Evaluation of extracts used in traditional Chinese medicine for antiviral potential against herpes simplex virus type 1

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A Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology

Middle Tennessee State University
December 2013

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ACKNOWLEDGEMENTS

This thesis would not have been possible without the support, advice, encouragement, and instruction from Dr. Stephen Wright. His guidance over the course of my studies has been invaluable. Thanks also go to my committee, Dr. Mary Farone and Dr. Anthony Newsome for their advice and support.

Special thanks to Mom, Dad, NDH, AKH, MH, WRM, and GEB for putting up with my crazy stress level, loving me anyways, and keeping me sane.

Thanks also to LPH, REL, and AEJ for making it so much fun to be a graduate student.

ABSTRACT

Herpes Simplex Virus type 1 (HSV-1) is a common pathogen that causes disease throughout the world. The need for new methods for the control and prevention of the virus is vital to reduce the number of people affected by HSV-1. A recent review concluded that natural products represent an important source for new antiviral activity and that Traditional Chinese Medicines (TCMs) are good sources for these agents. The objective of this study was to determine if any TCM plant extracts have potential active compounds capable of antiviral activity against HSV-1, with the ultimate goal identifying anti-HSV-1 drug candidates. After performing a cytotoxicity screen for each of 140 TCM extracts, Vero cells were exposed to HSV-1 and one of the extracts simultaneously to determine antiviral potential. Antiviral potential was determined by fluorescence readings from a spectrophotometer taken after a period of virus, cell, and extract incubation. Cell viability was determined using the fluorescent dye PrestoBlue which is reduced to a fluorescent red color by viable cells. Ten extracts showed potential antiviral activity by maintaining cell viability though cells were infected with HSV-1. The most effective four extracts inhibited HSV-1 by 80% and included *Mussaenda pubescens*, *Antirrhinum majus*, *Bidens biternata*, and *Gnetum parvifolium*. Further testing will be done to isolate active compounds from these extracts.

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I. INTRODUCTION

Herpes viruses

Herpes Simplex Virus type 1 (HSV-1) is a common pathogen that causes disease throughout the world. Herpes Simplex Virus type 1 is a large double stranded DNA virus in the family *Herpesviridae*. Eight herpes viruses within this family infect humans, including Herpes Simplex Viruses type 1 and type 2 (HSV-2), Varicella-Zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpes viruses (HHV) 6, 7, and 8. Within the family *Herpesviridae*, these eight herpes viruses are also classified into the subfamilies alpha, beta, and gamma. Classified as alpha herpes viruses are HSV-1, HSV-2, and VZV. Traditionally, HSV-1 is the primary cause of oral lesions, eye infections, and other skin related diseases, such as Herpes Whitlow. Herpes Simplex Virus type 2 commonly causes genital lesions and is responsible for most cases of meningoencephalitis and neonatal disease, which is when an infant is infected with HSV during birth. Although historically most cases of genital infection and neonatal disease are caused by HSV-2, both types of HSV can cause genital infection, and currently almost 33% of all new genital HSV diagnoses are caused by HSV-1 (Anzivino et al. 2009). Varicella-Zoster virus causes chicken pox and, after setting up latency, can be reactivated in adults as shingles. Other members of the family *Herpesviridae* can also cause serious illness in individuals. While most individuals infected with CMV remain asymptomatic, those showing symptoms can develop pneumonia and mononucleosis.

CMV is able to cross the placenta and cause a fetal infection resulting in organ defects, blindness, and even death in infants. Epstein-Barr virus is the primary cause of mononucleosis and is also associated with cancer, especially in Africa and China. Both CMV and EBV, like the alpha herpes viruses, are able to set up latency and become reactivated later in life.

Herpes virus life cycle

All herpes viruses consist of double stranded DNA and a protein capsid, tegument, and lipid bi-layer envelope (Whitley et al. 1997). The icosahedral capsid containing the viral DNA is covered by the tegument, which in turn is covered by an envelope containing embedded proteins (Akhtar and Shukla 2009).

Alpha herpes viruses, including HSV-1, infect host cells by attaching to a cell surface receptor (Fig. 1), heparan sulfate, via a specific protein, glycoprotein C (Geraghty et al. 1998). Heparan sulfate is found on many of our body's cells, including epithelial, pancreatic, and neural cells. Upon attachment, the virus particle undergoes receptor mediated endocytosis or fusion of the envelope with the host cell membrane and the viral DNA inside the capsid is released into the cell (Akhtar and Shukla 2009). The viral DNA is transported to the host nucleus, where the DNA is replicated (Whitley et al. 1997).

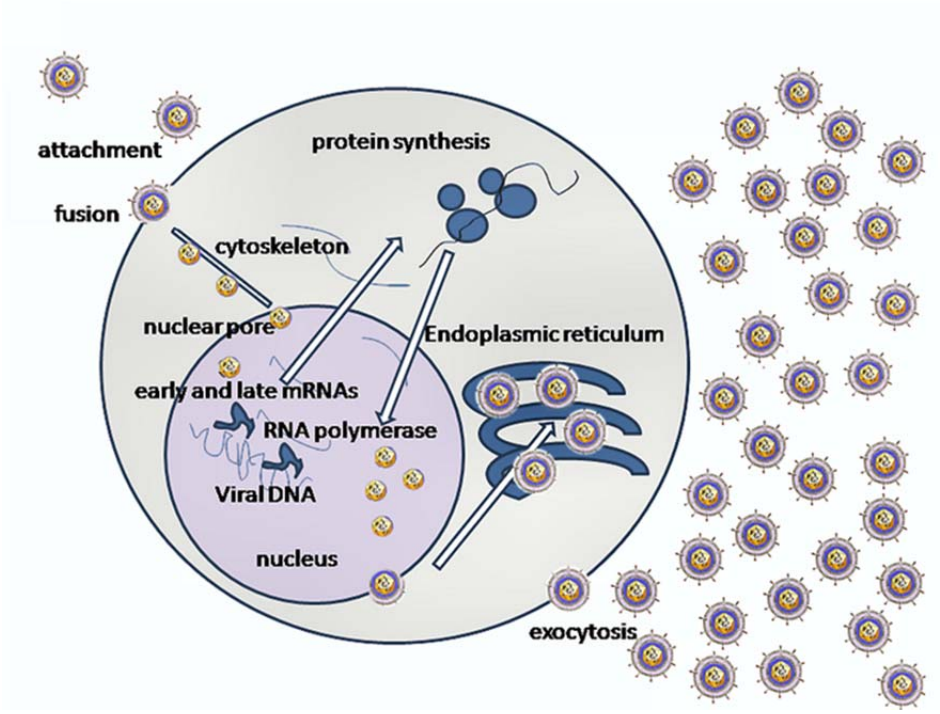

Fig. 1 – Mechanism of HSV-1 Infection. (Image by Graham Colm). After the virus attaches to the host cell via proteins on its surface, it undergoes receptor mediated endocytosis or fusion to release its viral DNA and capsid into the host cell. Viral DNA is transported to the nucleus where it is replicated, and reassembled HSV-1 virions then bud out of the host cell and spread.

After the viral DNA is transported to the nucleus, its genome is transcribed using host supplied DNA dependent RNA polymerase. Viral genes are expressed in a stepwise pattern, with first immediate early (IE), then early (E), and finally late (L) proteins being expressed (Pei et al. 2011). Immediate early proteins, once translated in the cytoplasm on free ribosomes, return to the nucleus to up-regulate the expression of early genes. Early proteins are also translated in the cytoplasm on free ribosomes, and then return to the nucleus to assist in viral replication. These proteins include those that help

circularize the DNA and also a DNA dependent DNA polymerase with a unique herpes thymidine kinase (TK). While viral DNA replication is occurring, late genes are being expressed and leave the nucleus to be translated into capsid and tegument proteins. Capsid proteins then return to the nucleus and assemble around the newly replicated viral genome, eventually budding out of the cell in order to acquire its attachment glycoproteins. The virus then is capable of spreading cell to cell, eventually invading neurons in the CNS (Akhtar and Shukla 2009).

Latency

Each of the herpes viruses is capable of setting up latency in different cells. Both CMV and EBV can become latent in lymphocytes and epithelial cells, and reactivation of the virus is common. Because alpha herpes viruses are able to replicate in the central nervous system (Whitley et al. 1997), during a primary infection the virus can set up lifelong latency in neurons and reactivate sporadically (Liu et al. 2008), usually due to physical stress or immune depression (Akhtar and Shukla 2009). The result of these reactivations may result in symptoms such as genital or oral lesions or may be asymptomatic (Piret and Boivin 2011). Varicella Zoster Virus sets up latency in the dorsal root ganglia, and HSV-1 and 2 set up latency in the trigeminal ganglia and lumbosacral ganglia, respectively (Anzivino et al. 2009).

Whereas during a lytic infection with HSV-1, all viral genes are expressed, during latency the viral genome is maintained, though most of the genes remain unexpressed

(Pinnoji et al. 2007). Once the HSV-1 genome has entered a neuron, most gene expression ceases (Jones 2003). Latency-associated transcript (LAT) RNA is the only RNA expressed in large quantities and is used by HSV-1 to inhibit productive infection (Jones 2003). The virus remains "silent" during latency, but can be reactivated to cause disease and transmit virus (Jones 2003).

Transmission of herpes viruses

Herpes viruses are transmitted human to human and are spread by direct contact with the virus, which may be present in skin lesions and also in respiratory secretions. The virus can enter through mucosal surfaces or a wound in the skin (Whitley et al. 1997). Herpes Simplex Virus type 2 is generally transmitted sexually and the earliest infections occur in early teens and young adulthood. The age of primary infection with HSV-1 is 1-4 and is generally through non-sexual contact. Though HSV-1 is primarily transmitted non-sexually, it is causing an increasing number of cases of genital herpes in the US and other developed countries due in part to oral-genital contact (Anzivino et al. 2009). About 30% of HSV infections can remain asymptomatic, but even if an individual shows no symptoms of HSV infection, he/she can still spread the virus by asymptomatic shedding (Akhtar and Shukla 2009). Herpes Simplex Virus type 1 can also be transmitted vertically during childbirth. Because HSV-1 is causing more cases of genital herpes, and because transmission can still occur even during asymptomatic shedding of the virus, the risk of infecting newborns with HSV-1 may be increasing.

Significance of HSV-1

Disease

Primary symptomatic infections of HSV-1 may include mouth and tongue lesions, other skin lesions such as those associated with Herpes Whitlow and Herpes gladiatorum, pharyngitis, and keratoconjunctivitis (Zu et al. 2009). Herpes Simplex Virus type 1 can also be responsible for genital lesions. Because HSV-1 can be reactivated to cause repeated infections, it is especially dangerous for immunocompromised individuals, such as infants and HIV/AIDS and organ transplant patients (Kuo et al. 2002). Even primary infections of HSV-1 can be opportunistic and can take advantage of immunocompromised people (Tolo et al. 2006). Herpes Simplex Virus type 1 can also cause more serious illnesses such as pneumonia and meningoencephalitis in immunocompromised individuals (Piret and Boivin 2011). Due to HSV-1's capability to set up latency in the central nervous system, several studies have even suggested a link between HSV-1 and Alzheimer's disease because the same regions of the brain are involved (Jones 2003). Recurring HSV-1 infections can cause oral lesions and also lead to corneal scarring (Liu et al. 2008). The scarring resulting from these eye infections causes blindness in individuals worldwide (Kuo et al. 2002).

Additionally, HSV-1 can cause neonatal herpes, which may be a direct result of infection through maternal genital secretions during birth, leading to viremia, central nervous system disease, and oral and ocular infections in the infant (Pepose et al. 2006). The biggest risk factor in spreading the disease to newborns is a pregnant mother

acquiring HSV-1 for the first time during pregnancy (Anzivino et al. 2009). Because most HSV infections occur in women of reproductive age, there is a greater risk of spreading HSV to newborns (Anzivino et al. 2009). Infections in infants and newborns can manifest as eye, mouth, and skin lesions, encephalitis, seizures, and multi organ dysfunction, resulting in nearly 30% mortality (Anzivino et al. 2009).

