Assessment Of Traditional Chinese Herbal Medicine Plant Extracts' Potential To Inhibit Activity Of Herpes Simplex Virus Type 1

by

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ABSTRACT

Herpes simplex virus type 1 (HSV-1) is associated with oral and genital lesions as well as more serious, even fatal, infections in immunocompromised patients or when transmitted to infants. Currently, there is no cure or preventative vaccine available for HSV. Acyclovir is used for treatment of HSV infections but resistance to this drug is common in immunocompromised patients and severe side effects can develop when used by pregnant mothers and infants. The lack of a preventative option and limited treatments demonstrate the need for more effective treatment measures. Many studies have demonstrated the effectiveness of TCM plants against various illnesses, but little has been done to evaluate TCM plant extracts against HSV-1. This study tested 51 TCM extracts from 13 different plants for their potential to inhibit HSV-1. Extracts were separated into fractions and dissolved in the solvents petroleum ether, ethyl acetate, 95% ethanol or water. Vero cells were used to evaluate plant extracts for anti HSV-1 activity. Extracts were combined with virus and protection of cells was determined by using PrestoBlue, a cell viability fluorescent dye. Extracts were tested for toxic effects on host cells and were diluted to non-toxic levels prior to antiviral testing. A total of 51 extracts from 13 different plants were tested. Out of these 51 extracts, 14 were found to have at least 50% viral inhibition with 3 of them showing above 95% viral inhibition. The ultimate goal of this study is to isolate and identify a pure compound that can combat HSV-1.

ACKNOWLEDGEMENTS

I would like to thank the Guangxi Botanical Garden of Medicinal Plants in China and the Tennessee Center for Botanical Medicine Research for providing TCM plant extracts for research. I also would like to thank the Honors College and the members of my committee for dedicating their time and expertise towards the evaluation of my thesis. Finally, I extend my greatest gratitude to Dr. Stephen Wright for his tremendous patience, mentorship, and expertise in advising my research, and aiding me in the completion of this entire process.

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INTRODUCTION

A. Herpes Viruses

Herpes simplex virus type 1 (HSV-1) is a common pathogen transmitted through direct contact with an infected person or by contaminated secretions. Herpes simplex virus type 1 and type 2 (HSV-2), varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, and human herpes viruses 6, 7, and 8 are all herpes viruses within *Herpesviridae* that infect humans. While HSV-1 is more commonly associated with oral lesions and HSV-2 with genital lesions, both are interchangeable (1). It is estimated that HSV-1 is responsible for about one-third of new genital herpes cases (1). HSV-1 infections are widespread among the human population with a seroprevalence ranging from 60 to over 95% in some places (2). The high prevalence of HSV-1 infections may be due to the virus's ability to be asymptomatic in its host during the latent stage, where the virus is dormant in the host's neurons (3). Besides causing cold sores, more serious outcomes of infection include encephalitis, aseptic meningitis, and corneal scarring leading to blindness (4). HSV-1 is also a concern among HIV patients, as the virus takes advantage when the host's immune system is stressed, causing pneumonia, esophagitis, hepatitis, or meningoencephalitis (5). In other cases, mother-to-child transmission of the herpes virus is possible during birth, which can cause oral or ocular infections, central nervous system disease, and possibly other fatal infections to the infant (6). All herpes viruses set up latency and may reactivate periodically throughout life.

B. Herpes Virus Life Cycle

The structure of HSV-1 is composed of linear, double-stranded DNA that is packed inside its icosahedral shaped capsid (7). On the outermost layer of the virus, surrounding the capsid is an envelope made of a lipid bi-layer with glycoproteins and other embedded proteins attached to it (7). HSV-1 uses these proteins, such as glycoprotein C, to bind to a receptor called heparan sulfate that is present on the surface of the host cells (8). Heparan sulfate is a glycosaminoglycan present on cell surfaces and extracellular matrix of virtually every animal cell (9). Both glycoprotein C and heparan sulfate are not absolutely required for virus entry, but efficiency of entry is reduced if either is absent (10).

After attaching to a receptor on the host cell, the virus fuses its envelope with the host cell membrane so that viral DNA can be released and transported to the host nucleus (7). Once viral DNA is inside the host nucleus, it will be replicated as the host cell replicates its own DNA (7). The viral DNA will then be transcribed and translated into capsids and proteins that will assemble and package the replicated viral genome. These new herpes viruses will eventually bud out of the host cell to obtain attachment glycoproteins and infect other cells (7). Because of this, herpes virus can spread cell to cell, and eventually infect the neurons in the central nervous system where it remains latent until an opportune time (7). Figure 1 below demonstrates the mechanism of HSV-1 infection of host cells.

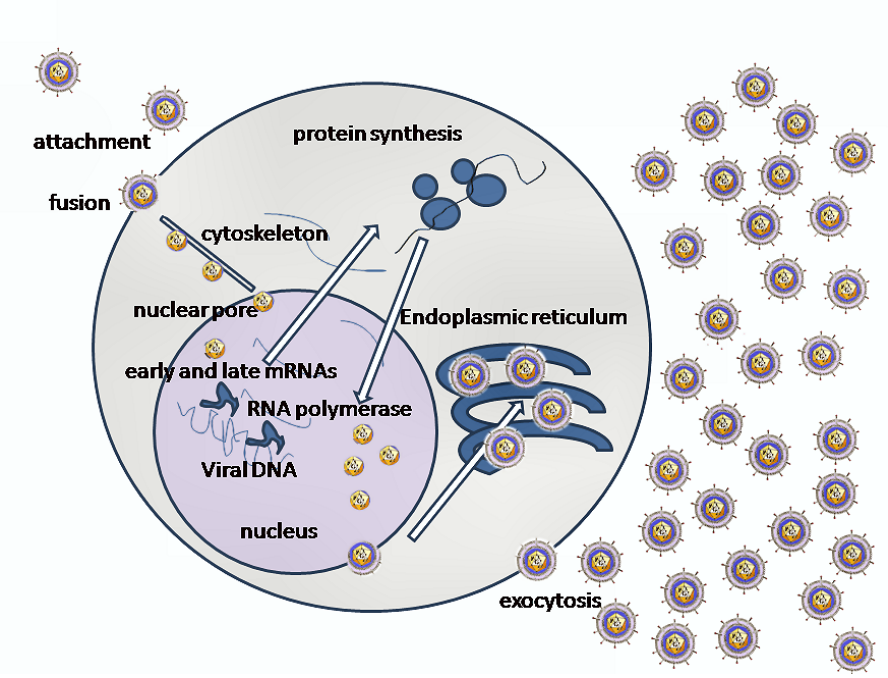

Figure 1. HSV-1 Mechanism of Infection. The virus will release viral DNA and capsid into the host cell after successful fusion with the host cell membrane by using proteins present on its surface. Viral DNA will then be transported to the nucleus where replication occurs. Afterwards, new viruses will assemble and bud out of the host cell, obtaining its envelope and spread to other cells. Image by Graham Colm (11).

C. Latency

All herpes viruses have the ability to remain latent and may reactivate periodically throughout life. Reactivation of HSV-1 and HSV-2 can manifest as oral or genital lesions or may be asymptomatic (5). Following infection, HSV-1 enters the nucleus of trigeminal ganglia sensory neurons through rapid axonal transport and becomes latent (12). HSV-2 is commonly found in the lumbosacral ganglia in its latent stage (1). Once HSV-1 has entered the neurons, most viral gene expression stops, with the exception of latency-associated transcript (LAT) (13). During latency, viral gene expression required for

productive infection does not occur (13). It is hypothesized that LAT RNA is expressed in large quantities during latency and is used to inhibit productive infection by HSV-1 (13). LAT may also be responsible for acting against apoptotic stimuli during maintenance of latency since it is the only gene that is abundantly expressed during this stage (13). A variety of factors, such as stress, hormones, ultraviolet radiation, or being immunocompromised can increase the risk of reactivation (14).

D. Transmission

Herpes viruses can be transmitted through direct contact with a person's skin lesions or respiratory secretions. Another major factor in transmission of herpes is through asymptomatic shedding, which occurs when one does not show any symptoms but is contagious (15). HSV-1 is usually spread through non-sexual transmission with the peak age of infection during childhood (1). Although HSV-1 can also cause genital herpes, HSV-2 is more commonly transmitted through sexual contact (1). In the U.S., there has been an increase in cases of genital herpes caused by HSV-1 among college students (1). Both herpes simplex viruses type 1 and 2 can be acquired vertically through mother-to-child transmission during childbirth (16). Mothers who acquire primary HSV genital infection during pregnancy are at greater risk of transmitting the disease to their infants than those with recurrent infections (16). HSV infections in neonates can cause long-term complications and may even be fatal (16).

E. Significance

1. Disease

Although both HSV-1 and HSV-2 commonly cause herpetic lesions, they also lead to more serious consequences like blindness, aseptic meningitis, and encephalitis (4). Pregnant women can transmit the disease to their infants during birth causing complications such as oral or ocular infections, and other disorders of the central nervous system (6). In rare but serious cases, the child may acquire disseminated neonatal herpes infection, which has a mortality rate of 85% if left untreated (17). Survivors may display sequelae including mental retardation, autism, epilepsy, cerebral palsy, and other neurological disorders (18). A link between HSV-1 and Alzheimer's disease has been suggested since similar regions of the brain are involved and because of HSV-1's ability to stay latent in the neurons of the central nervous system (13). The biggest concern with HSV-1 infections is in patients who are immunocompromised, such as HIV/AIDS patients, burn victims, and organ transplant patients (19).

2. Impact

HSV-1 currently affects around 70% of the world population, with a prevalence of 60 to over 95% in various places (2). HSV-1 seropositivity was found to be greater than 97% across all age groups in South African countries (20). In sub-Saharan African countries, such as Uganda and Eritrea, HSV-2 infections affect as high as 80% of the sexually active population (20). This is a concern because HSV-2 infections can more than double the risk for acquiring HIV infections (21). Immunocompromised individuals,

such as those with HIV/AIDS, are at an increased risk for recurrent HSV infections with severe outcomes (19).

