

Original Articles

## Screening for Various Herb Medicines Extracts against HSV-1,2

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**Objective :** This study was undertaken for discovering the characteristics of sleep in ordinary symptoms based on the Sasang Constitution. The result of this study could be helpful to understand and to identify patients such as Taeumin, Soyangin Soeumin or Taeyangin.

**Methods :** There were 1,229 patients (529 men), who answered the questionnaire about their ordinary sleeping patterns. They were diagnosed, including their clinical Sasang Constitution, by the Sasang Constitution specialist at Bundang Oriental Hospital of Dongguk University. By applying the multinomial and binary logistic regression analysis to those collected materials, we can measure the characteristics and the influence of ordinary sleeping patterns to the dependent variable (Sasang Constitution).

**Results :** In order of the item's influence that had decided one's constitution, between Taeumin and Soeumin, Taeumin snored frequently or well more than Soeumin, Soeumin had more dreams and more sleeping times than Taeumin, and Taeumin struggled frequently or well more than Soeumin. Between Soyangin and Soeumin, Soeumin dreams more frequently than Soyangin, Soyangin snored frequently or well more than Soeumin, and Soeumin has more sleeping times than Taeumin. Between Taeumin and Soyangin, Taeumin snored frequently or well more than Soyangin. Between Taeyangin and a group of the other constitutions, Taeyangin felt unwell after sleeping more than the other constitutions, the other constitutions awakened frequently more than Taeyangin during sleeping.

**Conclusion :** This study will be used for identifying patients as Taeumin, Soyangin, Soeumin or Taeyangin by contrast with each other.

**Key Words:** Anti-viral effect, Herb Medicines, Screening, Herpes simplex virus type I (HSV-1), Herpes simplex virus type II (HSV-2)

### Introduction

In oriental medicine, yukbyung(疫病-lemologii), ondock(溫毒-epidemic febrile toxicity), balchang(發瘡-

sore carbucle), yukgin(疫疹-lemologic erythma), mokyowhadan(纏腰火丹-herpes zoster), sakwanchang(蛇串瘡-scab over a boil) etc. appear to be related to viral infection. As described in Exploration to pathogenesis conversation(內經素問), oyukbyung(五疫病-five lemologii diseases) are very contagious and have very similar clinical features, irrespective of the of the infected. 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(益氣涼血), restored spleen-

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recuperate stomach(健脾養胃), removing fever and toxic material from the body(清熱解毒), removing fever and heat from the blood(清熱涼血), promoting blood circulation to remove blood stasis(活血化癥), 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)<sup>1,2)</sup>.

Recently several studies reported the anti-viral effects herb remedies. 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)<sup>3)</sup>, *Radix Scutellariae*(黃芩) and *Rhizoma Pinellia*(半夏) are reported to be effective in constrain virus activity and DNA replication<sup>4)</sup>. *Rhizoma Polygoni Cuspidatis*(虎杖根) and *Herba Prunellae*(夏枯草) are reported to have antiviral effect in screening test<sup>5)</sup>, and a refined extract of *Fructus Arctii*(牛蒡子) and *Radix Astragali*(黃芩) was effective for anti-viral action through screening test against HIV infection<sup>6)</sup>. *Descalzo AM and Coto C*<sup>7)</sup> reported that the extract of *Melia toosendan Sieb* leave inhibited the multiplication of pseudorabies, a variant of herpes virus and *Sakagami et al.*<sup>8)</sup> found the antiviral effect against herpes virus of pine cone extract. Also the anti-viral effects of combined prescription were studied<sup>8,9)</sup>. Guo et al<sup>9)</sup> reported that *soshihotang* has a therapeutic effect on viral hepatitis by antagonizing DNA replication of DHBV and Tang et al.<sup>5)</sup> reported the results of screening tests for an anti-viral effect of *soshihotang* against HIV. In Korea, Woo et al.<sup>11)</sup> reported the outcome of screening tests for anti herpes virus effect of several extracts of natural substance and *Kang et al.*<sup>14)</sup> published the study of anti-HSV activity of Korean traditional prescriptions, and *Park et al.*<sup>15)</sup> reported the study on the anti-HSV activity of natural complex products. However, all the studies on complex herbal treatments of virus disease are less significant than studies on single herbal treatments. We