Impact

Worldwide, HSV-1 is a significant problem, infecting the majority of the population in some areas and overall HSV-1 has a seroprevalence of 70% (Faral-Tello et al. 2012). In countries in Africa, such as Eritrea and Uganda (where there is also a high seroprevalence of HIV), seroprevalence of HSV-1 in young adults is greater than 90%, and continues to increase with age. In several countries in Europe and the Middle East, seroprevalence of HSV-1 is above 50% for all age ranges (Smith and Robinson 2002).

Herpes Simplex Virus type 1 is a major problem in the US as well. The CDC reported (2013) 24.1 million new and existing cases of HSV-2 in the US in 2008, and while HSV-2 seroprevalence in the US is only 17% (Wilson et al 2010), HSV-1 seroprevalence is 57.7% (Xu et al. 2006), suggesting that there could be three times as many cases of HSV-1. Additionally, there are nearly 60,000 new and repeated episodes of HSV related eye disease in the US each year (Wilson et al. 2010). While overall seroprevalence is down from 62% in previous years, the number of cases of genital herpes caused by HSV-1 has increased (Xu et al. 2006). Because fewer children in the US are becoming infected with HSV-1 at an early age, this provides the opportunity for

more women to get HSV-1 for the first time during pregnancy (Anzivino et al. 2009). In fact, around 21% of pregnant women in the US have never been infected with HSV-1 (Pepose et al. 2006), increasing the risk of passing the disease on to newborns (Anzivino et al. 2009).

Treatment for HSV-1

Vaccine potential

While drugs are available for treatment of HSV, there is not yet a vaccine for prevention of infection. Some attempts at vaccines have been made; however, none have shown clear benefit or significant positive results. For example, one vaccine advanced to phase II trials, but could not protect against recurrences of the virus, and another vaccine provided protection against genital herpes for women, but not for men (Wilson et al. 2010). The difficulty in finding a functional vaccine is finding a way to deliver an effective dose at a local mucosal site (Wilson et al. 2010). One recent live attenuated vaccine was able to prevent recurrence in 43.5% of patients (Wilson et al. 2010), but much work remains to be done to deliver an effective anti-herpes vaccine.

Drugs

Because the HSV life cycle has several stages (such as attachment, entry, etc.), anti-HSV drugs can target any of these stages to inhibit the virus (Kuo et al. 2002). The need for new methods for the control and prevention of the virus is vital to reduce the number of people affected by HSV-1. Many antiviral agents have originated from natural

sources, including the most widely used anti-herpes drug, acyclovir (ACV), which is a structural analogue of a compound from the sea sponge *Cryptotethya crypta* (Dang et al. 2010).

Herpes Simplex Virus type 1 infections are usually treated with ACV, a drug that acts by blocking specific enzymes of HSV that trigger disease (Chattopadhyay et al. 2009). Acyclovir is a nucleotide analog and works by being targeted specifically by thymidine kinase (Kuo et al. 2002). When phosphorylated by thymidine kinase, it stops the viral DNA polymerase and therefore viral DNA replication (Tolo et al. 2006). Also currently used are the drugs Valaciclovir (VCV), which is converted in the body to ACV (Wilson et al. 2010) and Famciclovir (FCV), converted in the body into penciclovir, both of which work in a similar fashion to ACV (Wilson et al 2010).

Acyclovir is normally administered orally but it is also available as a topical cream to treat some herpes lesions (Wilson et al. 2010). In general, both ACV and VCV are used to treat keratitis, and ACV is used to treat neonatal herpes infections (Wilson et al. 2010). Acyclovir, VCV, and FCV are all used to treat oral and genital lesions, with FCV and VCV being the most effective for genital herpes (Wilson et al. 2010). Treating with ACV cannot cure HSV-1, but the goal of treatment is to lessen the frequency and duration of lesions in order to stop transmission of the virus from person to person (Piret and Boivin 2011).

Drug resistance

Resistance to ACV occurs because of genetic mutations in viral genes for thymidine kinase (Christophers et al. 1998). Because it is so widely administered, there is growing worldwide resistance to ACV, and resistance to ACV and other drugs is expected to increase (Tolo et al. 2006). Some efforts at drugs to target ACV resistant HSV have been made, but both of the most widely used drugs can be toxic. Cidofovir is an anti-herpes drug used on ACV resistant strains of HSV, but it is nephrotoxic and must be taken in conjunction with other drugs and intravenous hydration (Wilson et al. 2010). Foscarnet is also used to treat ACV-resistant HSV, but is only available intravenously and, like cidofovir, is also nephrotoxic (Wilson et al. 2010).

Most of the ACV resistance is found in immunocompromised individuals, due to the virus being able to replicate longer and because the individual's immune response is impaired (Piret and Boivin 2011). While ACV resistance is low in general among immunocompetent patients, ranging from 0.1-0.7% (Piret and Boivin 2011), one study reported 6.4% of immunocompetent individuals with herpes keratitis to have ACV resistant HSV-1 (Wilson et al. 2010). Resistance to ACV among immunocompromised individuals is commonly between 3.5-10% (Piret and Boivin 2011), although a study reported that in some transplant patients, ACV-resistant HSV-1 has been seen in as many as 30% of cases (Wilson et al. 2010). Immunocompromised patients, such as those with HIV/AIDS or who have undergone transplants, have a 5-10% chance of developing resistant HSV after being treated with ACV or its analogues (Shin et al. 2001).

It is important therefore to find new agents capable of inhibiting HSV-1 that could replace or complement ACV.

Natural products

Natural products have been used to treat disease historically and include such products as morphine, penicillin, and aspirin (Yasuhara-Bell et al. 2010). Many studies have investigated the use of single plants and herbs as antivirals, suggesting that natural products are a good possible source of new anti-viral drugs (Kuo et al. 2002). Herbs and plants are used as medicines because they are readily available and accepted in certain areas of the world (Tolo et al. 2006). For example, the majority of the population in India still uses plants and herbs as antioxidants, and some herbs, such as *Bixa orellana* have been shown to have antitumor effects (Vaidya and Devasagayam 2007). Additionally, Brindley et al. (2009) reported that *Prunella vulgaris*, used in Chinese and Native American medicine, was able to prevent lentiviruses from entering cells by preventing viral attachment.

Traditional Chinese Medicine

Some plants and herbs used in Traditional Chinese Medicine (TCM) have been reported to be antiviral, antifungal, antibacterial, and have numerous other health benefits. These TCMs have been used for thousands of years to treat infections and disease in China (Ojwang et al. 2005). For example, the TCM plant *Euphorbia hirta* has been widely studied for antibacterial, antifungal, anti-asthma, and even anti-HIV properties with positive results (Kamya et al. 2012; Gyuris et al. 2009; Kunwar et al.

2010; Rajeh et al. 2010). Another TCM, *Gnetum parvifolium* has been reported to be anti-inflammatory and anticarcinogenic (Cheng et al. 2008). Since these herbs and plants in TCM have such a history of medicinal use in China, it would seem more likely to find antiviral compounds from these sources rather than a random screening of chemical compounds (Ting and Tao 2013). Currently, 10% of prescribed drugs in China are traditional Chinese herbal medicines (Ting and Tao 2013).

Many different plant and herb extracts used in TCM have been shown to be antiviral. One TCM, *Scutellaria radix*, has been used in China for thousands of years and has a major active component that effectively suppresses Hepatitis B Virus (HBV) antigens by potentially inhibiting HBV DNA polymerase and terminating DNA elongation (Guo et al. 2007). This active component, called wogonin, was found to have long lasting antiviral effects, and is under development as an anti-hepatitis B drug candidate (Guo et al. 2007). Other plant extracts used in TCM have been tested against influenza virus strains (Pleschka et al. 2009; Tian et al. 2011). Pleschka et al. (2009) reported that the widely used herb *Echinacea purpurea* inhibited influenza virus by preventing viral entry into cells. Similarly, an active component of *Agastache rugosa* (which has been used in TCM as both an antiviral and an antifungal) was shown to be chemotherapeutic and chemo-preventative against respiratory syncytial virus (RSV) by interfering with viral attachment and internalization (Wang et al. 2009). Ojwang et al. (2005) also reported anti-RSV activity from the plant *Lonicera deflexicalyx*, historically used in China to treat respiratory infections. While antiviral activity in an isolated compound of this plant

against RSV was reported, it was ineffective against HSV. A more promising study undertaken by Li et al. (2004) screened 21 extracts used in TCM against both RSV and HSV-1 and found three extracts that successfully inhibited HSV-1 and six that inhibited RSV.

Natural products and herpes virus inhibition

Some work has been done to test herbs and plants from areas outside of TCM specifically for HSV inhibition. In an early study done in the late 1990s, Benencia and Courreges (1999) reported that sandalwood oil showed antiviral activity against HSV-1 and 2, although it proved less antiviral at higher virus concentrations. Extracts from marine microorganisms have also been tested against HSV (Yasuhara-Bell et al. 2010). One study tested 20 extracts from marine organisms against HSV-1 and three other viruses using Vero cells and found seven that were broadly antiviral (Yasuhara-Bell et al. 2010). This study reported, however, that these extracts had little lasting effect on viral inhibition and no isolation of active components was done (Yasuhara-Bell et al. 2010). Kambizi et al. (2007) tested two South African plants, commonly used in that country to treat genital herpes, against HSV-1. Both exhibited some lasting antiviral activity, but mostly at very high (>1000 μg/mL) concentrations. Tolo et al. (2006) tested the plant *Carissa edulis*, used in Kenya for treatment of skin infections and sexually transmitted diseases. It was reported to be effective against both wild type and ACV resistant strains of HSV-1, but no active compounds were isolated.

More promising work has been done investigating antiviral properties specifically from TCM plant extracts against herpes viruses. *Ganoderma lucidum* has been used in TCM as a treatment for sickness ranging hypertension to ulcers and one study reported inhibition of HSV attachment to cells (Liu et al. 2004). Berberine, a compound from *Coptidis rhizome* which is used in traditional Chinese herbal medicine, was shown to exhibit anti HSV activity by interfering with the replicative cycle (Chin et al. 2010). While the researchers were able to determine that this interference occurred at some point after viral entry into the host cell but before viral DNA replication, they were not able to give an exact mechanism (Chin et al. 2010). Chiu et al. (2004) reported that a fraction of *Prunella vulgaris* was successfully able to reduce the amount of both wild type and ACV resistant strains of HSV-1 antigens expressed by Vero cells.

Other Chinese herbal extracts have been tested, such as *Limonium sinense,* and have demonstrated anti-herpetic activity by inhibiting viral replication at the immediate early and early stages of gene expression, which could significantly affect viral replication in both lytic and latent infections (Kuo et al. 2002). *Tripterygium hypoglaucum* is also a plant from TCM, commonly used to treat tumors and inflammation. It was found to have anti-HSV-1 activity against Vero cells with an IC50 (half maximal inhibitory concentration) of 6.5 μg/mL, lower than ACV's IC50, which is 15.4 μg/mL (Ren et al. 2010).

Based on the number of extracts and their widely accepted and historically demonstrated anti-HSV properties, there is enormous potential for finding an extract

capable of inhibiting HSV among plants used in TCM. TCMs have been screened on a very broad scale against H1NI Influenza virus (Chang et al. 2011) and Hepatitis B (Zhan et al. 2011), but little work has been done to broadly screen extracts from TCM specifically with HSV-1 (Li et al. 2004). Further, Kurokawa et al. (1995) reported that 4 extracts not investigated in this study, but used in TCM, when taken in combination with ACV, reduce HSV-1 virus yield and skin lesions. These facts demonstrate the need for a specific and more current study of TCM extracts against HSV-1.

A recent review concluded that "natural products are one of the most important sources of novel antiviral agents" and that TCMs may be good sources for these agents (Chattopadhyay et al. 2009). The growing resistance to HSV drugs worldwide, the lack of a vaccine for HSV, and the abundance and potential for drug candidates to fight HSV from a wide-ranging variety of TCMs make this an important area of study.

Current Study

This study was undertaken to screen plant extracts used in TCM for HSV-1 inhibition. Traditional Chinese Medicine plant extracts were provided through a partnership between the Tennessee Center for Botanical Medicine Research (TCBMR) and the Guangxi Botanical Garden of Medicinal Plants in China. Over 140 TCM extracts were screened for cytotoxicity and HSV-1 inhibition in Vero cells using a fluorescence based assay and spectrophotometry.