Besides being a major problem in African countries, herpes simplex type 1 also affects a large amount of the U.S. population. It was reported in 2006 that HSV-1 has a seroprevalence of 57.7% in the U.S., which is three times more than the percentage of HSV-2 infection (21). Out of the 110 million sexually transmitted infections (STIs) diagnosed in the U.S., 24 million of them are caused by HSV-2 (22). The CDC estimates that about 20 million new STIs occur each year and about 45% of these are HSV-2 infections (22). With high numbers of HSV infections all over the world, there is a need for appropriate treatment and prevention.

F. Treatment

There is currently no cure or preventative vaccine available for HSV (3). The primary drug of choice is acyclovir (ACV), which can be administered orally or topically (23). Although ACV works to treat some herpes lesions, immunocompromised patients can easily become resistant to this drug (24). A ten-year survey in France found that resistance to ACV in immunocompromised patients has risen significantly from 3.8% during 2002-2006 to 15.7% in 2007-2011 (25). In addition, ACV is not recommended for neonates or pregnant women due to its unfavorable drug reactions and possible serious side effects (26). Due to the many drawbacks of using ACV as a drug, there is a need to discover new agents that can fight against herpes simplex virus without the side effects and resistance that comes with ACV.

G. Traditional Chinese Medicine

Some studies done to find new agents that can fight against HSV-1 have evaluated Traditional Chinese Medicine (TCM). TCM has been used throughout China for hundreds of years to cure diseases, and some TCM have been reported to have antiviral activity (27). One example is Berberine, a compound extracted from a TCM plant named *Coptidis rhizome* (28). Berberine was suggested to have antiviral activity by inhibiting HSV replication, but further research needs to be done to confirm its mechanism (28). Another example of an effective TCM is *Tripterygium hypoglaucum*, which demonstrated activity against HSV-1 in addition to being used to treat inflammation and tumors (29). However, additional research to identify the active compound and its mechanism has not been carried out (29). *Gnetum parvifolium* is another TCM plant that has been found to have both anticarcinogenic and antiinflammatory properties (30). One study found that the TCM plant, *Scutelleria radix*, could be used to effectively suppress hepatitis B virus (31). A study that looked at the effects of phloroglucinol glycosides from *Eucalyptus maideni* on HSV-1 found 3 compounds showing weak antiviral activity against the virus (32).

H. Current Study

Many studies have demonstrated the effectiveness of TCM plants against various illnesses, but little has been done to test TCM extracts specifically against HSV-1 on a wider scale. The basis of this current study will be to test TCM extracts for their activity against HSV-1. Traditional Chinese Medicine plant extracts have been provided through partnership between the Guangxi Botanical Garden of Medicinal Plants in China and the

Tennessee Center for Botanical Medicine Research (TCBMR). Purification of extracts and evaluation of HSV compounds was undertaken through collaborative effort with faculty and students in the Chemistry department at MTSU. A total of 51 extracts from 13 different TCM plants were tested. The long-term goal is to identify antiviral compounds for treatment of HSV-1 infections.

MATERIALS AND METHODS

A. Laboratory Standards

All work involving cells, virus, and extracts was done under a biological safety cabinet. Bottles and flasks were wiped with 70% ethanol and flamed under the hood before use to prevent any contamination. Cytotoxicity testing was done on all extracts before continuing with testing against cells and viruses. Based on our laboratory standards, extracts must show less than 20% reduction in cell viability to be considered non-toxic. If greater than 20% of cells die, the extract was diluted 2-fold until no more than 20% cell loss occurred.

Positive control wells containing only virus and cells showing approximately 50 to 75% cell death were acceptable. Extracts exhibiting above 50% viral inhibition were considered moderately effective. The half maximal inhibitory concentration (IC50) was determined for those extracts that showed above 95% viral inhibition.

Additional standards that had been established previously in our laboratory were also applied to this study. Extracts were resuspended in dimethyl sulfoxide (DMSO, Sigma, Chemical Company, St. Louis, MO). Dimethyl sulfoxide was tested alone on cells without extract to verify that DMSO had no toxic effect on cells.

To ensure that our process for determination of anti-viral activity was valid, the known anti-HSV-1 drug acyclovir (Sigma) was also tested. Previous studies in our laboratory indicated that acyclovir was effective at inhibiting virus and the concentration was in good agreement with reported acyclovir anti-herpes activity (33).

B. Media Preparation

Phosphate Buffered Saline (PBS) was prepared by mixing 991 mL deionized water (dH_2O), 8 g of NaCl (Fisher Scientific, Suwanee, GA), 1.15 g of Na_2HPO_4 (Fisher Scientific), 0.2 g of KCl (Fisher Scientific), and 0.2 g of KH_2PO_4 (Fisher Scientific). The solution was then sterilized by autoclaving.

To prepare fresh supplemented M199 Hanks' or M199 Earle's medium, approximately 90 mL of M199 Hanks' or M199 Earle's media (Sigma) was poured into an autoclaved glass bottle. In addition, 8 mL of fetal bovine serum (Gibco Life Technologies, Grand Island, NY), 1 mL of glutamine (Sigma), 1 mL of penicillin-streptomycin (Sigma), and 0.5 mL of fungizone (Invitrogen Life Technologies) were added to the solution. Prepared supplemented media in glass bottles were labeled, dated, and stored at 4°C along with unsupplemented media.

C. Cell Maintenance

Host cells used to grow HSV-1 were Vero cells (African Green Monkey Kidney, American Type Culture Collection certified cell line #81). Vero cells were incubated at 37°C (Revco Scientific Inc., Asheville, NC) and were maintained in 25 cm^2 tissue culture flasks (Corning Costar Corp., Cambridge, MA) with M199 Hanks' and M199 Earle's medium. After about a week, cells grew to a confluent monolayer on the bottom of the flask. These cells can be passed into new flasks or used in new plates. Cells were passed by washing twice in 5 mL of PBS for a minute each time, decanting PBS after each wash. Following washing, 5 mL of 0.1% trypsin (Sigma) was added and incubated at 37°C for about 5-10 minutes to begin detachment of cells from the bottom of flask. Afterwards, the

trypsin solution was discarded and the empty flask containing cells was incubated again at 37°C for about 15-20 minutes. Once cells were able to easily slide off the flask, 5 mL of M199 Hanks' media was added to flask and triturated using a pipette, allowing clumps of cells to break up. At this point, a plate can be made from the re-suspended cells. Cells can also be passed onto new flasks by adding 1 mL of re-suspended cells and 4 mL of M199 Hanks' media. After 2 or 3 days, M199 Hanks' media in the new flask was discarded and 5 mL M199 Earle's media, containing a stronger buffer, was added.

D. Preparing a Plate

To prepare a plate, cells in a confluent monolayer in a 25 cm^2 culture flask were washed twice with PBS, detached with 0.1% trypsin, then triturated with 5 mL of M199 Hanks' media as described previously. After trituration, 2 mL of cell suspension and 8 mL of M199 Earle's media were added to a sterile plastic tray (Aquafill, Swedesboro, NJ).

Using a micropipette, 100 μL of the cell suspension was added to each well that required cells on a 96 well plate (Corning). The plate was then incubated for 24 hours in 5% CO_2 at 37°C (Fisher Scientific). Following incubation, fresh media containing virus or extract was added to the cell monolayer for testing.

E. Cytotoxicity Testing

Whole plants or plant parts had been previously ground up and separated into fractions that were dissolved into different solvents with increasing polarity (petroleum ether, ethyl acetate, 95% ethanol or water). Dried extracts were resuspended in DMSO at

a concentration of 10 mg/mL. A 96 well plate was prepared using a suspension of cells followed by incubation for 24 hours as described previously. After incubation, extracts were added to wells, in sets of 3 (Fig. 2). Extracts were tested starting at 100 µg/mL concentration by adding 396 µL of M199 Earle's and 4 µL of extract into a 1.5 mL Eppendorf microfuge tube. After extracts were used, they were removed from the hood and stored in the freezer. Stock virus was then removed from the freezer and thawed under the hood.

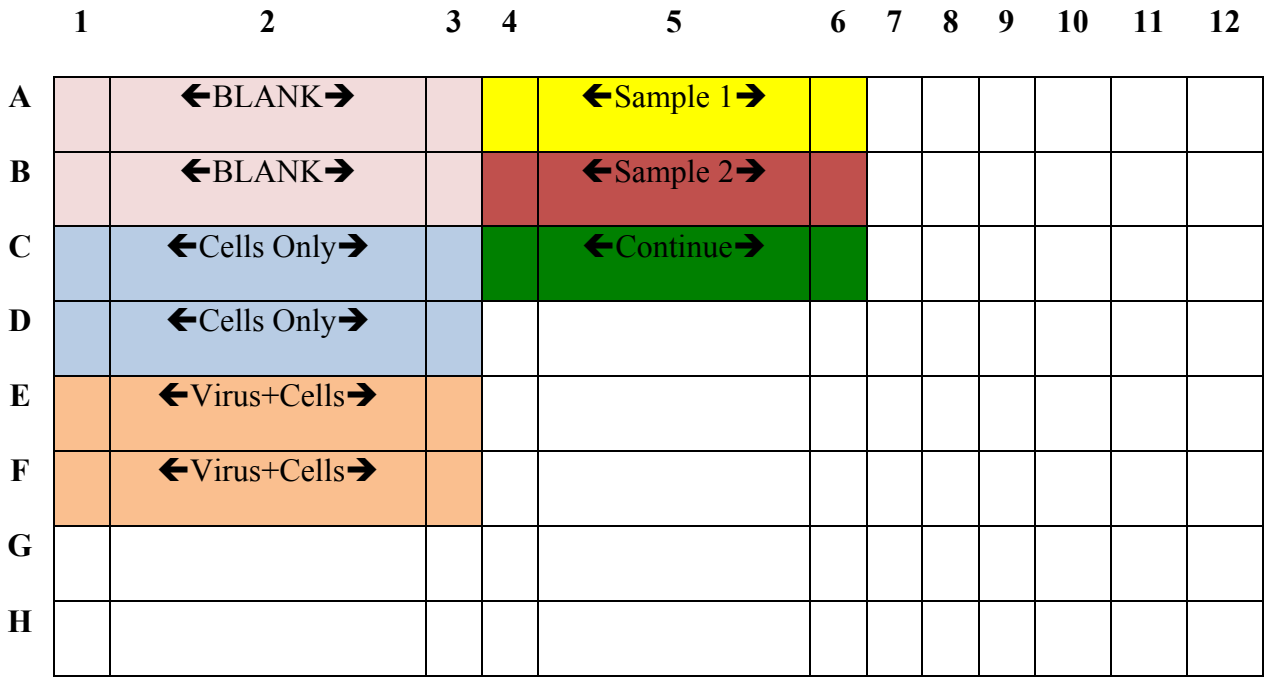

Figure 2. Plate Setup. Example of how each plate was set up for cytotoxicity testing, virus testing, and extract testing. Each extract and control was screened in triplicate as shown by the colors in the table above. Twenty-four different samples could be tested using this configuration.