have chosen 44 recipes of herbal medicines as possible candidates for development of anti-viral agents and tested cytotoxicity and anti-viral activity against *Herpes simplex virus type I* (HSV-1) and *Herpes simplex virus type II* (HSV-2). We tried to point out the problems associated with the development of effective anti-viral agents using herb remedies with fewer side effects and to show the guidelines in screening in selecting appropriate remedies for the development process.

## Materials and Methods

### 1) Compound prescription of forty four 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 牡丹皮 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* 苦參 15 大黃酒炒 防風 枳殼 玄蔘 獨活 黃連 8 黃芩 梔子 菊花 4  
*Homasan* 胡麻子 苦參 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 玄麥 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 玄麥 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 豬苓 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 苦參 鷄內金 6 黃連 白  
 礬 3  
*Chesuptang* 黃柏 蟬退 苦參 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 芒草 6 牧丹皮 桃仁 瓜樓  
 仁 8  
*Galkunwoobangjatang* 牛蒡子 升麻 葛根 麻黃 連翹 玄  
 麥 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 三稜酒  
 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 人參 白朮 黃芪蜜  
 炙 4 肉桂 2 甘草炙 4 熟地黃 五味子 防風 3 遠志 2 生  
 薑 3片 大棗 2個  
*Naetakwhangisan* 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* 人參 白朮 白茯苓 甘草 熟地黃 白芍藥 川芎  
 當歸 4  
*Gamroum* 熟地黃 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* 人參 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* 人參 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 人參 半夏 4 甘草 2 生薑 3片  
*Soshihitang plus hagocho* 柴胡 12 黃芩 8 人參 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 神麩 香附子 枳實 玄胡索 海粉  
 4 赤茯苓 陳皮 青皮 砂仁 麥芽 山楂 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

## 2) 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 5mg each were dissolved in water, then diluted to appropriate concentrations(500 $\mu$ g/ $\mu$ l, 166.67 $\mu$ g/ $\mu$ l, 55.56 $\mu$ g/ $\mu$ l, 18.52 $\mu$ g/ $\mu$ l, 6.17 $\mu$ g/ $\mu$ l) and filtered with microfilter prior to testing.

## 3) 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), *Herpes simplex virus type I* (HSV-1) and *Herpes simplex virus type II* (HSV-2) strain MS (ATCC VR-734) were purchased from American Type Culture Collection (ATCC).

#### 4) 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 4µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. Vero cell was subcultured twice a week. The stock of *Herpes simplex virus type I* (HSV-1) and *Herpes simplex virus type II* (HSV-2) prepared from culture supernatant of HSV-1,2 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.

#### 5) Quantification of the titer of HSV-1,2

In order to determine the titer of the virus, a MTT assay<sup>16)</sup> was carried out as follows (Pauwel et al., 1988, Franccois et al., 1986) : Vero cells (3 × 10<sup>3</sup> 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, 100µl 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 100µl of culture medium was added, then incubated for three days. After removing the culture medium, 50µl of 0.3% MTT soln.(3mg MTT was dissolved in 1µl DME medium supplemented with 2% FBS) was added then incubated for 120min at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. The acidified isopropanol/6% triton X-100 solution, 100µl, 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 wave length.