II. METHODS

Extracts

Plants and plant parts were ground up and separated into fractions using different solvents (water, butanol, ethyl acetate, petroleum ether, chloroform, ethanal, and ethanol) by our partner in China. The resulting dry crude extracts were re-suspended in pure dimethylsulfoxide (DMSO, Sigma, St. Louis, MO) to a concentration of 10 mg/mL by the TCBMR.

Media

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

Fresh supplemented M199 Hanks medium and fresh supplemented M199 Earle's medium were prepared under the hood. To prepare the supplemented media, all bottles were wiped with 70% ethanol soaked onto a paper towel and flamed under the hood before use to prevent contamination. Approximately 90 mL of unsupplemented M199 Hanks or M199 Earle's media (Sigma) was added to an autoclaved glass bottle. To this media was then added 8 mL Fetal Bovine Serum (Gibco by Life Technologies, Grand Island, NY), 1 mL glutamine (Sigma), 1 mL penicillin-streptomycin (Sigma), and 0.5 mL

fungizone (Invitrogen by Life Technologies). Supplemented media in glass bottles were labeled and dated and this, along with the unsupplemented media, were stored at 4°C.

Overlay medium was prepared and stored in autoclaved glass bottles. Overlay medium was made by adding together 25 mL sterile dH_2O, 10 mL 10X 199 Earle's medium, 10 mL Fetal Bovine Serum, 50 mL methylcellulose, 1 mL glutamine, 1 mL penicillin-streptomycin, 0.5 mL fungizone, and 2.9 mL of sodium bicarbonate (Sigma). Methylcellulose was prepared by adding 243 mL dH_2O to 7.5 g methylcellulose (Sigma). This mixture was heated to 90°C to allow it to dissolve, autoclaved, and stored at 4°C before being used to prepare overlay medium.

Cell maintenance

Vero cells (African Green Monkey Kidney, American Type Culture Collection certified cell line #81) were grown in a 25 cm^2 culture flask (Corning Costar Corp., Cambridge, MA) and incubated at 36°C (Revco Scientific Inc., Asheville, NC). When grown to a confluent monolayer on the bottom of the flask, cells were passed into new flasks as needed. All work was done under a laminar flow hood (Nuaire, Plymouth, MN) and all bottles and flasks were wiped with a paper towel soaked in 70% ethanol and then flamed under the hood to prevent contamination.

To pass cells, existing culture medium was decanted and cells were washed twice with 5 mL sterile PBS for a minute each time, decanting PBS after each wash. After the last wash of PBS, 5 mL 0.1% trypsin (Sigma) was added and cells were placed into the

incubator at 36°C for 5-10 minutes. Cells were visually inspected with an inverted microscope (Southern Micro Instruments, Atlanta, GA) every 2-3 minutes to look for cells to round up but not detach. When cells began to round up, the trypsin was decanted under the hood and the cells were returned to the incubator for an additional 10-15 minutes until cells completely detached and slid off the bottom of the flask. This was checked every 4-5 minutes by gently tapping the flask in the palm of hand. Once cells had detached, they were returned to the hood and cells were washed off the flask bottom with 5 mL of fresh M199 Hanks. Cells and media were triturated with a pipette for 2-3 minutes to break up cell clumps. After trituration, 0.5 mL of cell suspension was transferred to as many new flasks as needed for experimentation and maintenance along with 4.5 mL of fresh M199 Hanks for a final volume of 5 mL in each flask. Flasks were stored in a 36°C incubator with flask caps on tight. As medium became orange 2 days later, old medium was decanted and 5 mL fresh M199 Earle's was added to cells. Cells were passed every seven days. Cells were quantified using a haemocytometer (Fisher Scientific) and were examined using a brightfield microscope (Olympus Optical Co. Ltd., Japan). Using a trypan blue exclusion stain, viable cells (non-stained) were counted in each of 10 grids on the haemocytometer. The cell count equaled the number of viable cells divided by the number of squares counted times the cell dilution times 10,000.

Virus preparation

To prepare HSV-1 stock virus, cells from one 25 cm^2 flask were passed as described previously through the trituration step. After trituration, the entire 5 mL cell suspension was added to a 75 cm^2 flask (Corning) with 20 mL fresh M199 Hanks and incubated at 36°C for 2 days. After two days, the old medium was decanted and 15 mL fresh M199 Earle's along with 1 mL HSV-1 (ATCC® VR-1383™) was added to the flask and the flask was returned to the incubator. After two days, the flask was removed from incubation and placed in a freezer at -20°C for 15-20 minutes. When frozen, the flask was removed from the freezer and thawed for 5-10 minutes. After thawing, media was taken out of the flask, placed in equal amounts in two centrifuge tubes (Fisher Scientific), and spun for 5 minutes at 2000xg rpm in a centrifuge (Eppendorf, Hamburg, Germany) until a pellet had formed. The supernatant containing the virus was removed and aliquoted into 15 cryovials (Fisher Scientific) containing 1 mL each and stored in an ultra-low freezer at -70°C. Tubes containing virus were removed from the ultra-low freezer and stored in a -20°C freezer 2 tubes at a time for viral inhibition testing. Tubes were used twice and then thrown away to ensure virus potency.

Viral plaque assays were performed on HSV-1 using Vero cells (ATCC® VR-1383™). Vero cells were set up on a 24 well plate (Corning) in a confluent monolayer across the bottom of the wells. All work was performed under a laminar flow hood. Stock virus was diluted in M199 Earle's to $2x10^4$, $4x10^4$, $8x10^4$, $1.6x10^5$, and $3.2x10^5$ with each dilution being repeated on the plate 4 times. First, medium from the 24 well plate

was decanted onto adsorbent paper. After media was decanted, 0.2 mL of appropriate virus dilution was added to wells. After virus was allowed to adhere for 2 hours in a 5% CO_2 incubator (Fisher Scientific) at 37°C, medium was removed with a sterile pipette and 0.5 mL overlay medium was added to each well. The plate was returned to a 5% CO_2 incubator for 2-3 days until appropriate size plaques had formed. After plaques had formed, overlay medium was decanted onto adsorbent paper. After the overlay medium had completely run out of wells (approximately 10 minutes), 0.5 mL methanol (Sigma) was added to each well for 5 minutes. After 5 minutes, the methanol was decanted and 0.5 mL Giemsa stain (Sigma) was added to each well for 10 minutes. After 10 minutes, the stain was decanted and wells were allowed to air dry for 10 minutes. Plaques were evaluated using an inverted microscope and virus titer was calculated by multiplying the number of plaques by the dilution.

Cytotoxicity testing

The cytotoxicity of extracts was tested using a spectrophotometer to measure cell viability. All work was performed under a laminar flow hood.

Cells in a confluent monolayer in a 25 cm^2 tissue culture flask were detached with 0.1% trypsin and triturated using 5 mL of supplemented Hanks 199 media as described previously. After triturating, 1 mL of cell suspension was added to 11.5 mL of fresh 199 Earle's in a sterile plastic tray (Aquafill, Swedesboro, NJ). Using a multi-channel pipettor, 100 µL of cell suspension was added to each well that required cells on

a 96 well plate (Corning) so that each well contained 5,000 cells. The plate was then incubated at 37°C in 5% CO_2 (Fisher Scientific) for 24 hours.

After 24 hours of cell incubation, extracts were removed from a freezer and quickly spun down using a centrifuge (Fisher Scientific) at 10,000 x g for 30 seconds. One and a half mL Eppendorf tubes were labeled with names of all media, extracts, and controls being used. Fresh 199 Earle's was used for blank wells and media only controls.

Extracts were screened in triplicate (Fig. 2) starting at a concentration of 100 µg/mL and diluted as necessary down to 6.25 µg/mL (and retested) if found to be cytotoxic. To test extracts at 100 µg/mL, 396 µL of fresh 199 Earle's and 4 µL of extract was added to each appropriate Eppendorf tube. After extracts were removed from under the hood and replaced in freezer, the titered virus was removed from the freezer and placed under the hood to thaw.

	1	2	3	4	5	6	7	8	9	10	11	12
A		←BLANK→			←Extract 1→							
B		←BLANK→			←Extract 2→							
C		←Cells only→										
D		←Cells only→										
E		←Virus+cells→										
F		←Virus+cells→										
G												
H												

Fig. 2 – Example of a Plate Setup for Cytotoxicity Testing. Extracts and controls were screened in triplicate as indicated by the colors on the plate.

The virus only control wells were prepared by diluting the viral stock solution to achieve a multiplicity of infection (MOI) of 0.1, or 1 virus particle for every 10 cells. To each of the virus control wells was added 100 µL.

The prepared 96 well plate was removed from incubation after 24 hours, when cells were confluent. Using a vacuum (Cole Parmer, Vernon Hills, IL), medium was aspirated from wells 3 columns at a time. Finally, 100 µL of appropriate extract/control was added to each well and the plate was incubated 48 hours at 37°C in 5% CO_2.

After a 48 hour incubation, 1.1 mL PrestoBlue dye (Invitrogen) was added to a plastic tray. Using a multi-channel pipettor, 11.1 µL dye was added to each well on the plate. The plate was then incubated at 37°C in 5% CO_2 for 30 minutes. After this 30

minute incubation, the plate was read for fluorescence using a spectrophotometer, (Molecular Devices, Sunnydale, CA) which measures the intensity of light using Softmax Pro software. The cell permeable dye being used, PrestoBlue, measured cell viability by changing color from blue to red when resazurin in the dye was reduced to resorufin by a viable cell.

The above steps were repeated as necessary, diluting extracts 2-fold in succession to 6.25 µg/mL until a working concentration of extract was found to inhibit less than 20% of cell growth. This working concentration of extract was then used for virus testing.

Virus inhibition screen

The anti-viral potential of extracts at was tested using PrestoBlue and a spectrophotometer to measure cell viability. All work was performed under a laminar flow hood.

Cells in a confluent monolayer in a 25 cm^2 tissue culture flask were detached with 0.1% trypsin and triturated using 5 mL of fresh Hanks 199 as previously described. After triturating, 1 mL of cell suspension was added to 11.5 mL of fresh 199 Earle's in a plastic tray. Using a multi-channel pipettor, 100 µL of cell suspension was added to each well on a 96 well plate that required cells. The plate was then incubated at 37°C in 5% CO_2 for 24 hours.

After 24 hours, extracts being tested were removed from a freezer and quickly spun down using a centrifuge at 10,000 x g for 30 seconds. Eppendorf tubes were labeled with names of all media, extracts, and controls being used. For blank wells and media only controls, fresh 199 Earle's was used.

To test extracts not cytotoxic at 100 µg/mL, tubes were prepared containing 392 µL fresh M199 Earle's and 4 µl of appropriate extract. The virus to be used in the control and in tubes containing extract 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.

The prepared 96 well plate was removed from incubation. Using a vacuum, medium was aspirated from wells 3 columns at a time. Finally, 100 µL of appropriate extract/virus or control was added to each well and the plate was incubated 48 hours at 37°C in 5% CO_2.

After a 48 hour incubation, 1.1 mL PrestoBlue dye was added to a plastic tray. Using a multi-channel pipettor, 11.1 µL dye was added to each well on the plate. The plate was then incubated at 37°C in 5% CO_2 for 30 minutes. After this 30 minute incubation, the plate was read for fluorescence using a spectrophotometer, looking for at least 50% virus inhibition with respect to controls.

This experiment was done for all extracts at their appropriate non-cytotoxic working concentrations. Dilutions of extracts showing antiviral activity were performed to verify results using the above method and to find an IC50.

Controls

Because extracts had been re-suspended in DMSO at 100 µg/mL and 50 µg/mL was tested with cells to ensure it had no negative impact on cell viability. As little as 2 µg/mL ACV has been reported to be 62% effective against HSV-1 in combination with Vero cells (Safrin et al. 1994), and so Acyclovir (Sigma) was also used as a positive control of virus inhibition at concentrations of 2, 4, and 8 µg/mL.

Statistics

Each set of triplicate wells in cytotoxicity and viral inhibition testing was repeated between 2-6 times. Fluorescence measurements were averaged using Microsoft Excel to yield a final percentage. For each set of percentages was calculated a mean ± standard error using Sigma Plot. IC50s were calculated using Microsoft Excel and a line of best fit.