A total of 6 wells were reserved as the virus only control. Virus control wells were prepared by adding 8 µL of virus and 792 µL of M199 Earle's media into a microfuge tube. The stock virus tube was then marked as used and put back in the freezer. When stock virus tubes were used for the second time to prepare the virus only control wells, 10 µL of virus and 790 µL of M199 Earle's media were added to the microfuge tube. Stock virus tubes were discarded after the second use.

After all microfuge tubes were prepared with the appropriate volume of viruses and extracts, the 96 well plate which had been prepared 24 hours previously, was removed from incubation. Medium was aspirated from 6 wells at a time using a Pasteur pipette and vacuum (Cole Parmer, Vernon Hills, IL), then 100 µL of appropriate extract/control was added to each well and the plate was incubated for 48 hours at 37°C in 5% CO_2.

After the 48 hour incubation period, the plate was ready to be read. About 1 mL of PrestoBlue dye (Invitrogen) was added to a sterile plastic tray and 11.1 µL of the dye was pipetted into each well on the plate. PrestoBlue is a cell permeable dye that changes color from blue to red when resazurin in the dye is reduced to resorufin by viable cells. The plate was then incubated for 30 minutes at 37°C in 5% CO_2. After incubation, the plate was read for fluorescence using a spectrophotometer (Molecular Devices, Sunnydale, CA) that measured the intensity of light by using Softmax Pro software.

This procedure was repeated as necessary. Extracts were diluted 2-fold until the working concentration was found that caused less than 20% cell death. The working concentration of extract was then used for virus testing.

F. Virus Dilution Testing

A plate was prepared using a confluent monolayer of cells and incubated for 24 hours as described previously. Virus was added to cells at a multiplicity of infection (MOI) of 0.1, meaning 1 virus particle per every 10 cells. Each well was estimated to contain 5000 cells, so a dilution containing 500 HSV-1 was added to each well. Following incubation, PrestoBlue was added to verify appropriate exposure to virus. Any virus dilution that killed more than 50% but less than 75% of cells was considered acceptable.

G. Extract Screening

To test an extract's ability to inhibit HSV-1, the appropriate amount of virus and a non-toxic concentration of the extract were added to wells with a confluent monolayer. The non-toxic or working concentration of extracts was determined by performing cytotoxicity testing as described.

To test for the antiviral potential of extracts, a 96 well plate was prepared using a suspension of cells and incubated for 24 hours as described previously. After the 24-hour incubation period, extracts were removed from the freezer. Microfuge tubes were labeled with names of all media, extracts, and controls being used. Fresh M199 Earle's was used for blank wells and cells only controls.

For extracts that were not toxic at 100 μg/mL, 4 μL of the appropriate extract was added to a microfuge tube. For extracts with working concentration of 50 μg/mL, 2 μL was added and for those with working concentration of 25 μg/mL, 1 μL was added to

tubes. Virus to be used in controls and in tubes with extracts was prepared by diluting the viral stock solution to an appropriate level to achieve an MOI of 0.1 when added to extract and control tubes. M199 Earle's media was added to sample tubes until the total volume of fluid was 400 µL, and for control tubes, M199 Earle's media was added until the total volume was 800 µL.

The prepared 96 well plate was then removed from incubation. Medium was aspirated from 6 wells at a time using a vacuum. Next, 100 µL of appropriate extract/virus or control was added to each well and the plate was incubated for 48 hours at 37°C in 5% CO_2.

Following 48 hours of incubation at 37°C in 5% CO_2, the plate was ready to be read. About 1 mL of PrestoBlue dye was added to a sterile plastic tray and 11.1 µL of the dye was pipetted into each well on the plate. The plate was then incubated for 30 minutes at 37°C in 5% CO_2. After incubation, the plate was read for fluorescence using a spectrophotometer and the percent of virus inhibition was calculated. Extracts had to inhibit at least 50% of virus to be considered effective.

This experiment was done for all extracts at their appropriate non-cytotoxic concentrations at least three times to ensure valid results. IC50s were performed on extracts showing above 95% viral inhibition by diluting the extract 2-fold to show antiviral activity.

RESULTS

A total of 51 extracts from 13 different plants were evaluated for their cytotoxicity on cells and their potential to inhibit HSV-1. A list of all extracts evaluated appears in Appendix 1. All extracts were screened for cytotoxicity starting at a concentration of 100 μg/mL. Table 1 below summarizes the results of cytotoxicity studies.

Table 1. Non-Cytotoxic Working Concentrations of Extracts. While a majority of extracts were used at working concentrations of 100 μg/mL, others had to undergo subsequent 2-fold dilutions to result in less than 20% cell death.

Working concentration (μg/mL)	**# of extracts**
100	30
50	14
25	5
12.5	2

After determining the non-toxic working concentrations, all extracts were tested for their potential to inhibit HSV-1. Data for all evaluations are included in Appendix 2. Out of the 51 extracts tested, 14 extracts exhibited above 50% viral inhibition and were considered effective at inhibiting HSV-1. A total of 11 out of these 14 extracts showed

from 50 to 95% viral inhibition, while the other three extracts inhibited over 95% of virus. Table 2 lists the extracts that showed between 50 to 95% viral inhibition and their working concentrations. Table 3 lists the three extracts that inhibited over 95% of virus and their working concentration. Extract 32B displayed unusual results. Three separate evaluations were done. Its antiviral average includes two numbers over 100%, and one below 40%. With those three numbers averaged together, 32B still demonstrated antiviral activity of 98% and is included in Table 2 below.

Table 2. Extracts Showing Moderate Antiviral Activity. These extracts showed between 50 to 95% viral inhibition. Their working concentration, cytotoxicity, percentage of viral inhibition, and their standard error are shown below.

Extract	Working Concentration at µg/mL	% Cytotoxicity at Working Concentration	% Viral Inhibition and Standard Error
23D	100	-2*	50 ± 25
26B	100	16	57 ± 3
27B	100	20	70 ± 1
28B	100	11	75 ± 7
28C	100	-7*	62 ± 19
29B	50	4	55 ± 22
31C	100	6	58 ± 17
32C	50	1	50 ± 25
33A	100	17	62 ± 7
33B	50	5	51 ± 21
33C	100	-1*	78 ± 14

* Negative values for cytotoxicity indicate that the extract resulted in greater cell growth than cells grown in the absence of extract.

Table 3. Extracts Showing Highest Antiviral Activity. These extracts inhibited over 95% of HSV-1. Their working concentration, cytotoxicity, percentage of viral inhibition and their standard error are shown below.

Extract	**Working Concentration at µg/mL**	**% Cytotoxicity at Working Concentration**	**% Viral Inhibition and Standard Error**
22B	25	12	107 ± 13
23B	25	-3*	99 ± 27
32B	100	-58*	98 ± 29

* Negative values for cytotoxicity indicate that the extract resulted in greater cell growth than cells grown in the absence of extract.

Direct images of cells were taken to show the qualitative effects of virus and extracts on the cells. Figure 3 shows the negative control well containing only viable cells. Figure 4 shows the positive control well containing both viruses and cells. Very few intact cells can be seen in Figure 4 as most host cells have been destroyed by HSV-1. In Figure 5, the protective effect of an extract can be seen as the cell destruction has been reduced.

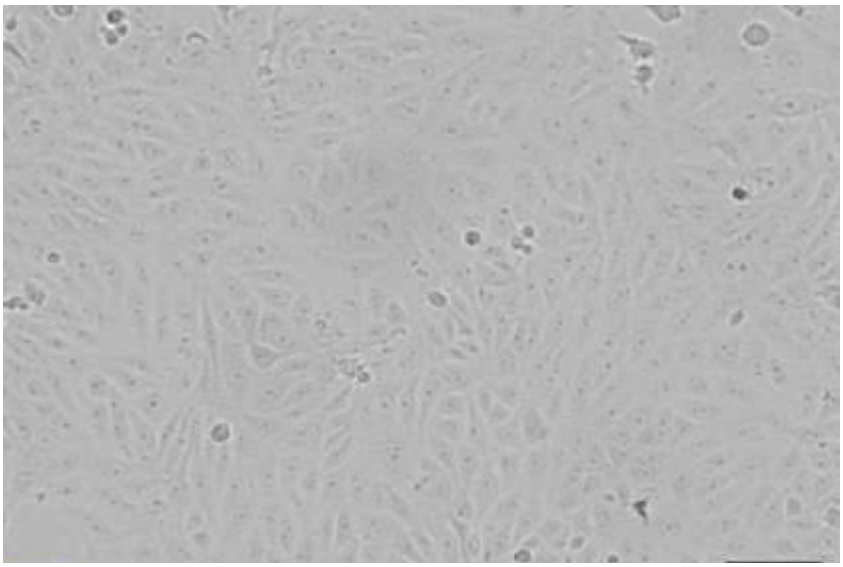

Figure 3. Negative Control. This is an image of a negative control containing only cells and media. Only healthy cells can be seen in this image.

