced CPE 50%

c) Selective index(SI)=CC_{50}/EC_{50}

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