III. RESULTS

A total of 140 extracts obtained from 34 plants were evaluated for cytotoxic effects on Vero cells and potential anti-HSV-1 activity. All plants and extracts from those plants are listed in Appendix A. A cytotoxicity screen was performed on all extracts beginning with a concentration of 100 µg/mL. Prior to conducting any testing, parameters were established to determine minimum acceptable cytotoxicity and viral inhibition. To be used against virus at its tested concentration, extracts had to show less than 20% cell inhibition. To exhibit antiviral activity, extracts had to inhibit the virus by at least 50%, or show at least a half maximal inhibitory response.

As summarized in Table 1, the majority of the extracts were used at a starting concentration of 100 µg/mL, and 36 only needed to be diluted once, to 50 µg/mL to achieve less than 20% cell inhibition.

Table 1 – Dilutions of Extracts for Cytotoxicity. While 86 extracts were used at working concentrations of 100 µg/mL, others had to undergo subsequent 2-fold dilutions to achieve less than 20% cell inhibition.

Working concentration (µg/mL)	**# of extracts**
100	86
50	36
25	12
12.5	4
6.25	2

As shown in Table 2, 86 extracts exhibited less than 20% Vero cell inhibition and were tested against HSV-1 at a working concentration of 100 µg/mL. Many extracts displayed negative cell inhibition at this concentration, meaning that no inhibition was evident and cell fluorescence was higher than control cells not exposed to extract. All other extracts were diluted 2-fold and tested again for cytotoxicity until less than 20% cell death was achieved. Two extracts, mdz-1 and mdz-5 (Table 2), were toxic to cells at all concentrations tested (6.25-100 µg/mL) and are not included in subsequent tables or figures.

Presented in Table 3 are the 86 extracts that inhibited virus at 100 µg/mL. Presented in Table 4 are the 36 extracts that inhibited virus at 50 µg/mL. This was the lowest concentration from which were found extracts that inhibited virus. Tables 5-7 display the final Vero cell toxicity results for the remaining extracts.

Table 2 - Percent Vero Cell Inhibition by All Extracts. Extracts were evaluated at 100 µg/mL. If cell inhibition was greater than 20%, extracts were diluted until a working concentration non-toxic to cells was achieved. Extracts with cytotoxicity less than 20% were used at a working concentration of 100 µg/mL. Numbers in parentheses indicate higher fluorescence values when compared with control cells not exposed to extract.

Extract	%	Extract	%	Extract	%	Extract	%	Extract	%	Extract	%
2B	99	15C	47	5A	24	sb6	9	BE-B	(1)	21A	(10)
16B	99	ct3	45	8C	24	5C	9	RI-D	(1)	20D	(13)
3C	99	AN-D	44	5D	22	5B	8	19D	(1)	PA-C	(15)
1A	98	ct5	44	ct2	21	7D	8	mdz 2	(2)	13A	(16)
12B	83	DE-B	42	18C	21	1D	8	16A	(2)	RI-C	(17)
10B	80	ct4	41	3B	21	19A	8	11A	(2)	cp6	(20)
mdz 5	71	6A	41	7B	20	17A	7	15D	(3)	PA-A	(20)
15B	71	4C	41	17B	18	PA-D	7	RI-B	(3)	cp1	(21)
9B	70	10A	40	4A	15	14D	7	12A	(3)	20C	(22)
FL-D	69	3A	38	cp4	17	21C	6	14A	(3)	11B	(23)
DE-D	68	EU-D	38	BE-C	15	14C	6	MA-C	(3)	13D	(24)
MA-D	67	10D	37	4B	18	ct1	6	7C	(3)	FL-B	(24)
BE-D	66	19B	37	12D	15	1C	5	PA-B	(4)	FL-A	(24)
11D	64	1B	37	20A	15	DE-A	4	mdz 6	(4)	lp5	(27)
mdz 4	63	9C	36	2C	15	sb2	4	BE-A	(4)	13C	(29)
6B	61	6C	35	16D	13	13B	3	21D	(4)	lp3	(29)
9D	59	11C	35	lp1	13	AN-C	3	7A	(4)	mdz 3	(30)
17D	59	DE-C	34	10C	12	16C	2	cp2	(4)	AN-B	(36)
sb5	56	cp5	33	8B	12	9A	2	AN-A	(6)	lp4	(40)
18B	56	2D	30	3D	11	18A	1	MA-A	(6)	8D	(43)
12C	56	21B	30	sb3	11	EU-A	(0)	ct6	(7)	6D	(49)
cp3	55	EU-C	29	EU-B	11	RI-A	(0)	FL-C	(8)	14B	(52)
4D	55	sb1	28	15A	10	2A	(0)	lp6	(8)		
mdz1	54	MA-B	26	17C	10			19C	(9)		
		20B	26								
		18D	25								

Table 3 - Cytotoxicity Results at 100 µg/mL. This table shows only extracts that exhibited less than 20% cell inhibition at a concentration of 100 µg/mL.

Extract	Working conc. (µg/mL)	% Cytotoxicity at working conc.	Extract	Working conc. (µg/mL)	% Cytotoxicity at working conc.	Extract	Working conc. (µg/mL)	% Cytotoxicity at working conc.
17B	100	18	1C	100	5	AN-A	100	(6)
4A	100	15	DE-A	100	4	MA-A	100	(6)
cp4	100	17	sb2	100	4	ct6	100	(7)
BE-C	100	15	13B	100	3	FL-C	100	(8)
4B	100	18	AN-C	100	3	lp6	100	(8)
12D	100	15	16C	100	2	19C	100	(9)
20A	100	15	9A	100	2	21A	100	(10)
2C	100	15	18A	100	1	20D	100	(13)
16D	100	13	EU-A	100	(0)	PA-C	100	(15)
lp1	100	13	RI-A	100	(0)	13A	100	(16)
10C	100	12	2A	100	(0)	RI-C	100	(17)
8B	100	12	BE-B	100	(1)	cp6	100	(20)
3D	100	11	RI-D	100	(1)	PA-A	100	(20)
sb3	100	11	19D	100	(1)	cp1	100	(21)
EU-B	100	11	mdz 2	100	(2)	20C	100	(22)
15A	100	10	16A	100	(2)	11B	100	(23)
17C	100	10	11A	100	(2)	13D	100	(24)
sb6	100	9	15D	100	(3)	FL-B	100	(24)
5C	100	9	RI-B	100	(3)	FL-A	100	(24)
5B	100	8	12A	100	(3)	lp5	100	(27)
7D	100	8	14A	100	(3)	13C	100	(29)
1D	100	8	MA-C	100	(3)	lp3	100	(29)
19A	100	8	7C	100	(3)	mdz 3	100	(30)
17A	100	7	PA-B	100	(4)	AN-B	100	(36)
PA-D	100	7	mdz 6	100	(4)	lp4	100	(40)
14D	100	7	BE-A	100	(4)	8D	100	(43)
21C	100	6	21D	100	(4)	6D	100	(49)
14C	100	6	7A	100	(4)	14B	100	(52)
ct1	100	6	cp2	100	(4)			

Table 4 – Cytotoxicity Results at 50 µg/mL. This table shows only extracts that exhibited less than 20% cell inhibition at a concentration of 50 µg/mL.

Extract	Working concentration (µg/mL)	% Cytotoxicity at working concentration
2D	50	17
3C	50	15
15C	50	15
9C	50	13
EU-D	50	13
11C	50	13
DE-C	50	11
11D	50	9
9D	50	9
cp-3	50	8
6A	50	8
18B	50	7
DE-B	50	5
EU-C	50	4
3A	50	3
21B	50	3
5D	50	3
4D	50	3
4C	50	2
ct-5	50	1
3B	50	1
18C	50	1
10D	50	(1)
sb-1	50	(3)
DE-D	50	(4)
20B	50	(5)
5A	50	(5)
8C	50	(6)
cp-5	50	(6)
AN-D	50	(6)
12C	50	(7)
18D	50	(7)
BE-D	50	(8)
7B	50	(12)
sb-5	50	(14)
17D	50	(14)

Table 5 – Cytotoxicity Results at 25 µg/mL. This table shows only extracts that exhibited less than 20% cell inhibition at a concentration of 25 µg/mL.

Extract	Working concentration (µg/mL)	% Cytotoxicity at working concentration
16B	25	19
ct-2	25	17
10B	25	16
FL-D	25	15
9B	25	12
6B	25	9
MA-D	25	8
MA-B	25	7
12B	25	(2)
2B	25	(3)
10A	25	(3)
1B	25	(9)

Table 6 - Cytotoxicity Results at 12.5 µg/mL. This table shows only extracts that exhibited less than 20% cell inhibition at a concentration of 12.5 µg/mL.

Extract	Working concentration (µg/mL)	% Cytotoxicity at working concentration
ct-3	12.5	9
ct-4	12.5	9
1A	12.5	9
19B	12.5	2

Table 7 - Cytotoxicity Results at 6.25 µg/mL. This table shows only extracts that exhibited less than 20% cell inhibition at a concentration of 6.25 µg/mL.

Extract	Working concentration (µg/mL)	% Cytotoxicity at working concentration
15B	6.25	7
mdz-4	6.25	2

After finding nontoxic working concentrations, extracts were tested for their ability to inhibit HSV-1. Ten extracts demonstrated antiviral potential. All extracts displaying antiviral potential were found in the groups of extracts with working concentrations of either 50 or 100 μg/mL. Presented in Table 8 are six extracts that were capable of inhibiting virus by at least 50%, but no more than 80%. The results of the four extracts that showed antiviral activity of at least 80% are shown in Table 9. For example, extract 16C, which only displayed 2% cytotoxicity at its working concentration of 100 μg/mL, inhibited virus by nearly 97% at this concentration. Out of these four extracts, all had working concentrations of 100 μg/mL. Results for all ten extracts represent an average of 2-6 tests of each extract and do not include one plate (Plate 9, Appendix B) on which inconclusive results were evident. Extract 14B displayed some unusual results. Included in its antiviral average are three antiviral numbers well over 100% and three less than 25%. Even with this number included in its results, 14B still demonstrated antiviral activity of 76.6%.

Controls were run on each plate. A series of direct images taken of cells also shows qualitatively the effects of extracts or virus on cells. Viable cells are visible in the negative control well containing cells only (Fig. 3). Destroyed cells are seen in the positive control well containing only virus and cells (Fig. 4). After determining that extract 16C alone at 100 μg/mL did not harm cells (Fig. 5), it was shown that the same extract, 16C, at its working concentration of 100 ug/mL, was able to protect cells against virus (Fig. 6).

Table 8 – Extracts Showing Moderate Antiviral Activity. These extracts inhibited virus greater than 50%, but less than 80%.

Extract	Working concentration (µg/mL)	% Cytotoxicity at working concentration	% Viral inhibition at working concentration (± SD)
cp2	100	(4)	52.3 ± 8.1
cp4	100	17	51.4 ± 8.3
14B	100	(52)	76.6 ± 28.9
7B	50	(12)	53.4 ± 18.6
AND	50	(6)	58.3 ± 19.9
ANB	100	(36)	54.3 ± 15.8

Table 9 – Extracts Showing the Highest Antiviral Activity. These extracts inhibited HSV-1 by at least 80%. Dilutions were run on these 4 extracts to determine an IC50.

Extract	Working concentration (µg/mL)	% Cytotoxicity at working concentration	% Viral inhibition at working concentration (± SD)
18A	100	1	83.2 ± 4.4
16C	100	2	96.9 ± 7.7
5B	100	8	92.7 ± 1.9
19A	100	8	100.1 ± 14.6

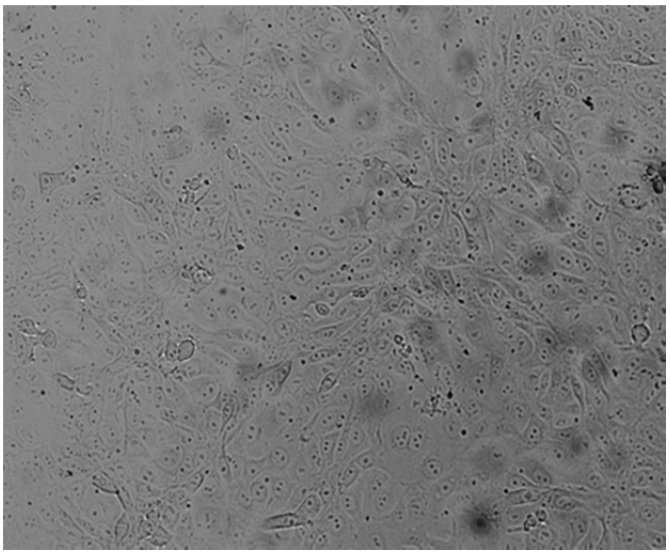

Fig. 3 – Control Vero Cells. This image is a negative control well containing cells only. This well is shown after dye has been added containing only cells and media after a 48 hour incubation. Cells are spread out as a confluent monolayer. This picture was taken using an inverted Olympus, model IX71.