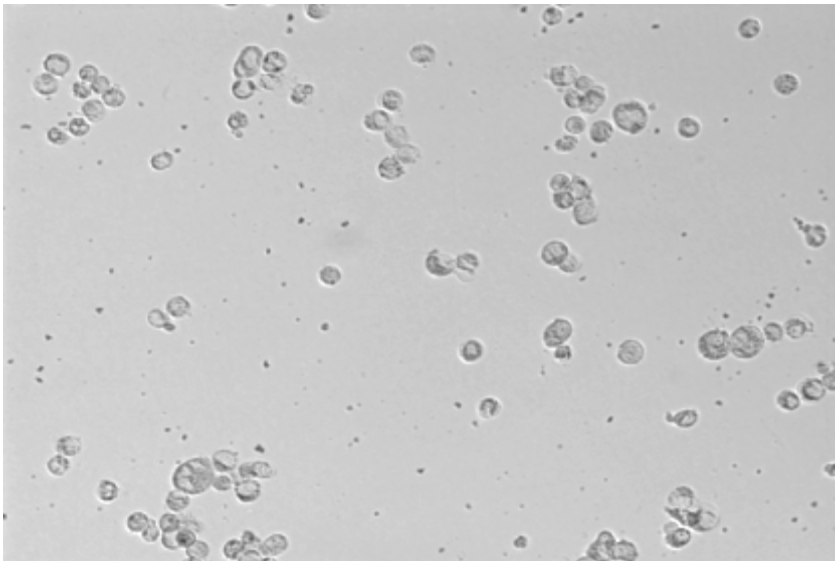

Figure 4. Positive Control. This is an image of a positive control containing cells, media and HSV-1. Very few intact cells can be seen in the image due to the destructive effects of the virus.

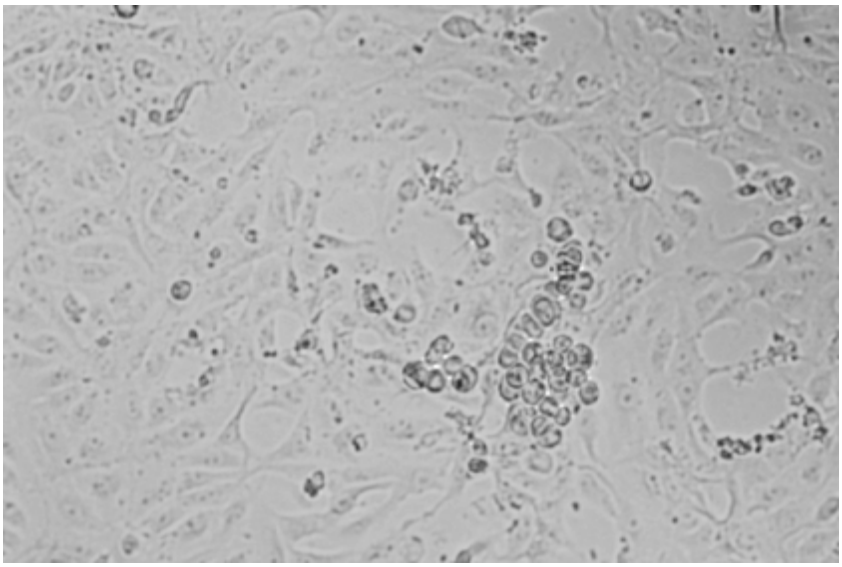

Figure 5. Cells Exposed to Extract and HSV-1. The protective effects of an extract can be seen in this image, as the cell destruction has been reduced.

Two out of the three extracts showing above 95% viral inhibition were serially diluted and graphed to determine each extract's IC50 using a line of best fit (Fig. 6, 7). Extract 22B displayed the lowest IC50 at 4.39 μg/mL, and extract 23B showed an IC50 at 5.15 μg/mL. Both extracts have a working concentration at 25 μg/mL. The IC50 curve for extract 32B is not presented here due to laboratory errors and the extreme variability of virus inhibition.

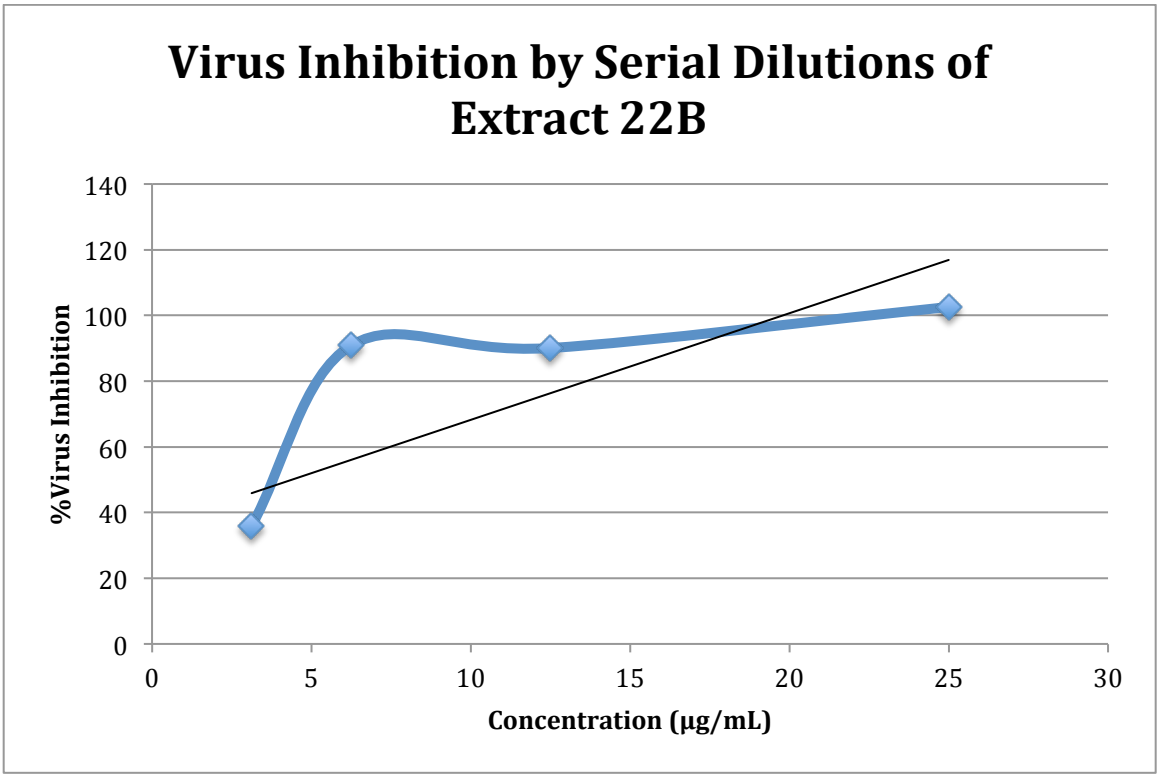

Figure 6. Graph Depicting the Serial Dilution of Extract 22B. Extract 22B was diluted 2-fold starting at 25 µg/mL concentration and ending with 3.125 µg/mL concentration. Percentage of viral inhibition was determined at each concentration. The IC50 was determined to be 4.39 µg/mL.

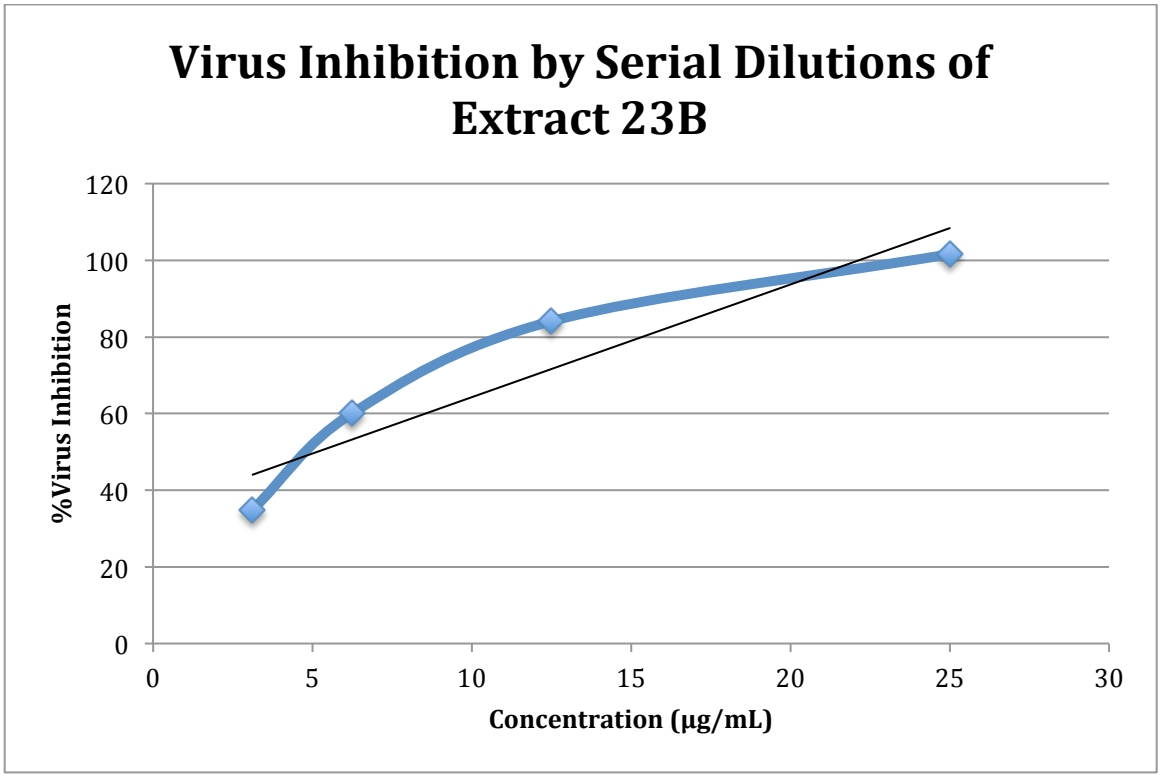

Figure 7. Graph Depicting the Serial Dilution of Extract 23B. Extract 23B was diluted 2-fold starting at 25 µg/mL concentration and ending with 3.125 µg/mL concentration. Percentage of viral inhibition was determined at each concentration. The IC50 was determined to be 5.15 µg/mL.