The evaluation of anti-herpetic activity by CPE (cytopathic effect) inhibition assay : Vero cells (3x10<sup>3</sup> 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, 100µl of the virus solution, diluted with DME medium supplemented with 2% FBS, which was equivalent to 50% cell culture inhibitory dose (CCID<sub>50</sub>) was added to each well and incuabted for 60 min at 37°C. After absorption of the virus, the culture medium was removed and 100µl 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<sub>2</sub> 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<sub>50</sub> (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:

$$\frac{(A_T)_{\text{HSV 1,2}} - (A_C)_{\text{HSV 1,2}}}{(A_C)_{\text{mock}} - (A_C)_{\text{HSV 1,2}}} \times 100$$

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

(A<sub>T</sub>)<sub>HSV 1,2</sub> are the Optical density (OD) of the cell, treated with the and samples

(A<sub>C</sub>)<sub>HSV 1,2</sub> are the OD of the cell, treated with the virus (virus control).

(A $\tau$ )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 measles became available and presently, vaccines are being developed utilizing interferons or to attack the specific enzymes which are used for viral multiplication<sup>17)</sup>. The basic principles of treatment to viral diseases are invigorating qi-recuperate yin(補氣養陰), replenishing qi-removing heat from the blood(益氣涼血), restored spleening-recuperate stomach(健脾養胃), removing fever and heat from the blood(清熱涼血), promoting blood circulation to remove blood stasis(活血化癥), regulating the flow of qi to alleviate mental depression(理氣解鬱), which inhabits the strength of infection and encourages the spirit and energy of the patients(扶正祛邪)<sup>1,2)</sup>.

In the present study, we carried out a convenient and rapid CPE (cytopathic effect) inhibition assay to evaluated anti-viral activity against 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 HSV-1,2 were determined by a MTT assay. A diluted HSV -1,2 were concentration at 100  $\mu$ l which was equivalent to 50% cell culture inhibitory dose (CCID<sub>50</sub>) was used as a seeding virus throughout the experiment. ACV and

Arc-C which are clinically used for the treatment of HSV disease<sup>18,19)</sup>, were used as a positive control under this assay system. Abstractions of decocting herbs were prepared by solvent fractionation from forty-four 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<sub>2</sub>O extracts of *Sanpoongkosamwhan* against HSV-1,2 was 18.5, *Chonyolchesuptang* against HSV-1,2 was 13.1, *Whangyonhaedoktang* against HSV -1,2 was 15.3, *Hyongbangpaedoksan* against HSV-1,2 was 9.3. 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<sub>50</sub>/EC<sub>50</sub>. The other herbal medicines were unable to showed potent 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 1.** Screening for Extracts of Group-1\* Herbal Medicines against HSV-1,2