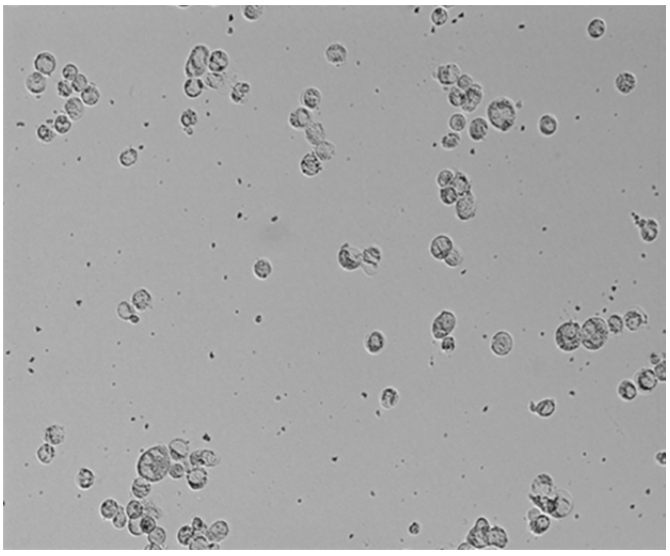

Fig. 4 – Vero Cells with HSV-1. These cells were exposed to HSV-1. This well is shown after dye has been added containing cells, virus, and media after a 48 hour incubation. The majority of cells have been destroyed and only a few round cells are still intact. This picture was taken using an inverted Olympus, model IX71.

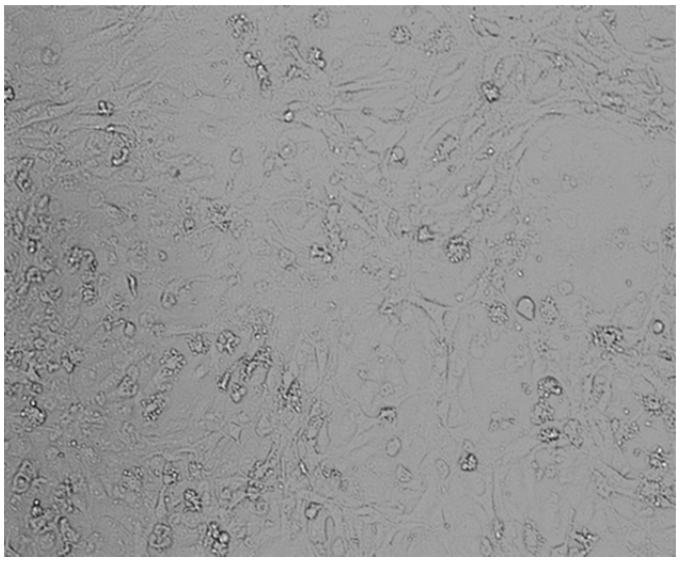

Fig. 5 – Vero Cells Exposed to Extract. This well is shown after dye has been added containing Extract 16C, cells and media after a 48 hour incubation and shows cells not harmed by the extract. Slight rounding is evident, but the monolayer is intact. This picture was taken using an inverted Olympus, model IX71.

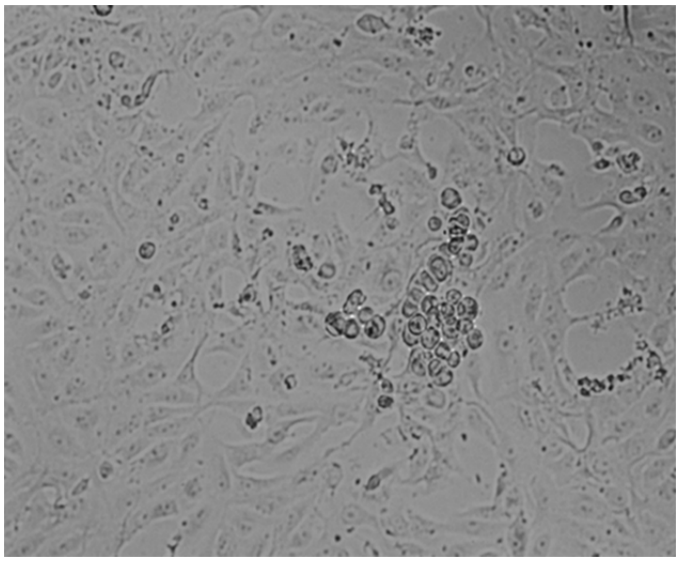

Fig. 6 – Vero Cells Exposed to Extract and HSV-1. This well is shown after dye has been added, containing extract 16C , cells, virus, and media after a 48 hour incubation and shows cells not harmed by the virus. Near the center of the image, a cluster of cells demonstrated viral presence, although the typical viral effect on cells has been minimized. This picture was taken using an inverted Olympus, model IX71.

The four extracts that showed the greatest antiviral activity were serially diluted. Results were graphed as shown in Figs. 7-10 to determine each extract's IC50, listed in Table 9, using a line of best fit. Extract 5B displayed the lowest IC50 at 10.15 µg/mL, and 16C had the highest at 72.65 µg/mL. Table 10 presents virus inhibition results and IC50 for ACV as a point of comparison to the extracts exhibiting antiviral potential. ACV was able to inhibit the virus more than 50% at concentrations of greater than 4 µg/mL, and its established IC50 ranges between 0.2-13.8 µg/mL. Most notable of the extracts tested is 5B, which as a crude extract, has an IC50 in the range of ACV and 19A, which also has a low IC50 at 18.61 µg/mL and shows virus inhibition of greater than 100%.

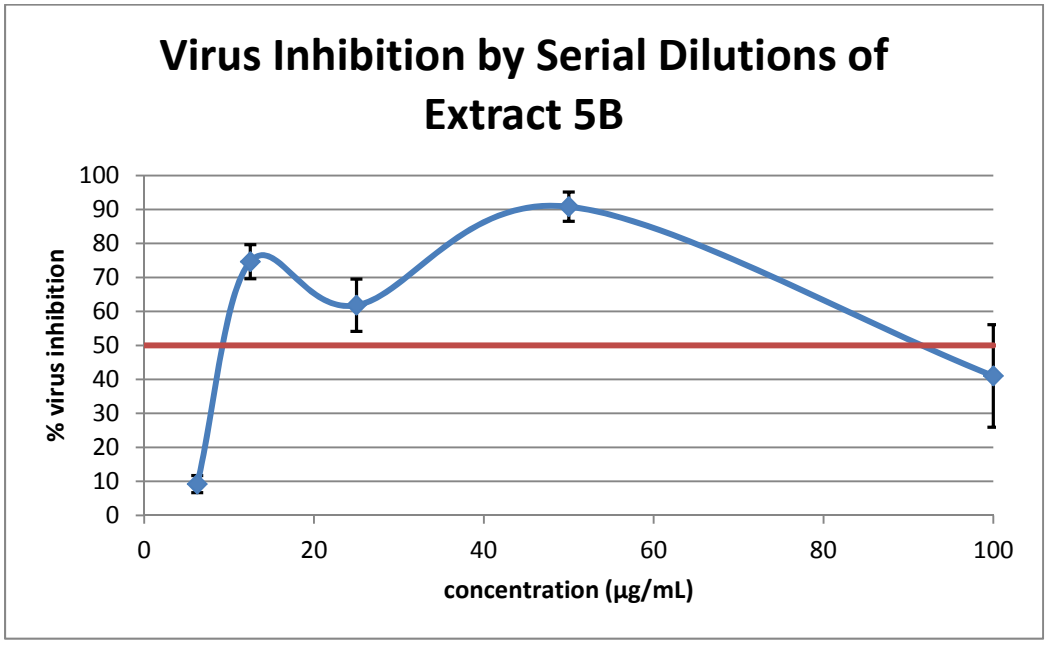

Fig. 7 – Graph Depicting the Serial Dilution of Extract 5B. The extract 5B was diluted 2 fold from a concentration of 100 µg/mL to a concentration of 6.25 µg/mL and virus inhibition was determined at each concentration. Though the dilution crosses the IC50 line, depicted as a red line at 50% virus inhibition, twice, the second time was most likely due to error and the IC50 was calculated to be 10.15 µg/mL.

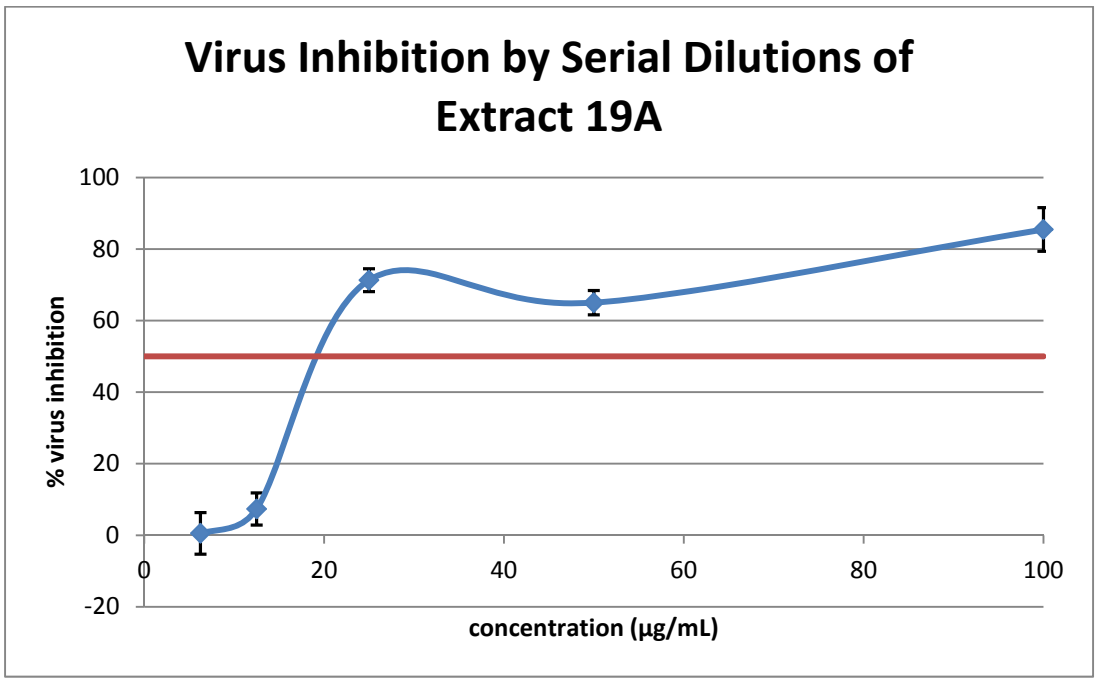

Fig. 8- Graph Depicting the Serial Dilution of Extract 19A. The extract 19A was diluted 2 fold from a concentration of 100 µg/mL to a concentration of 6.25 µg/mL and virus inhibition was determined at each concentration. The IC50, depicted as a red line at 50% virus inhibition, was calculated to be 18.61 µg/mL.