DISCUSSION

Herpes simplex virus type 1 can cause a significant range of diseases worldwide and is especially troublesome for immunocompromised individuals and neonates. After attaching to its specific host cell receptor, HSV-1 commonly enters the cell through receptor-mediated endocytosis or by fusion. The virus then enters the nucleus and begins replication. New viruses are assembled and eventually bud out of the cell to spread the infection to other cells. The virus may become latent in neurons. The HSV-1 life cycle presents many opportunities where the virus could be inhibited.

Extracts exhibiting antiviral activity may inhibit the virus through several mechanisms such as blocking specific proteins for viral entry or by working directly on the virus itself. The current drug of choice, acyclovir, works by acting as a substitute for a nucleotide. Acyclovir is activated by the viral enzyme, thymidine kinase, which stops viral DNA polymerase in infected cells (34). Although successful at inhibiting HSV-1, increasing resistance to acyclovir, especially in immunocompromised individuals, demonstrates a need for new agents that can combat HSV-1. TCM plants have been historically successful in treating antiviral infections and provide potential as anti-HSV-1 drugs. The results of this study support this statement. Out of the 51 Traditional Chinese Medicine (TCM) extracts tested, 11 were shown to be moderately effective at inhibiting HSV-1 and two, perhaps three, other extracts are highly effective against the virus.

The majority of the extracts tested did not show any anti-HSV-1 activity. Although most of these plants have not been previously tested for antiviral activity, some have been reported to have other therapeutic effects. There were no reports regarding the

antiviral activity of *Tinospora sinensis* but this plant has been reported to be effective against protozoan infection of Leishmania (35). Studies on *Sarcandra glabra* extract have reported it to have both antiinflammatory and antiinfluenza properties (36,37). Extract from the fruit of *Melaleuca leucadendra* has been reported to have anti-HSV-1 activity (38), but in this study, extract from the stem of the same plant was inactive against the virus. Lastly, there have been no reports of any therapeutic properties associated with the plant *Mappianthus iodoides*. Extracts of *M. iodoides* failed to show any anti-HSV-1 activity in this study.

Extracts that were moderately effective against HSV-1 showed between 50-78% viral inhibition. The ethyl acetate extract of the plant *Ramulus uncariac* inhibited HSV-1 at 57% but has not been previously evaluated for its antiviral properties. Extract 27B, the ethyl acetate fraction of the plant *Wedelia calendulacea*, showed 70% viral inhibition. This plant has not been previously tested for its antiviral activity but one study stated it has neuroprotective effects (39). The ethyl acetate and ethanol extract of *Uncaria macrophylla* showed moderate antiviral activity at 75% and 62% respectively. Besides *R. uncariac*, this, too, was the first study that evaluated *U. macrophylla* for antiviral activity.

A wide range of studies have been done on *Plantago major* and *Syzygium cumini*. Extract 29B is the ethyl acetate fraction of *P. major*, which exhibited 55% viral inhibition. One study evaluated *P. major* against HSV-1, HSV-2, and adenoviruses and found slight antiviral activity against all three viruses (40), which is in agreement with this study. Another extract showing moderate antiviral activity at 58% was extract 31C, which is the ethanol fraction of *S. cumini*. Although no previous antiviral work has been done on this plant, it is reported to have many other therapeutic properties such as being

antidiabetic, antiulcer, hepatoprotective, an antioxidant, and other protective activities (41).

Extracts 32B and 32C are the ethyl acetate and ethanol fraction of *Rubus reflexus*. These extracts inhibit HSV-1 at 98% and 50% respectively. Unfortunately, perhaps due to laboratory errors, an IC50 curve was not obtained for extract 32B. Results suggest that the IC50 for 32B should be somewhere in between 50 μg/mL and 100 μg/mL. Further investigation is needed to determine the exact IC50 concentration. No previous studies were found on this plant. Lastly, extracts 33A, 33B, and 33C all inhibit HSV-1 at 62%, 51%, and 78%. These are the petroleum ether, ethyl acetate, and ethanol fraction of the plant *Cissus pteroclada*. This plant has not been tested for antiviral activity, but it is reported to have antioxidant and hepatoprotective properties (42).

Extract 22B showed the highest HSV-1 inhibition in this study at 107%. This is the ethyl acetate fraction of the plant *Bidens pilosa*. Studies on *B. pilosa* have reported this plant to be useful in the treatment of more than 40 disorders including cancer, immunological disorders, wounds, digestive disorders, bacterial infections, and other maladies (43). Although much research has been done on the beneficial properties of *B. pilosa*, only one study was found evaluating its antiviral activity (44). This investigation evaluated a hot water extract of *B. pilosa* against HSV-1 and HSV-2 (44). The extract was found to significantly inhibit HSV replication at a concentration of 100 μg/mL, a concentration that was cytotoxic for Vero cells (44). The positive antiviral results of the previous study support the evaluation of the current research on *B. pilosa*. The difference is that the ethyl acetate fraction *of B. pilosa* was used in this study, which has a working concentration at 25 μg/mL. Extract 22B presents the lowest IC50 in this study at 4.39

μg/mL, suggesting it may be more effective at lower concentrations. These properties of 22B suggest that this is a promising extract for future investigation.

The second extract that was highly effective, inhibiting HSV-1 at 99%, was 23B. This is the ethyl acetate fraction of the plant *Mangifera persiciformis*. Extract 23D, the water fraction from this same plant, also shows moderate inhibition at 50%. Not many previous studies have been done on this plant, and none were found that evaluated its antiviral properties. The limited study of this plant may be due to *M. persiciformis* being currently labeled as a threatened species (45). This may limit future investigation of extract 23B in spite of its effective anti-HSV-1 activity and IC50 at 5.15 μg/mL.

Out of 51 extracts evaluated, 14 showed moderate to high antiviral activity. From the 14 extracts, the majority of the fractions that were effective were extracts containing ethyl acetate solvent. Figure 8 below shows the proportion of extracts in different solvents that were effective.

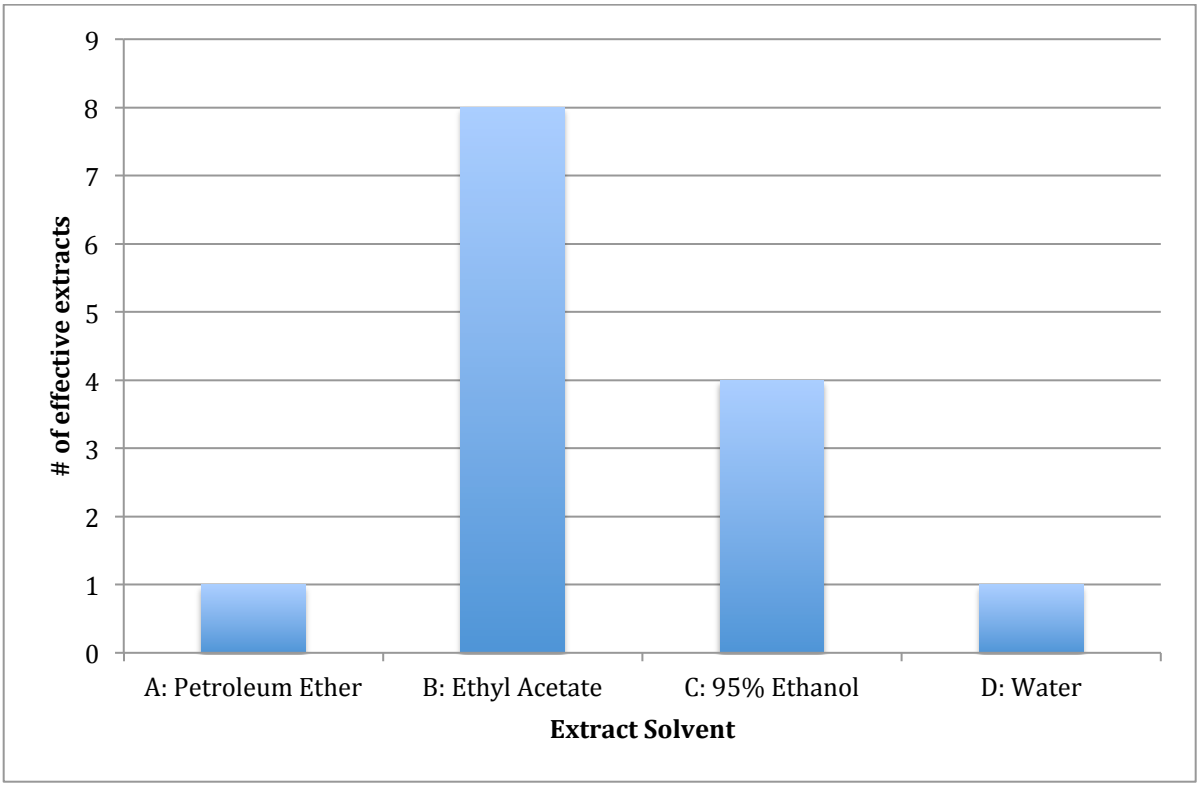

Figure 8. Proportion of Solvents with Number of Effective Extracts. Out of the 14 effective extracts, 8 were the ethyl acetate fraction, 4 were in 95% ethanol, 1 was in petroleum ether, and 1 was in water.

The majority of the effective extracts were present in the ethyl acetate fraction, including the two that showed the highest viral inhibition. The water and petroleum ether fraction had the fewest anti-HSV-1 activity. The disproportional distribution of solvents with effective extracts suggests that ethyl acetate may be binding to some active compound in plants, allowing it to more effectively inhibit HSV-1. A study that investigated the chemical composition of the ethyl acetate fraction of *B. pilosa* using High Performance Liquid Chromatography reported the presence of two flavonoids, quercetin and iso-okanin (46). One study evaluated the chemical composition of *M. persiciformis*, and the same flavonoid, quercetin, was also isolated from this plant (47).

These are just two molecules that *B. pilosa* and *M. persiciformis* have in common. There may be many other active compounds from these two plants that are present in the ethyl acetate fraction.

This study has reported several effective antiviral extracts with potential for drug development. Additional investigation that includes guided fractionation and identification of active compounds is needed for further development. Three of the effective extracts exhibited above 95% inhibition, making them great candidates for future investigation. Overall, these extracts merit further study to identify the active compounds that may act as novel agents against HSV-1.