| No | Herbal Medicin                          | Toxicity $CC_{50}$ <sup>a)</sup> | Antiviral activity( $EC_{50}$ ) <sup>b)</sup> |         | Selective index <sup>c)</sup> |        |
|----|---|----------------------------------|---|---------|-------------------------------|--------|
|    |   |                                  | III BC  | BL20    | III B                         | CBL20  |
| 1  | <i>Tanglisodogeum</i>                   | >100.00                          | >100.00                                       | >100.00 | ND                            | ND     |
| 2  | <i>Jungdokbusaengtang</i>               | 112.9                            | >112.92                                       | >112.92 | <1                            | <1     |
| 3  | <i>Naetagsan</i>                        | 104.9                            | >104.88                                       | >104.88 | <1                            | <1     |
| 4  | <i>Wanvaetang</i>                       | >300.00                          | >300.00                                       | >300.00 | ND                            | ND     |
| 5  | <i>Sunbanghwalmyungeum</i>              | >300.00                          | >300.00                                       | >300.00 | ND                            | ND     |
| 6  | <i>Naesooksultang</i>                   | >300.00                          | >300.00                                       | >300.00 | ND                            | ND     |
| 7  | <i>Sunbanghwalmyungeumplus daewhang</i> | 182.5                            | >182.49                                       | >182.49 | <1                            | <1     |
| 8  | <i>Yongdamsagantangplus daewhang</i>    | 107.7                            | >107.72                                       | >107.72 | <1                            | <1     |
| 9  | <i>Euinbujapaejangsan</i>               | >100.00                          | >100.00                                       | >100.00 | ND                            | ND     |
| 10 | <i>Euiintang</i>                        | >300.00                          | >300.00                                       | >300.00 | ND                            | ND     |
| 11 | <i>Sanpoongkosamwhan</i>                | 18.5                             | >18.50  | >18.50  | <1                            | <1     |
| 12 | <i>Homasan</i>                          | >300.00                          | >300.00                                       | >300.00 | ND                            | ND     |
| 13 | <i>Chungyolyanghyulhaed-doktang</i>     | 27.5                             | >27.53  | >27.53  | <1                            | <1     |
| 14 | <i>Injinhotahg</i>                      | 150.8                            | >150.82                                       | >150.82 | <1                            | <1     |
| 15 | <i>Whadoktang</i>                       | 101.2                            | >101.21                                       | >101.21 | <1                            | <1     |
| 16 | <i>Sodokeum</i>                         | 203.5                            | >203.5  | >203.52 | <1                            | <1     |
| 17 | <i>Gilkyungsakantang</i>                | 63.5                             | >63.46  | >63.46  | <1                            | <1     |
| 18 | <i>Gilkyungtang</i>                     | 130.0                            | >130.04                                       | >130.04 | <1                            | <1     |
| 19 | <i>Dohongsamultang plusgeumjacgeun</i>  | 352.5                            | >352.46                                       | >352.46 | <1                            | <1     |
| 20 | ACV                                     | >10.00                           | 0.2746  | 0.7823  | >36.42                        | 712.78 |
| 21 | Ara-C                                   | 3.841                            | >3.841  | >3.841  | <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 2.** Screening for Extracts of Group-2\*\* Herbal Medicines against HIV

| No | Herbal Medicin               | Toxicity $CC_{50}$ <sup>a)</sup> | Antiviral activity( $EC_{50}$ ) <sup>b)</sup> |         | Selective index <sup>c)</sup> |       |
|----|------------------------------|----------------------------------|---|---------|-------------------------------|-------|
|    |                              |                                  | III BC  | BL20    | III B                         | CBL20 |
| 1  | <i>Yongdamsagantang</i>      | >100.00                          | >100.00                                       | >100.00 | ND                            | ND    |
| 2  | <i>Chesupwilyungtang</i>     | >500.00                          | >500.00                                       | >500.00 | ND                            | ND    |
| 3  | <i>Chongyolchesuptang</i>    | 13.1                             | >13.14  | >13.14  | <1                            | <1    |
| 4  | <i>Chesuptang</i>            | 274.1                            | >271.38                                       | >271.38 | <1                            | <1    |
| 5  | <i>Whangyonhaedoktang</i>    | 15.3                             | >15.3   | >15.3   | <1                            | <1    |
| 6  | <i>Daewhangmokdanpi-tang</i> | >300.00                          | >300.00                                       | >300.00 | ND                            | ND    |
| 7  | <i>Galkamwoobangjatang</i>   | 323.3                            | >323.34                                       | >323.34 | <1                            | <1    |
| 8  | <i>Chongkihaedoktang</i>     | 45.0                             | >45.03  | >45.03  | <1                            | <1    |
| 9  | <i>Galkunhaegitang</i>       | 316.4                            | >316.43                                       | >316.43 | <1                            | <1    |
| 10 | <i>Yikoyunkyosankintang</i>  | 183.4                            | >183.42                                       | >183.42 | <1                            | <1    |
| 11 | <i>Sammyosan</i>             | 165.8                            | >165.83                                       | >165.83 | <1                            | <1    |
| 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 3.** Screening for Extracts of Group-3\*\*\* Herbal Medicines against HIV