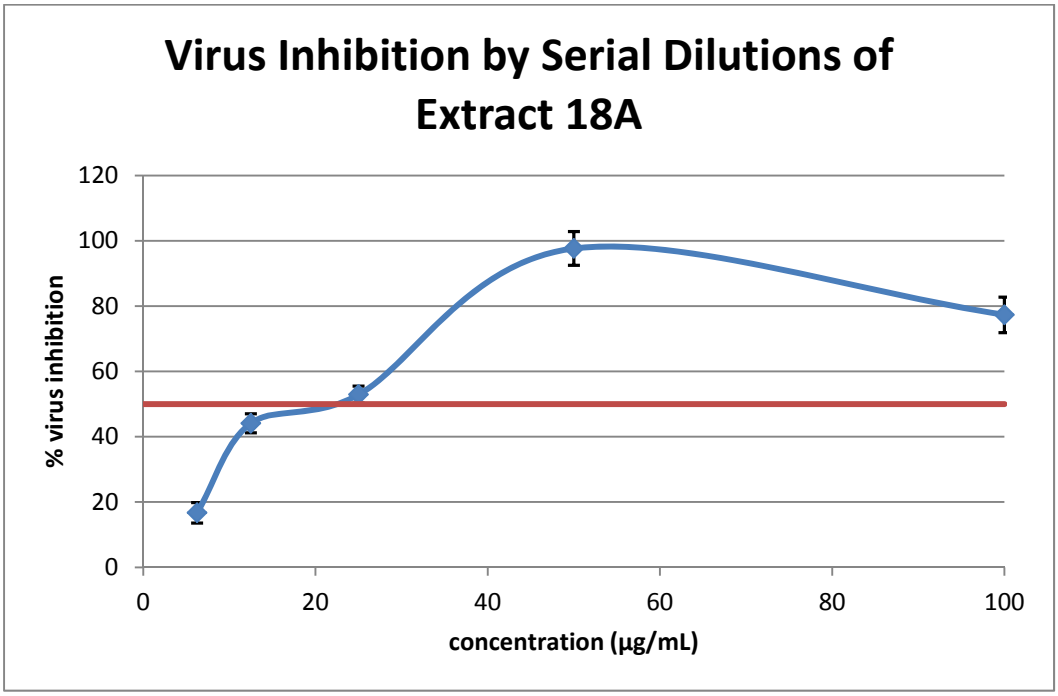

Fig. 9 - Graph Depicting the Serial Dilution of Extract 18A. The extract 18A was diluted 2 fold from a concentration of 100 µg/mL to a concentration of 6.25 µg/mL and virus inhibition was determined at each concentration. The IC50, depicted as a red line at 50% virus inhibition, was calculated to be 19.11 µg/mL.

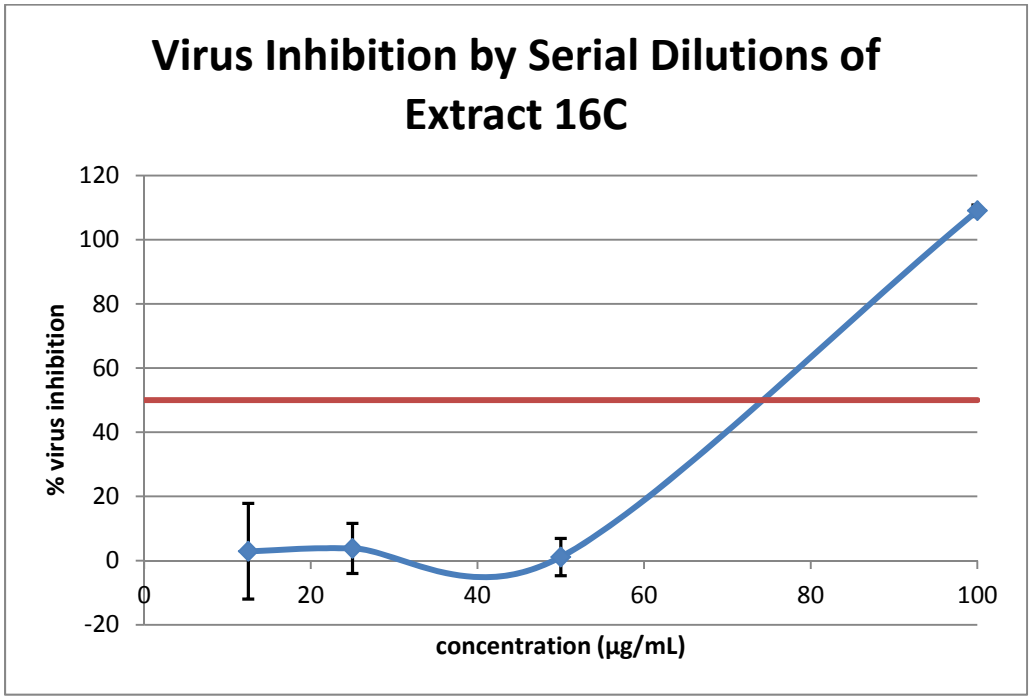

Fig. 10 - Graph Depicting the Serial Dilution of Extract 16C. The extract 16C was diluted 2 fold from a concentration of 100 µg/mL to a concentration of 12 µg/mL and virus inhibition was determined at each concentration. The IC50, depicted as a red line at 50% virus inhibition, was calculated to be 72.65 µg/mL.

Table 10 – Summary of the Most Effective Extracts. This table lists the most effective extracts, their virus inhibition results, and their IC50.

Extract	% Viral inhibition at working concentration	IC50 (µg/mL)
18A	83.2 ± 4.4	19.11
16C	96.9 ± 7.7	72.65
5B	92.7 ± 1.9	10.15
19A	100.1 ± 14.6	18.61

Table 11 – Acyclovir as an Antiviral. This table shows the viral inhibition results for ACV at three concentrations, as well as the established IC50.

ACV concentration (µg/mL)	% Viral inhibition	ACV IC50 (µg/mL)
2	31.7 ± 17.1	0.2-13.8
4	51.4 ± 17.2	
8	59.4 ± 15.9	

IV. DISCUSSION

Herpes simplex virus type 1 causes a significant range of disease worldwide such as oral and genital lesions and even neonatal disease. Like the other alpha herpes viruses, HSV-1 infects its host cell by attaching to receptors on the surface, undergoing receptor mediated endocytocis, and injecting its DNA into a host cell. Once the viral DNA is inside the host cell, the virus uses host cell machinery to produce viral proteins, replicate, and package new virions before budding out of the host cell and/or potentially becoming latent in the host. The viral life cycle provides many opportunities where the virus could potentially be inhibited.

Extracts demonstrating antiviral activity may either inhibit virus by working on the host cell, blocking specific proteins for viral entry, or may work directly on the virus itself. Antivirals found in plant compounds have many different modes of action, including inhibiting viral DNA and RNA formation and replication (Jassim and Naji 2003; Ting and Tao 2013;). Potential drug candidates may target viral entry into host cells by interacting with the cell's membrane, blocking virus binding, or interacting with the viral envelope (Jassim and Naji 2003; Reichling et al. 2009; Ting and Tao 2013). Potential drugs may also target viral assembly and exocytosis by inhibiting viral protein synthesis and function or by inhibiting viral DNA packaging or capsid synthesis and budding out (Jassim and Naji 2003; Ting and Tao 2013).

Acyclovir, the current drug of choice for HSV-1, itself an analogue of a natural product, works by acting as a substitute for a nucleotide and stopping viral DNA polymerase and thus viral replication. Though successful, increasing resistance is occurring to ACV, especially in immunocompromised individuals. It is important to search for new methods to control HSV-1. The traditional historical success and abundance of plants used in TCM provide potential for anti HSV-1 drugs. The results from this study support that fact by reporting six extracts prepared from plants used in TCM that are moderately HSV-1 inhibitory and four that are highly HSV-1 inhibitory. Before this study, many of these plants, while studied on a broader scale, had not been evaluated for antiviral or anti HSV-1 potential.

Of the 140 extracts tested in this study, the majority did not inhibit HSV-1. Though most of these plants have not been previously investigated for antiviral properties, some have been reported to be antiviral. Aqueous extracts of *Mallotus repandus* have been reported to be antiinflammatory (Lin et al. 1992) and while this study presents no anti HSV-1 effect of *M. repandus*, one study did report that a compound originally found in *M. repandus* inhibited HIV-1 reverse transcriptase by up to 90% (Ogata et al. 1992). Conflicting antiviral investigations exist for the plant *Scutellaria baicalensis*. Xin et al. (2011) reported that a fraction from *S. baicalensis* had antiviral activity against influenza viral pneumonia while Lu et al. (2011) reported that while it had antimicrobial effects, *S. baicalensis* showed little antiviral effects against Hepatitis A virus. No other study has been done with *S. baicalensis* against HSV-1 and the current

study showed no significant antiviral effects against HSV-1. Only two plants in this study had been previously tested against HSV-1: *Mussaenda pubescens* (discussed below) and *Paeonia suffruticosa*. This study concluded that none of the extracts prepared from *Paeonia suffruticosa* inhibited HSV-1. Conflicting with this result is one study by Hsiang et al. (2001), who reported that both methanol and aqueous extracts of the plant *Paeonia suffruticosa* showed anti HSV-1 activity by preventing viral attachment. This could be because while Hsiang et al. used the entire plant in the extraction, this study investigated only the seeds.

Of the extracts that were moderately effective against HSV-1, most were 50-59% inhibitory. Both the petroleum ether and the ethyl acetate extracts from the plant *Cyclocarya paliurus* displayed moderate HSV-1 inhibitory activity between 51-53%. In other studies, *C. paliurus* has displayed antioxidant (Xie et al. 2012) and moderate anti HIV potential (Zhang et al. 2010) but has not been tested against HSV-1. Extract 7B, the ethyl acetate extract of the plant *Achras zapota* showed some anti HSV-1 potential, inhibiting the virus by 53%. This was the first reported study to evaluate *A. zapota* for antiviral activity.

Also displaying moderate HSV-1 inhibitory activity were the extracts AN-B and AN-D, the petroleum ether and ethyl acetate extracts from *Antirrhinum majus*. While 19A, also a petroleum ether extract from *A. majus*, showed highly inhibitory HSV-1 activity, the extractions were done in a different sequential order for AN-B and AN-D,

perhaps affecting which active compounds were extracted in the fractions. This plant, as discussed below, has not been well studied for its antiviral capabilities.

Most interesting among the extracts that moderately inhibited virus because of its higher HSV inhibition was extract 14B, the ethyl acetate extract of *Euphorbia hirta*. *Euphorbia hirta* has been widely studied and has been reported to be antibacterial, selectively antifungal, antioxidant, and anti-inflammatory (Mohamed et al. 1996; Nelofar et al. 2006; Rajeh et al. 2010; Shih et al. 2010; Subramanian et al. 2011; Kamya et al. 2012). It has even been tested against HIV-1 and HIV-2 with positive results (Gyuris et al. 2009). Compounds from some *Euphorbia* species have shown antiviral activity after viral entry into a cell (Forero et al. 2008). Forero also reported that *Euphorbia pulcherrima* and *Euphorbia cotinifolia* were capable of inhibiting influenza A virus, however, *Euphorbia hirta* has not been investigated for any antiviral activity other than against HIV. In this study, the ethyl acetate extract of *E. hirta* inhibited HSV-1 by 76.6%. Included in this average are three antiviral numbers well over 100% and three less than 25%. While these results are unusual, because this extract displayed some high antiviral potential and because this particular species of *Euphorbia* has never been studied against HSV-1, extracts from *E. hirta* merit additional study for anti HSV-1 potential.

Extract 5B, the ethyl acetate extract of the plant *Mussaenda pubescens* exhibited anti-HSV-1 activity at 92.7%. This number reflects the average of its performance over several tests, and does not include some unusual results from several plates. For

example, on Virus Plate 9 (Appendix B), 26 extracts that had previously shown high anti HSV-1 activity were tested, but this plate only showed 2 extracts that inhibited virus. This plate was run 6 months after receiving the initial tubes of extracts, and all of these extracts had undergone repeated freeze-thaw cycles for testing. Murias et al. (2005) found that repeated freeze-thaw cycles reduced antioxidant capability of certain enzymes. If any active compounds in the extracts from this study were sensitive to freeze-thaw cycles, it could have affected their antiviral ability. After this plate, new tubes of extract were acquired to continue testing. Extract 5B also displayed some low antiviral numbers spread over a wide range on 2 different plates when used at the 100 µg/mL concentration not included in its final average. It was concluded that there was probably variation in the number of cells in these wells, thus affecting how much of the dye, PrestoBlue was able to be reduced. Extract 5B still requires further study, as upon visual inspection of the cells, 5B provided protection from the virus. Additionally, 5B also had the lowest IC50 of all extracts, at 10.15 µg/mL, meaning it may be more effective at lower concentrations. This is the only plant extract displaying promising HSV-1 inhibition from this study that has already been tested previously against HSV-1. The results from this study conflict with those reported by Li et al. (2004). In that study, 21 extracts were tested against both RSV and HSV-1. Li reported that while an aqueous extract of *M. pubescens* showed no anti HSV-1 inhibition, it was able to inhibit RSV. This suggests that this extract may target a protein for viral attachment or penetration that is specific to RSV. The positive antiviral results from the current study may conflict with

those reported by Li et al. due to differences in extraction, as the *M. pubescens* extract that inhibited HSV-1 in this study was extracted using ethyl acetate.