Appendix 1 – List of TCM Plants Used.

Name of the plant 植物名	**Materials 取材部位**	**Extraction solvent and weight (g)**	**Code**
Bidens pilosa L. 百花鬼针草	whole plant	petroleum ether: 0.06	22A
		ethyl acetate: 0.07	22B
		95% ethanol: 0.01	22C
		water: 0.10	22D
Mangifera persiciformis C. Y. Wu et T. L. Ming 扁桃	branch, leaf	petroleum ether: 0.13	23A
		ethyl acetate: 0.06	23B
		95% ethanol: 0.05	23C
		water: 0.03	23D
Tinospora sinensis(Lour)Merr. 青九牛	stem	petroleum ether: 0.05	24A
		ethyl acetate: 0.08	24B
		95% ethanol: 0.04	24C
		water: 0.04	24D
Mappianthus iodoides Hand—Mazz. 铜钻	stem	petroleum ether: 0.04	25A
		ethyl acetate: 0.05	25B
		95% ethanol: 0.06	25C
		water: 0.08	25D
Ramulus Uncariac Rhynchophyllae Cum Uncis. 钩藤	branch, leaf	petroleum ether: 0.03	26A
		ethyl acetate: 0.03	26B
		95% ethanol: 0.05	26C
		water: 0.11	26D
Wedelia calendulacea Less. 蟛蜞菊	whole plant	petroleum ether: 0.04	27A
		ethyl acetate: 0.06	27B
		95% ethanol: 0.07	27C
		water: 0.04	27D
Uncaria macrophylla Wall. 大叶钩藤	branch, leaf	petroleum ether: 0.04	28A
		ethyl acetate: 0.07	28B
		95% ethanol: 0.14	28C
		water: 0.04	28D

Name of the plant 植物名	**Materials 取材部位**	**Extraction solvent and weight (g)**	**Code**
Plantago major Linn.　大叶车前	whole plant	petroleum ether: 008	29A
		ethyl acetate: 0.08	29B
		95% ethanol: 0.05	29C
		water: 0.08	29D
Sarcandra glabra (Thunb.) Nakai 九节风	whole plant	petroleum ether: 0.06	30A
		ethyl acetate: 0.08	30B
		95% ethanol: 0.07	30C
		water: 0.06	30D
Syzygium cumini(L.) Skeels.　海南蒲桃	dried fruit	petroleum ether: 0.07	31A
		ethyl acetate: 0.11	31B
		95% ethanol: 0.08	31C
		water: 0.08	31D
Rubus reflexus Ker var. lanceolobus metc 七爪风	stem	petroleum ether: 0.05	32A
		ethyl acetate: 0.06	32B
		95% ethanol: 0.04	32C
Cissus pteroclada Hayata 四方钻	stem	petroleum ether: 0.03	33A
		ethyl acetate: 0.0595%	33B
		ethanol: 0.11	33C
		water: 0.04	33D
Melaleuca leucadendra Linn. 百千层	stem	petroleum ether: 0.04	34A
		ethyl acetate: 0.0795%	34B
		ethanol: 0.08	34C
		water: 0.05	34D

Appendix 2 – Raw Data from Extract and Virus Testing

Plate 1

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	6374....	7448.803	862.796	11.5...
	C2	8523....			
	C3	7414....			
	D1	8277....			
	D2	6598....			
	D3	7503....			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	1678....	1957.421	261.365	13.3...	73.722
	E2	2232....				
	E3	2107....				
	F1	1622....				
	F2	2185....				
	F3	1918....				

22A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	6368....	6226.994	225.196	3.616	16.403	2...	77.750
	A5	5967....						
	A6	6345....						

22B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	9527....	9187.693	1198.212	13.0...	-23.345	-3...	131.666
	A8	7856....						
	A9	10179...						

22C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	4678....	3399.684	1119.049	32.9...	54.359	7...	26.264
	A11	2597....						
	A12	2923....						

22D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	3630....	3240.259	657.614	20.2...	56.500	7...	23.361
	B5	3608....						
	B6	2481....						

23A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	5444....	4854.051	585.588	12.0...	34.834	4...	52.749
	B8	4273....						
	B9	4844....						

23B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	10122...	10390.029	920.074	8.855	-39.486	-5...	153.561
	B11	11414...						
	B12	9633....						

23C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	6770....	6246.825	747.931	11.9...	16.137	2...	78.112
	C5	6579....						
	C6	5390....						

23D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	6420....	7435.858	887.060	11.9...	0.174	0....	99.764
	C8	8059....						
	C9	7827....						

24A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	4258....	3738.124	846.614	22.6...	49.816	6...	32.427
	C11	2761....						
	C12	4194....						

24B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	2127....	2049.476	101.066	4.931	72.486	9...	1.676
	D5	2084....						
	D6	1935....						

24C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	2179....	2214.049	172.387	7.786	70.276	9...	4.673
	D8	2061....						
	D9	2401....						

24D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	2143....	1990.620	137.195	6.892	73.276	9...	0.605
	D11	1948....						
	D12	1879....						

Plate 2

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	15361...	17366.549	1449.731	8.348
	C2	16652...			
	C3	17956...			
	D1	17040...			
	D2	19715...			
	D3	17472...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	5866....	6706.950	756.976	11.2...	61.380
	E2	6689....				
	E3	6950....				
	F1	5826....				
	F2	7131....				
	F3	7776....				

22A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	10917...	10219.221	759.738	7.434	41.156	6...	32.949
	A5	9410....						
	A6	10330...						

22B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	15027...	16543.392	1456.590	8.805	4.740	7....	92.278
	A8	17931...						
	A9	16671...						

22C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	4087....	3916.496	157.273	4.016	77.448	1...	-26.178
	A11	3778....						
	A12	3883....						

22D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	4908....	4916.345	76.328	1.553	71.691	1...	-16.798
	B5	4996....						
	B6	4844....						

23A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	8578....	8328.245	430.463	5.169	52.044	8...	15.210
	B8	8574....						
	B9	7831....						

23B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	13745...	14883.408	1081.406	7.266	14.298	2...	76.705
	B11	15007...						
	B12	15897...						

23C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	9996....	9776.859	656.094	6.711	43.703	7...	28.799
	C5	9039....						
	C6	10295...						

23D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	8806....	8802.547	227.342	2.583	49.313	8...	19.659
	C8	9027....						
	C9	8573....						

24A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	2673....	3070.139	705.853	22.9...	82.322	1...	-34.118
	C11	2651....						
	C12	3885....						

24B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	3418....	2887.369	495.003	17.1...	83.374	1...	-35.832
	D5	2438....						
	D6	2805....						

24C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	4015....	4001.859	261.809	6.542	76.957	1...	-25.377
	D8	4256....						
	D9	3733....						

24D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	7474....	7560.953	173.636	2.296	56.463	9...	8.012
	D11	7447....						
	D12	7760....						

Plate 3

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	12013...	14790.678	1772.524	11.9...
	C2	15014...			
	C3	16259...			
	D1	13271...			
	D2	16311...			
	D3	15873...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	5041....	5401.440	1198.137	22.1...	63.481
	E2	7295....				
	E3	5560....				
	F1	3553....				
	F2	5541....				
	F3	5414....				

22A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	6733....	7614.065	785.833	10.3...	48.521	7...	23.566
	A5	7863....						
	A6	8245....						

22B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	13806...	14506.302	1082.465	7.462	1.923	3....	96.971
	A8	15753...						
	A9	13959...						

22C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	3843....	3983.204	296.222	7.437	73.069	1...	-15.105
	A11	3782....						
	A12	4323....						

22D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	5105....	4932.198	457.346	9.273	66.653	1...	-4.998
	B5	5277....						
	B6	4413....						

23A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	5133....	5823.713	711.194	12.2...	60.626	9...	4.497
	B8	5784....						
	B9	6553....						

23B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	10292...	11777.414	2862.009	24.3...	20.373	3...	67.907
	B11	9962....						
	B12	15076...						

23C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	7676....	7360.510	394.824	5.364	50.235	7...	20.865
	C5	6918....						
	C6	7486....						

23D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	9447....	8336.018	1006.840	12.0...	43.640	6...	31.255
	C8	8074....						
	C9	7486....						

24A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	1983....	2070.435	210.591	10.1...	86.002	1...	-35.477
	C11	1917....						
	C12	2310....						

24B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	2244....	2412.610	190.066	7.878	83.688	1...	-31.833
	D5	2618....						
	D6	2374....						

24C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	2615....	3422.719	742.014	21.6...	76.859	1...	-21.074
	D8	3577....						
	D9	4074....						

24D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	5501....	5920.712	444.307	7.504	59.970	9...	5.530
	D11	6386....						
	D12	5873....						

Plate 4

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	20391...	20614.561	713.063	3.459
	C2	21708...			
	C3	20581...			
	D1	19847...			
	D2	19986...			
	D3	21172...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	3561....	3668.451	301.288	8.213	82.205
	E2	3248....				
	E3	3838....				
	F1	4128....				
	F2	3717....				
	F3	3516....				

22B 1

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	15945...	18567.537	2457.265	13.2...	9.930	1...	87.920
	A5	18940...						
	A6	20816...						

22B .5

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	21818...	20879.746	1307.877	6.264	-1.286	-1...	101.565
	A8	21435...						
	A9	19385...						

22B .25

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	20883...	18128.784	2705.352	14.9...	12.058	1...	85.331
	A11	18027...						
	A12	15475...						

22B .125

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	7816....	8715.315	779.991	8.950	57.723	7...	29.782
	B5	9120....						
	B6	9209....						

23B 1

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	20162...	21405.263	1569.275	7.331	-3.836	-4...	104.666
	B8	23168...						
	B9	20884...						

23B .5

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	21146...	19496.958	1645.993	8.442	5.421	6....	93.405
	B11	19490...						
	B12	17854...						

23B .25

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	12162...	15639.291	5156.174	32.9...	24.135	2...	70.641
	C5	13192...						
	C6	21563...						