| No | Herbal Medicin            | Toxicity(CC <sub>50</sub> ) <sup>a)</sup> | Antiviral activity(EC <sub>50</sub> ) <sup>b)</sup> |         | Selective index <sup>c)</sup> |        |
|----|---------------------------|---|---|---------|-------------------------------|--------|
|    |                           |   | III BC  | BL20    | III B                         | CBL20  |
| 1  | <i>Insanyangyoungtang</i> | 413.7                                     | >413.68   | >413.68 | <1                            | <1     |
| 2  | <i>Naetakwanggisan</i>    | 384.8                                     | >384.81   | >384.81 | <1                            | <1     |
| 3  | <i>Gamibaenongtang</i>    | >500.00                                   | >500.00   | >500.00 | ND                            | ND     |
| 4  | <i>Paljintang</i>         | >100.00                                   | >100.00   | >100.00 | ND                            | ND     |
| 5  | <i>Gamroum</i>            | 111.5                                     | >115.0  | >115.0  | <1                            | <1     |
| 6  | <i>Samginaetaksan</i>     | 400.5                                     | >400.48   | >400.48 | <1                            | <1     |
| 7  | <i>Toonongsan</i>         | >500.00                                   | >500.00   | >500.00 | ND                            | ND     |
| 8  | ACV                       | >10.00                                    | 10.2746   | 0.7823  | >36.42                        | >12.78 |
| 9  | Arc-C                     | 3.841                                     | >3.84   | >3.84   | <11                           | <1     |

\*\*\* Treatment methods of herbal medicines are invigorating qi and recuperate yin.

a) 50% Cytotoxic Concentration (CC<sub>50</sub>) is the concentration of the 50% cytotoxic effect

b) 50% Effective Concentration (EC<sub>50</sub>) is the concentration of the sample required to inhibit virus-induced CPE 50%

c) Selective index(SI)=CC<sub>50</sub>/EC<sub>50</sub>

**Table 4.** Screening for Extracts of Group-4\*\*\*\* Herbal Medicines against HIV

| No | Herbal Medicin                  | Toxicity(CC <sub>50</sub> ) <sup>a)</sup> | Antiviral activity(EC <sub>50</sub> ) <sup>b)</sup> |         | Selective index <sup>c)</sup> |       |
|----|---------------------------------|---|---|---------|-------------------------------|-------|
|    |                                 |   | III BC  | BL20    | III B                         | CBL20 |
| 1  | <i>Hyungbangpaedoksan</i>       | 9.3                                       | >9.3  | >9.3    | <1                            | <1    |
| 2  | <i>Soshihotang</i>              | >100.00                                   | >100.00   | >100.00 | ND                            | ND    |
| 3  | <i>Soshihotang plus hagocho</i> | >300.00                                   | >300.00   | >300.00 | ND                            | ND    |
| 4  | <i>Jungsihoum</i>               | >100.00                                   | >100.00   | >100.00 | ND                            | ND    |
| 5  | <i>Sogonjunwonsan</i>           | >300.00                                   | >300.00   | >300.00 | ND                            | ND    |
| 6  | <i>Chunghwajiyangtan I</i>      | 155.8                                     | >155.77   | >155.77 | <1                            | <1    |
| 7  | <i>Chunghwajiyangtan II</i>     | 14.2                                      | >14.20  | >14.20  | <1                            | <1    |
| 8  | ACV                             | >250                                      | 0.77  | 1.27    | >36.42                        | >197  |
| 9  | Arc-C                           | 1   | 0.48  | 0.88    | 20.75                         | 11.32 |

\*\*\*\* Treatment methods of herbal medicines are reducing fever by reconciliation.

a) 50% Cytotoxic Concentration (CC<sub>50</sub>) is the concentration of the 50% cytotoxic effect

b) 50% Effective Concentration (EC<sub>50</sub>) is the concentration of the sample required to inhibit virus-induced CPE 50%

c) Selective index(SI)=CC<sub>50</sub>/EC<sub>50</sub>

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