Much work has been done on the chemical composition of *M. pubescens* and this genus and species in particular has been studied as being a source of triterpenoid saponins. Saponins are a type of glycoside made by plants and have been reported to show anti HIV, HCV, and HSV activity (Lee et al. 2012). Many studies have tested saponins for antiviral activity, and more specifically against HSV-1. One study reported inhibition of HSV-1 entry by a triterpenoid saponin isolated from the seed of a fruit (Gosse et al. 2002). The saponin was able to inhibit virus with an IC50 of 8.5 µg/mL (Gosse et al. 2002). *Mussaenda pubescens* is used in Chinese medicine as a diuretic, antiinflammatory, and antipyretic (Xu et al. 1996). One study identified 18 saponins in *M. pubescens* whose chemical structures were determined and one of these saponins reportedly demonstrated "immunopromotive and hemolytic" activities (Xu et al. 1996).

Extract 18A, the petroleum ether extract from the plant *Gnetum parvifolium*, showed consistent anti HSV-1 activity in this study, with an average 83.2% virus inhibition. This genus has been studied because of certain polyphenols in many of its species. These polyphenols, called oligostilbenes, have been studied for antiinflammatory, anticarcinognic, and antioxidant activity (Cheng et al. 2008). One study tested a different species, *Gnetum macrostachyum*, which is commonly used in traditional medicine in Thailand for pain relief (Kloypan et al. 2012). This study also reported that the genus *Gnetum* is a good source of stilbenoids, and that *Gnetum*

macrostachyum contained compounds capable of anti-platelet activity (Kloypan et al. 2012). Polyphenols have been isolated specifically from *Gnetum parvifolium* and Piao et al. (2010) reported their ability to inhibit HIV-1, but no work has been done specifically on HSV.

Extract 16C, the 95% ethanol extract from the plant *Bidens biternata,* consistently showed 80% or greater HSV inhibition throughout the course of study and displayed the second highest HSV-1 inhibition at 96.9%. It has been less widely studied, with most studies having been done on its genome in terms of phylogenetic placement. Recent studies have broadened the investigation and one study acknowledged that *B. biternata* is commonly used in India to treat colds and hepatitis, but studied the plant solely to determine its nutritional value (Sukumaran et al. 2012). Others reported that a butanolic extract from *Bidens biternata* showed 88.9% antiradical activity (Durre et al. 2011), but no work has evaluated the plant for potential anti HSV activity. Both 16C and 18A have very low (2% or less) cytotoxicity effects as well, meaning they do little, if any, harm to the cells while inhibiting virus.

Extract 19A, the petroleum ether extract from the plant *Antirrhinum majus* (commonly known as snapdragon), has been widely studied, but not as an antiviral, and not against HSV-1. This extract exhibited the highest antiviral activity at 100.1% and the second lowest IC50. Its antiviral average does not include one low result from Virus Plate 9, although even with this number, *A. majus* still would have shown over 75% virus

inhibition. The strong HSV-1 inhibition and the lack of investigation done on this extract suggest *Antirrhinum majus* merits further study.

The IC50 of ACV can vary greatly when tested in cell culture, with IC50 values against HSV-1 ranging from 0.02-13.5 μg/mL (NIH 2011). Many studies doing similar testing use a control dose of ACV somewhere in the middle of this range. This study reported ACV to be at least 50% inhibitory at concentrations 4 μg/mL and greater. Of the four extracts exhibiting greater than 80% anti HSV-1 activity, most have IC50s greater than ACV. However, these numbers represent IC50s for crude extracts, and once active compounds from these extracts have been isolated, it can be expected that the IC50 will decrease into a range with greater potential for drug development.

This study has reported several effective antiviral extracts and suggests additional investigation that includes guided fractionation of promising extracts to determine active compounds and discovery of a mechanism of action. Because of the many stages of the viral life cycle that can be inhibited, time course studies would be useful. Several investigations utilizing time of addition studies on active compounds reported that plant extracts are able to inhibit the virus before it enters the host cell, disrupting viral attachment and penetration. Reichling et al. (2009) reported extract from the root and stem of *Rhus aromatica* exhibited anti HSV-1 activity. After performing a time course study, it was determined that the extract worked by preventing the virus from attaching to and entering host cells. No antiviral effect was seen when the extract was added to cells already infected with HSV-1 (Reichling et al.

2009). Another study (Schnitzler et al. 2001) reported that tea tree oil from Australia inhibited HSV-1 plaque formation by 98.2% and eucalyptus oil reduced virus titer by 57.9%. To determine how the extracts were inhibiting the virus, Schnitzler et al. did a time course study and found that the oils were able to inhibit the virus either before or during receptor mediated endocytosis, but not after the virus was already in the cell.

Acyclovir is a competitive inhibitor of viral DNA polymerase and terminates chain elongation as the virus is replicating inside the cell. Other drugs available that act in similar fashion as ACV do so by limiting chain extension (Jassim and Naji 2003). Since plant extracts may work differently than ACV by conferring protection outside the host cell at viral attachment or entry, they would be an excellent complement to ACV and a good replacement for individuals with ACV resistant HSV-1.

This study was undertaken to evaluate 140 plant extracts used in TCM for anti HSV-1 activity. Ten extracts showed some degree of antiviral activity, with four extracts demonstrating 80% or greater HSV-1 inhibition. These extracts merit further study to determine active compounds that could be used to provide new drug candidates for the control of HSV-1.

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APPENDICES

Appendix A - List of TCM Plants Used.

Plant	Part of plant	Code on tube	Solvent
Berchemia lineate (Linn.) DC. 铁包金	Root	BE-A	Water
		BE-B	Petroleum ether
		BE-C	Ethanal
		BE-D	Ethyl acetate
Antirrhinum majus Linn. 金鱼草	the whole plant	AN-A	Water
		AN-B	Petroleum ether
		AN-C	Ethanal
		AN-D	Ethyl acetate
Consolida ajacis L. 飞燕草	the whole plant	DE-A	Water
		DE-B	Petroleum ether
		DE-C	Ethanal
		DE-D	Ethyl acetate
Euphorbia lathyris Linn. 续随子	the whole plant	EU-A	Water
		EU-B	Petroleum ether
		EU-C	Ethanal
		EU-D	Ethyl acetate
Fluggea virosa (Willd.) Baill. 白饭树	stem	FL-A	Water
		FL-B	Petroleum ether
		FL-C	Ethanal
		FL-D	Ethyl acetate
Papaver rhoeas L. 虞美人	the whole plant	PA-A	Water
		PA-B	Petroleum ether
		PA-C	Ethanal
		PA-D	Ethyl acetate
Mallotus repandus (Willd.) Muell. Arg. 石岩枫	the whole plant	MA-A	Water
		MA-B	Petroleum ether
		MA-C	Ethanal
		MA-D	Ethyl acetate
Ricinus microcarpus G. M. Popova. 意大利蓖麻	the whole plant	RI-A	Water
		RI-B	Petroleum ether
		RI-C	Ethanal
		RI-D	Ethyl acetate

Plant	Part of plant	Code on tube	Solvent
Scutellaria baicalensis Georgi *黄芩*		Sb-1	Ethanol
		Sb-2	Petroleum ether
		Sb-3	Chloroform
		Sb-4	Ethyl acetate
		Sb-5	Butanol
		Sb-6	Water
Cyclocarya paliurus *青钱柳*		cp-1	Ethanol
		cp-2	Petroleum ether
		cp-3	Chloroform
		cp-4	Ethyl acetate
		cp-5	Butanol
		cp-6	Water
Lithocarpus polystachyus Rehd *多穗柯*		lp-1	Ethanol
		lp-2	Petroleum ether
		lp-3	Chloroform
		lp-4	Ethyl acetate
		lp-5	Butanol
		lp-6	Water
Catsia tora Linn *决明子*		ct-1	Ethanol
		ct-2	Petroleum ether
		ct-3	Chloroform
		ct-4	Ethyl acetate
		ct-5	Butanol
		ct-6	Water
Paeonia suffruticosa	seeds	mdz-1	92% Ethanol
		mdz-2	70% Ethanol
		mdz-3	Petroleum ether
		mdz-4	Chloroform
		mdz-5	Ethyl acetate
		mdz-6	Water
Garcinia paucinervis Chun et How	stem, branch, leaf	1A	petroleum ether
		1B	ethyl acetate
		1C	95% ethanol
		1D	water

Plant	Part of plant	Code on tube	Solvent
Elephantopus scaber Linn.	branch, leaf	2A	petroleum ether
		2B	ethyl acetate
		2C	95% ethanol
		2D	water
Psychotria rubra (Lour.) Poir.	branch, leaf	3A	petroleum ether
		3B	ethyl acetate
		3C	95% ethanol
		3D	water
Evodia lepta (Spreng.) Merr.	branch, leaf	4A	petroleum ether
		4B	ethyl acetate
		4C	95% ethanol
		4D	water
Mussaenda pubescens Ait. f	branch, leaf	5A	petroleum ether
		5B	ethyl acetate
		5C	95% ethanol
		5D	water
Glycosmis citrifolia (Willd.) Lindl.	stem	6A	petroleum ether
		6B	ethyl acetate
		6C	95% ethanol
		6D	water
Achras zapota Linn.	branch, leaf	7A	petroleum ether
		7B	ethyl acetate
		7C	95% ethanol
		7D	water
Polygonum perfoliatum L.	branch, leaf	8A	petroleum ether
		8B	ethyl acetate
		8C	95% ethanol
		8D	water
Stephania longa Lour.	the whole plant	9A	petroleum ether
		9B	ethyl acetate
		9C	95% ethanol
		9D	water
Belamcanda chinensis (Linnaeus) Redoute	the whole plant	10A	petroleum ether
		10B	ethyl acetate
		10C	95% ethanol
		10D	water

Plant	Part of plant	Code on tube	Solvent
Crocosmia crocosmiflora (Nichols.) N. E. Br.	the whole plant	11A	petroleum ether
		11B	ethyl acetate
		11C	95% ethanol
		11D	water
Pandanus tectorius Sol.	stem	12A	petroleum ether
		12B	ethyl acetate
		12C	95% ethanol
		12D	water
Eupatorium odoratum L.	aerial parts	13A	petroleum ether
		13B	ethyl acetate
		13C	95% ethanol
		13D	water
Euphorbia hirta Linn.	the whole plant	14A	petroleum ether
		14B	ethyl acetate
		14C	95% ethanol
		14D	water
Aristolochia tagala Cham.	the whole plant	15A	petroleum ether
		15B	ethyl acetate
		15C	95% ethanol
		15D	water
Bidens biternata (Lour.) Merr. et Schreff	aerial parts	16A	petroleum ether
		16B	ethyl acetate
		16C	95% ethanol
		16D	water
Cyperus rotundus L.	the whole plant	17A	petroleum ether
		17B	ethyl acetate
		17C	95% ethanol
		17D	water
Gnetum parvifolium (Warb.) C. Y. Cheng	vine, leaf	18A	petroleum ether
		18B	ethyl acetate
		18C	95% ethanol
		18D	water
Antirrhinum majus Linn.	the whole plant	19A	petroleum ether
		19B	ethyl acetate
		19C	95% ethanol
		19D	water

Plant	Part of plant	Code on tube	Solvent
Catharanthus roseus (Linn.) G. Don	the whole plant	20A	petroleum ether
		20B	ethyl acetate
		20C	95% ethanol
		20D	water
Microsorium fortuni (Moore) Ching	aerial parts	21A	petroleum ether
		21B	ethyl acetate
		21C	95% ethanol
		21D	water