23B .125

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	7884....	9017.438	1269.097	14.0...	56.257	6...	31.565
	C8	10388...						
	C9	8778....						

25A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	10744...	9747.165	900.095	9.234	52.717	6...	35.871
	C11	9500....						
	C12	8995....						

25B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	7548....	7511.216	407.139	5.420	63.564	7...	22.676
	D5	7086....						
	D6	7898....						

25C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	5536....	5961.945	400.734	6.722	71.079	8...	13.534
	D8	6015....						
	D9	6332....						

25D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	5817....	6123.725	316.776	5.173	70.294	8...	14.489
	D11	6103....						
	D12	6450....						

26A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E4	12540...	12932.970	812.239	6.280	37.263	4...	54.670
	E5	13866...						
	E6	12391...						

26B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E7	15311...	14236.665	1703.957	11.9...	30.939	3...	62.364
	E8	15126...						
	E9	12272...						

26C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E10	9484....	9710.684	742.135	7.642	52.894	6...	35.656
	E11	10539...						
	E12	9107....						

26D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F4	11171...	11143.776	102.914	0.924	45.942	5...	44.112
	F5	11229...						
	F6	11029...						

27A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F7	9354....	9197.641	137.039	1.490	55.383	6...	32.628
	F8	9137....						
	F9	9100....						

27B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F10	12847...	15298.171	2182.874	14.2...	25.789	3...	68.628
	F11	17033...						
	F12	16013...						

27C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G1	9459....	10267.087	922.662	8.987	50.195	6...	38.939
	G2	10069...						
	G3	11272...						

27D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	% virus inhibition
01	G4	4992....	5692.682	842.772	14.8...	72.385	Error
	G5	5457....					
	G6	6627....					

28A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G7	15146...	14058.297	1518.364	10.8...	31.804	3...	61.311
	G8	14704...						
	G9	12323...						

28B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G10	16170...	16008.435	1661.632	10.3...	22.344	2...	72.819
	G11	17583...						
	G12	14271...						

Plate 5

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	19112...	20400.499	1493.177	7.319
	C2	19288...			
	C3	22292...			
	D1	22092...			
	D2	20551...			
	D3	19064...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	4472....	5943.651	1544.325	25.9...	70.865
	E2	7254....				
	E3	5935....				
	F1	8311....				
	F2	4749....				
	F3	4938....				

22B 1

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	19938...	22101.615	1947.028	8.809	-8.339	-1...	111.767
	A5	23714...						
	A6	22652...						

22B 0.5

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	17935...	14794.720	2915.459	19.7...	27.479	3...	61.224
	A8	12175...						
	A9	14273...						

22B 0.25

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	20071...	20314.607	2404.209	11.8...	0.421	0....	99.406
	A11	22831...						
	A12	18041...						

22B 0.125

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	14515...	12890.519	2262.158	17.5...	36.813	5...	48.052
	B5	13849...						
	B6	10306...						

23B 1

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	19273...	19237.259	31.809	0.165	5.702	8....	91.954
	B8	19226...						
	B9	19212...						

23B 0.5

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	18498...	17212.654	2575.085	14.9...	15.626	2...	77.949
	B11	14247...						
	B12	18891...						

23B 0.25

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	15481...	11517.045	3436.934	29.8...	43.545	6...	38.552
	C5	9682....						
	C6	9386....						

23B 0.125

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	9852....	11911.714	3269.177	27.4...	41.611	5...	41.282
	C8	10201...						
	C9	15681...						

25A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	11140...	10524.330	726.042	6.899	48.411	6...	31.685
	C11	10708...						
	C12	9723....						

25B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	7103....	7192.897	83.858	1.166	64.742	9...	8.641
	D5	7205....						
	D6	7270....						

25C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	8438....	7890.343	649.892	8.237	61.323	8...	13.466
	D8	7172....						
	D9	8059....						

25D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	8577....	8412.951	165.489	1.967	58.761	8...	17.080
	D11	8414....						
	D12	8246....						

26A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E4	8078....	9271.791	1957.636	21.1...	54.551	7...	23.021
	E5	11531...						
	E6	8205....						

26B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E7	12043...	13618.464	1947.803	14.3...	33.244	4...	53.088
	E8	13015...						
	E9	15796...						

26C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E10	9429....	9490.456	723.832	7.627	53.479	7...	24.534
	E11	10242...						
	E12	8798....						

26D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F4	8639....	9737.967	985.071	10.1...	52.266	7...	26.246
	F5	10032...						
	F6	10542...						

27A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F7	9159....	9883.444	636.745	6.443	51.553	7...	27.252
	F8	10136...						
	F9	10354...						

27B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F10	15031...	15785.199	1690.074	10.7...	22.623	3...	68.075
	F11	17720...						
	F12	14602...						

27C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G1	8769....	8559.183	214.482	2.506	58.044	8...	18.092
	G2	8341....						
	G3	8566....						

27D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	% virus inhibition
01	G4	6546....	6810.500	248.854	3.654	66.616	Error
	G5	7041....					
	G6	6843....					

28A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G7	17635...	13665.615	3448.648	25.2...	33.013	4...	53.414
	G8	11956...						
	G9	11405...						

28B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G10	15972...	15188.733	924.458	6.086	25.547	3...	63.949
	G11	15424...						
	G12	14169...						

Plate 6

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	20527...	20962.529	1379.360	6.580
	C2	19139...			
	C3	21745...			
	D1	20080...			
	D2	21183...			
	D3	23097...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	12608...	10508.416	1105.107	10.5...	49.870
	E2	10304...				
	E3	10213...				
	F1	9532....				
	F2	10654...				
	F3	9736....				

22B 1

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	20633...	21804.438	1095.508	5.024	-4.016	-8...	108.053
	A5	21974...						
	A6	22805...						

22B .5

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	21678...	21738.789	378.848	1.743	-3.703	-7...	107.425
	A8	21393...						
	A9	22144...						

22B .25

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	20048...	19686.384	338.070	1.717	6.088	1...	87.793
	A11	19378...						
	A12	19631...						

22B .125

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	13515...	13583.287	560.693	4.128	35.202	7...	29.413
	B5	13059...						
	B6	14174...						

23B 1

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	22135...	21812.470	298.705	1.369	-4.055	-8...	108.130
	B8	21546...						
	B9	21755...						

23 B .5

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	20473...	18968.254	1350.676	7.121	9.514	1...	80.924
	B11	18568...						
	B12	17862...						

23 B .25

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	17531...	17931.198	348.598	1.944	14.461	2...	71.003
	C5	18089...						
	C6	18172...						

23B .125

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	13091...	13825.626	642.303	4.646	34.046	6...	31.731
	C8	14103...						
	C9	14282...						

25A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	14057...	15274.727	1321.123	8.649	27.133	5...	45.593
	C11	15086...						
	C12	16679...						

25B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	10126...	10417.207	255.164	2.449	50.306	1...	-0.872
	D5	10602...						
	D6	10523...						

25C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	12005...	13551.198	1342.660	9.908	35.355	7...	29.106
	D8	14216...						
	D9	14431...						

25D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	14385...	13584.401	1119.073	8.238	35.197	7...	29.424
	D11	14061...						
	D12	12305...						

26A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E4	13521...	13671.934	130.762	0.956	34.779	6...	30.261
	E5	13756...						
	E6	13737...						

26B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E7	16348...	16336.942	81.308	0.498	22.066	4...	55.753
	E8	16411...						
	E9	16250...						

26C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E10	11767...	13010.389	1076.194	8.272	37.935	7...	23.933
	E11	13648...						
	E12	13614...						

26D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F4	13440...	13541.244	1079.917	7.975	35.403	7...	29.011
	F5	14777...						
	F6	12162...						
	F7	13784...						

27A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F8	13363...	13111.863	355.409	2.711	37.451	7...	24.904
	F9	12860...						

27B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	F10	18634...	18063.967	558.388	3.091	13.827	2...	72.273
	F11	18039...						
	F12	17518...						

27C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G1	12065...	12218.915	571.698	4.679	41.711	8...	16.362
	G2	12851...						
	G3	11739...						

27D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	% virus inhibition
01	G4	11854...	11488.828	328.786	2.862	45.194	Error
	G5	11394...					
	G6	11217...					

28A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G7	14525...	14054.430	408.882	2.909	32.955	6...	33.920
	G8	13797...						
	G9	13839...						

28B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	G10	18027...	19779.227	2589.140	13.0...	5.645	1...	88.681
	G11	18556...						
	G12	22753...						

Plate 7

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	23555...	23795.700	1313.889	5.522
	C2	23469...			
	C3	25575...			
	D1	21837...			
	D2	23397...			
	D3	24938...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	9443....	9256.252	590.917	6.384	61.101
	E2	9553....				
	E3	8288....				
	F1	9127....				
	F2	10053...				
	F3	9071....				

28C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	21307...	23194.604	1662.651	7.168	2.526	4....	95.866
	A5	24445...						
	A6	23829...						

28D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	17983...	18385.856	713.437	3.880	22.735	3...	62.792
	A8	19209...						
	A9	17964...						

29A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	21198...	20685.704	861.443	4.164	13.070	2...	78.610
	A11	21167...						
	A12	19691...						

29B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	21039...	21652.515	999.079	4.614	9.007	1...	85.260
	B5	21113...						
	B6	22805...						

29C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	18505...	17751.547	903.044	5.087	25.400	4...	58.429
	B8	17998...						
	B9	16750...						

29D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	12287...	13742.900	1966.255	14.3...	42.246	6...	30.858
	B11	12961...						
	B12	15979...						

30A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	10943...	12586.097	1449.213	11.5...	47.108	7...	22.902
	C5	13684...						
	C6	13130...						

30B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	17553...	17582.203	426.981	2.428	26.112	4...	57.265
	C8	18022...						
	C9	17170...						

30C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	16590...	16686.567	94.514	0.566	29.876	4...	51.105
	C11	16779...						
	C12	16689...						

30D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	13223...	13787.634	719.745	5.220	42.058	6...	31.166
	D5	13541...						
	D6	14598...						

31A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	16101...	15650.120	410.607	2.624	34.231	5...	43.976
	D8	15299...						
	D9	15549...						

31B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	18148...	18717.101	496.331	2.652	21.343	3...	65.070
	D11	19065...						
	D12	18936...						

Plate 8

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	18472...	18132.361	353.340	1.949
	C2	18376...			
	C3	17635...			
	D1	18406...			
	D2	17776...			
	D3	18125...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	5258....	5718.357	366.620	6.411	68.463
	E2	5475....				
	E3	5995....				
	F1	5584....				
	F2	5723....				
	F3	6272....				

28C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	11973...	13271.154	1168.837	8.807	26.810	3...	60.841
	A5	14241...						
	A6	13598...						

28D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	8312....	8583.794	235.521	2.744	52.660	7...	23.082
	A8	8708....						
	A9	8730....						

29A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	9228....	9934.600	855.994	8.616	45.211	6...	33.964
	A11	10886...						
	A12	9688....						

29B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	15160...	14267.629	860.447	6.031	21.314	3...	68.868
	B5	14198...						
	B6	13443...						

29C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	8916....	9253.310	295.196	3.190	48.968	7...	28.476
	B8	9468....						
	B9	9374....						

29D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	8301....	8135.638	290.479	3.570	55.132	8...	19.472
	B11	7800....						
	B12	8305....						

30A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	4602....	5055.370	452.905	8.959	72.120	1...	-5.341
	C5	5056....						
	C6	5507....						

30B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	6890....	7248.111	316.251	4.363	60.027	8...	12.323
	C8	7489....						
	C9	7365....						

30C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	9988....	10223.198	563.906	5.516	43.619	6...	36.288
	C11	10866...						
	C12	9814....						

30D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	8629....	8305.146	421.869	5.080	54.197	7...	20.838
	D5	7828....						
	D6	8457....						

31A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	5913....	6159.359	225.300	3.658	66.031	9...	3.552
	D8	6355....						
	D9	6208....						

31B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	12772...	13230.507	400.054	3.024	27.034	3...	60.514
	D11	13407...						
	D12	13511...						

Plate 9

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	11686...	12613.452	569.294	4.513
	C2	13064...			
	C3	13013...			
	D1	12138...			
	D2	12791...			
	D3	12986...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	3724....	3716.934	390.815	10.5...	70.532
	E2	3655....				
	E3	3505....				
	F1	4435....				
	F2	3708....				
	F3	3271....				

28C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	6152....	6247.680	102.822	1.646	50.468	7...	28.446
	A5	6234....						
	A6	6356....						

28D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	3037....	3057.781	201.087	6.576	75.758	1...	-7.409
	A8	3268....						
	A9	2867....						

29A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	612.151	781.428	153.342	19.6...	93.805	1...	-32.996
	A11	821.091						
	A12	911.041						

29B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	4379....	4746.565	995.514	20.9...	62.369	8...	11.573
	B5	5873....						
	B6	3986....						

29C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	5393....	4937.643	414.280	8.390	60.854	8...	13.721
	B8	4584....						
	B9	4835....						

29D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	4098....	4795.984	671.761	14.0...	61.977	8...	12.129
	B11	4851....						
	B12	5438....						

30A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	412.136	883.534	542.426	61.3...	92.995	1...	-31.848
	C5	762.071						
	C6	1476....						

30B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	674.648	832.594	137.979	16.5...	93.399	1...	-32.421
	C8	929.683						
	C9	893.449						

30C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	5040....	5026.976	412.406	8.204	60.146	8...	14.725
	C11	4607....						
	C12	5432....						

30D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	5179....	4942.040	278.068	5.627	60.819	8...	13.771
	D5	5010....						
	D6	4636....						

31A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	940.071	2473.829	1344.503	54.3...	80.387	1...	-13.973
	D8	3448....						
	D9	3032....						

31B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	4072....	4497.067	367.945	8.182	64.347	9...	8.769
	D11	4713....						
	D12	4705....						

Plate 10

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	14696...	15873.306	1203.990	7.585
	C2	15680...			
	C3	17680...			
	D1	14444...			
	D2	16258...			
	D3	16481...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	5161....	5749.441	940.153	16.3...	63.779
	E2	5302....				
	E3	5255....				
	F1	4896....				
	F2	7121....				
	F3	6759....				

31C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	11175...	10747.612	442.461	4.117	32.291	5...	49.370
	A5	10776...						
	A6	10291...						

31D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	6693....	6429.185	281.774	4.383	59.497	9...	6.714
	A8	6461....						
	A9	6132....						

32A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	4042....	4022.993	90.295	2.244	74.656	1...	-17.053
	A11	3924....						
	A12	4101....						

32B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	7236....	9765.479	5455.338	55.8...	38.479	6...	39.669
	B5	6033....						
	B6	16026...						

32C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	6790....	7825.837	945.143	12.0...	50.698	7...	20.510
	B8	8043....						
	B9	8642....						

33A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	11527...	10749.012	717.661	6.677	32.282	5...	49.384
	B11	10606...						
	B12	10113...						

33B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	8355....	8308.452	820.632	9.877	47.658	7...	25.277
	C5	9104....						
	C6	7465....						

33C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	12861...	14939.787	1802.003	12.0...	5.881	9....	90.779
	C8	15882...						
	C9	16074...						

33D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	8762....	9183.799	408.158	4.444	42.143	6...	33.923
	C11	9577....						
	C12	9211....						

34A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	3317....	2715.736	581.717	21.4...	82.891	1...	-29.966
	D5	2673....						
	D6	2156....						

34B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	2120....	1898.632	203.672	10.7...	88.039	1...	-38.037
	D8	1720....						
	D9	1855....						

34C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	2009....	1780.592	308.910	17.3...	88.782	1...	-39.203
	D11	1903....						
	D12	1429....						

34D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E4	9342....	9568.154	371.219	3.880	39.722	6...	37.720
	E5	9365....						
	E6	9996....						

Plate 11

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	18944...	19923.328	1716.325	8.615
	C2	20457...			
	C3	22234...			
	D1	17274...			
	D2	19673...			
	D3	20954...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	4048....	4781.565	659.192	13.7...	76.000
	E2	4737....				
	E3	4423....				
	F1	6002....				
	F2	4666....				
	F3	4810....				

31C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	8362....	10112.864	1714.802	16.9...	49.241	6...	35.209
	A5	11789...						
	A6	10185...						

31D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	7501....	8872.603	2006.737	22.6...	55.466	7...	27.018
	A8	7940....						
	A9	11175...						

32A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	13312...	12066.863	1345.619	11.1...	39.433	5...	48.114
	A11	12249...						
	A12	10639...						

32B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	24216...	24661.781	2884.972	11.6...	-23.783	-3...	131.294
	B5	22025...						
	B6	27743...						

32C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	10620...	9382.146	1419.904	15.1...	52.909	6...	30.383
	B8	9693....						
	B9	7832....						

33A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	14430...	14492.863	363.536	2.508	27.257	3...	64.136
	B11	14164...						
	B12	14883...						

33B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	11509...	10363.266	1260.654	12.1...	47.984	6...	36.863
	C5	9013....						
	C6	10567...						

33C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	12788...	12342.286	614.393	4.978	38.051	5...	49.933
	C8	11641...						
	C9	12597...						

33D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	13401...	13306.479	852.616	6.408	33.212	4...	56.301
	C11	14107...						
	C12	12410...						

34A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	9701....	9787.837	424.522	4.337	50.872	6...	33.063
	D5	10248...						
	D6	9413....						

34B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	11680...	11554.512	899.678	7.786	42.005	5...	44.730
	D8	12384...						
	D9	10598...						

34C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	8688....	9899.815	1529.842	15.4...	50.310	6...	33.802
	D11	11618...						
	D12	9392....						

34D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E4	9894....	10680.838	828.983	7.761	46.390	6...	38.960
	E5	10601...						
	E6	11546...						

Plate 12

Cells and Media

Sample	Well	Values	MeanValue	Std.Dev.	CV%
01	C1	19301...	18576.768	1090.894	5.872
	C2	19235...			
	C3	19663...			
	D1	16722...			
	D2	17966...			
	D3	18571...			

Cells and Virus

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death
01	E1	6809....	7050.376	554.194	7.860	62.047
	E2	7986....				
	E3	7423....				
	F1	6873....				
	F2	6477....				
	F3	6730....				

31C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A4	17718...	17579.903	172.407	0.981	5.366	8....	91.351
	A5	17386...						
	A6	17634...						

31D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A7	12336...	13919.212	1564.875	11.2...	25.072	4...	59.592
	A8	15465...						
	A9	13956...						

32A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	A10	15456...	15542.973	190.574	1.226	16.331	2...	73.680
	A11	15411...						
	A12	15761...						

32B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B4	20091...	21263.219	1287.897	6.057	-14.461	-2...	123.307
	B5	21055...						
	B6	22642...						

32C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B7	19109...	18535.303	923.135	4.980	0.223	0....	99.640
	B8	19026...						
	B9	17470...						

33A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	B10	15700...	15370.938	414.719	2.698	17.257	2...	72.187
	B11	15506...						
	B12	14905...						

33B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C4	16392...	17611.294	1282.405	7.282	5.197	8....	91.624
	C5	18949...						
	C6	17491...						

33C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C7	18249...	17705.994	491.859	2.778	4.687	7....	92.445
	C8	17292...						
	C9	17576...						

33D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	C10	13883...	13279.100	523.558	3.943	28.518	4...	54.039
	C11	12964...						
	C12	12989...						

34A

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D4	13339...	13579.903	224.630	1.654	26.898	4...	56.648
	D5	13614...						
	D6	13785...						

34B

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D7	10344...	11152.006	707.279	6.342	39.968	6...	35.585
	D8	11450...						
	D9	11661...						

34C

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	D10	13436...	12599.018	856.711	6.800	32.179	5...	48.139
	D11	11724...						
	D12	12635...						

34D

Sample	Well	Values	MeanValue	Std.Dev.	CV%	% cell death	T	% virus inhibition
01	E4	11174...	11019.863	439.556	3.989	40.679	6...	34.438
	E5	11361...						
	E6	10523...						

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