Appendix B - Raw Data from Extract and Virus Testing.
Plate 1

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib-raw		% virus Inhib
cells+med. Only	10535	10524	10669							
cells+med. Only	10617	12472	11402	11036.5	777.103	317.251	2.874561487			
virus+cells	3149	4283	4036							
virus+cells	3577	3060	3866	3661.8333	489.8838	199.9942	5.461587621	66.82070101		
1C	3676	3365	3241	3427.3333	224.0989	129.3836	3.775051001	68.94546882	103.1798	-3.17980474
1D	4214	2791	3511	3505.3333	711.5169	410.7945	11.71912764	68.23872303	102.1221	-2.12212981
2C	4895	3557	4195	4215.6667	669.2394	386.3855	9.16546683	61.80250381	92.49006	7.509943952
3D	4711	4023	4227	4320.3333	353.3686	204.0174	4.722261287	60.85413552	91.07078	8.92921714
8B	9148	12887	10649	10894.667	1881.567	1086.323	9.971146807	1.285129646	1.923251	98.07674923
7A	5961	5672	5132	5588.3333	420.7854	242.9406	4.347280953	49.36498588	73.87679	26.12321461
7C	8477	10637	13627	10913.667	2586.123	1493.099	13.68100186	1.112973618	1.665612	98.33438799
7D	2568	3784	3144	3165.3333	608.2806	351.191	11.09491332	71.31940984	106.7325	-6.73250768
9A	6054	4857	5503	5471.3333	599.128	345.9067	6.322164598	50.42510458	75.4633	24.53670222
10C	3209	3387	3365	3320.3333	97.04295	56.02777	1.687414042	69.91497908	104.6307	-4.63071777
11A	5912	6458	5739	6036.3333	375.279	216.6674	3.589388192	45.30572796	67.80193	32.19806545
12A	5972	6784	6221	6325.6667	415.9956	240.1752	3.796835657	42.68412389	63.87859	36.12140662
12D	4776	3433	3454	3887.6667	769.3909	444.208	11.42608337	64.7744605	96.93771	3.062285301
14B	14375	14471	13467	14104.333	554.0301	319.8694	2.267880447	-27.7971579	-41.5996	141.5996203
14C	6682	5879	5801	6120.6667	487.6908	281.5684	4.600290132	44.54159682	66.65838	33.34161996
14D	4870	3664	4217	4250.3333	603.6906	348.5409	8.200319825	61.48839457	92.01998	7.980021696
15A	6323	4137	4113	4857.6667	1269.073	732.6994	15.08336148	55.98544224	83.78458	16.21542217
16A	9051	10434	7512	8999	1461.694	843.9094	9.377812594	18.46146876	27.62837	72.37163262
16C	11937	9830	7495	9754	2221.975	1282.858	13.15212095	11.62053187	17.39062	82.60938347
16D	6140	4310	4533	4994.3333	998.4219	576.4392	11.54186408	54.74712696	81.93139	18.06861327
17A	5669	5655	4933	5419	420.9466	243.0336	4.484842321	50.89928872	76.17293	23.82706563
17C	5471	4389	3637	4499	921.9349	532.2794	11.83105969	59.2352648	88.64807	11.35192551
19A	11502	13716	11125	12114.333	1399.834	808.1944	6.67138942	-9.76607922	-14.6153	114.6153498
20A	4228	3543	3107	3626	565.0903	326.255	8.99765617	67.14538123	100.4859	-0.48589767

Plate 2

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as % of mean	%Inhib: cells		% virus Inhib
cells+med. Only	11917	10643	12532							
cells+med. Only	12147	10958	12082	11713.167	741.8651	302.8651	2.585681148			
virus+cells	2102	1718	2471							
virus+cells	2120	1949	1995	2059.1667	248.1092	101.2901	4.918987444	82.42006858		
20C	3761	3626	3767	3718	79.7308	46.0326	1.238101053	68.25794334	82.81714	17.1828603
20D	2739	2458	3185	2794	366.6074	211.6609	7.575550906	76.1465018	92.3883	7.611698087
21A	3613	3610	3770	3664.3333	91.52231	52.84043	1.442020303	68.71611719	83.37304	16.62695946
21C	5482	4511	5225	5072.6667	503.1047	290.4676	5.726132816	56.69261088	68.78496	31.2150404
sb2	3819	4036	4082	3979	140.46	81.09459	2.038064714	66.0296817	80.1136	19.88640287
sb3	3745	3301	3264	3436.6667	267.6646	154.5362	4.496689342	70.6597988	85.7313	14.26869691
sb6	2418	2159	2511	2362.6667	182.4071	105.3128	4.457368582	79.8289674	96.85623	3.143774601
cp1	6892	6868	7043	6934.3333	94.87009	54.77327	0.789885161	40.79881615	49.50107	50.49892963
cp2	7436	7645	8554	7878.3333	594.4025	343.1784	4.355977711	32.73950967	39.72274	60.27725986
cp4	8200	8083	7251	7844.6667	517.4479	298.7487	3.808303136	33.0269355	40.07147	59.92852704
ct6	2854	2863	2649	2788.6667	121.0386	69.88165	2.505916064	76.1920346	92.44355	7.556453284
mdz2	2020	2358	2213	2197	169.5671	97.89961	4.456058646	81.24333016	98.57227	1.427732891
mdz3	3597	4190	3439	3742	395.9407	228.5964	6.108937522	68.05304572	82.56854	17.43146192
lp1	4521	4056	4370	4315.6667	237.2137	136.9554	3.173446808	63.15542338	76.62627	23.3737311
RID	5692	5417	4570	5226.3333	584.796	337.6321	6.460210104	55.38069694	67.19322	32.8067813
lp3	10229	7319	9110	8886	1467.875	847.478	9.537227361	24.13665533	29.28493	70.71507493
lp4	3706	3427	2636	3256.3333	555.0408	320.453	9.840914464	72.19937677	87.59927	12.40073199
lp5	2672	2886	2510	2689.3333	188.5983	108.8873	4.048858512	77.0400831	93.47248	6.527518818
lp6	3363	2618	2536	2839	455.6457	263.0672	9.266191091	75.76231876	91.92217	8.077826117
ANA	3113	2257	2268	2546	491.0672	283.5178	11.13581238	78.26377723	94.95719	5.042814723
BEA	2701	2618	1850	2389.6667	469.2039	270.895	11.33610065	79.59845758	96.57655	3.423451419
DEA	2474	2291	1153	1972.6667	715.725	413.224	20.94748452	83.15855376	100.896	-0.896001657
EUA	2161	1693	1112	1655.3333	525.5134	303.4053	18.32895506	85.86775566	104.1831	-4.183067468
FLA	1562	1431	1112	1368.3333	231.4527	133.6293	9.7658408	88.31798973	107.1559	-7.155928458

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	11726	12271	13014							
cells+med. Only	13005	14259	13595	12978.333	904.5471	369.2798	2.84535618			
virus+cells	5351	6320	5924							
virus+cells	5870	6503	5930	5983	400.6125	163.5494	2.733568125	53.90008989		
PAA	6906	7217	6863	6995.3333	193.1692	111.5263	1.594295434	46.09991011	85.5284	14.47155246
MAA	6263	6922	6218	6467.6667	394.107	227.5378	3.518081537	50.16566072	93.0716	6.928428476
RIA	7329	7647	6767	7247.6667	445.6022	257.2686	3.549674263	44.15564402	81.9213	18.07871915
ANB	9741	11379	12688	11269.333	1476.558	852.4909	7.564697042	13.16810068	24.4306	75.56942724
BEB	7692	6294	7447	7144.3333	746.5295	431.009	6.032879525	44.95184281	83.3985	16.60154389
EUB	13630	14280	14427	14112.333	424.1301	244.8716	1.735160415	-8.737639656	-16.211	116.2108072
FLB	12129	13834	12070	12677.667	1001.848	578.4175	4.562491603	2.316681649	4.2981	95.7018965
PAB	6860	8521	8137	7839.3333	869.5886	502.0572	6.40433556	39.59676384	73.4633	26.53673878
RIB	8162	10229	12737	10376	2291.04	1322.732	12.74799922	20.05136766	37.201	62.79900886
ANC	14316	11497	11196	12336.333	1721.035	993.6398	8.054579937	4.946706049	9.17755	90.82245306
BEC	7635	8269	7167	7690.3333	553.0799	319.3208	4.152236232	40.74483113	75.5933	24.40674736
FLC	6774	6797	9113	7561.3333	1343.832	775.8617	10.26091179	41.73879543	77.4373	22.56266082
PAC	12574	14521	14073	13722.667	1019.682	588.7139	4.290084144	-5.735199692	-10.64	110.640427
MAC	9547	13501	10527	11191.667	2059.093	1188.818	10.62235074	13.76653397	25.5408	74.45916325
RIC	9477	8025	21424	12975.333	7352.69	4245.078	32.71652083	0.023115449	0.04289	99.95711427
PAD	8351	7091	6161	7201	1099.136	634.5865	8.812477149	44.51521767	82.5884	17.41160774
2D	4856	5354	5316	5175.3333	277.2027	160.043	3.092420129	60.12328239	111.546	-11.54579243
3A	7151	6979	5764	6631.3333	756.0399	436.4998	6.58238425	48.90458456	90.7319	9.268083484
3B	14121	12696	10491	12436	1828.914	1055.924	8.49086335	4.178759471	7.75279	92.24721243
4C	8343	8475	5601	7473	1622.542	936.7753	12.53546527	42.41941698	78.7001	21.29991423
4D	7848	6548	4752	6382.6667	1554.608	897.5532	14.06235456	50.82059843	94.2867	5.713332698
5A	7516	6104	4689	6103	1413.5	816.0848	13.37186234	52.97547194	98.2846	1.715429334
5D	5929	5337	4522	5262.6667	706.4392	407.8629	7.75011765	59.45036599	110.297	-10.29734108
6A	4853	4167	4415	4478.3333	347.3576	200.547	4.478162181	65.49377167	121.51	-21.50957781

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	11271	10683	10283							
cells+med. Only	11577	11559	10891	11044	516.4676	210.847	1.909154491			
virus+cells	2533	3316	3112							
virus+cells	2201	2458	2487	2684.5	430.9481	175.9338	6.553691255	75.69268381		
7B	9781	9679	11212	10224	857.1517	494.8768	4.840343822	7.42484607	9.809199	90.19080089
8C	3776	3638	3646	3686.667	77.46827	44.72633	1.213191546	66.61837498	88.01164	11.98835656
9C	3383	4136	3399	3639.333	430.2003	248.3763	6.824774207	67.04696366	88.57787	11.4221345
9D	3071	2914	2370	2785	367.8736	212.3919	7.626281268	74.78268743	98.79777	1.202225013
10D	3448	3124	2814	3128.667	317.0258	183.0349	5.85025278	71.67089219	94.68668	5.313316187
15C	3516	3676	2481	3224.333	648.6974	374.5256	11.61559886	70.80466015	93.54228	6.457722751
18B	4590	5257	5088	4978.333	346.7598	200.2018	4.021463192	54.92273331	72.56016	27.43983891
18C	2295	2614	2822	2577	265.4411	153.2525	5.946934999	76.66606302	101.286	-1.28596208
18D	4137	4262	4708	4369	300.1616	173.2984	3.966545981	60.44005795	79.84927	20.15072672
20B	6389	6962	6743	6698	289.1384	166.9341	2.492297981	39.35168417	51.98876	48.01124469
sb5	3346	3276	3354	3325.333	42.91076	24.77454	0.745024225	69.89013642	92.33407	7.665928983
ct5	3367	2953	3681	3333.667	365.1429	210.8153	6.323828068	69.81468067	92.23438	7.765615966
DEB	5208	6478	6992	6226	918.3093	530.1861	8.515678298	43.62549801	57.63503	42.36497398
DEC	8966	9194	11174	9778	1214.334	701.0963	7.170139951	11.46323796	15.14445	84.85555356
EUC	3290	2634	3023	2982.333	329.8853	190.4594	6.386254147	72.99589521	96.43719	3.562812768
AND	7159	8385	7274	7606	677.0798	390.9122	5.139523811	31.13002535	41.12686	58.87313835
BED	4825	3445	2886	3718.667	998.0483	576.2234	15.49543114	66.32862489	87.62885	12.37115457
DED	10332	9851	8292	9491.667	1066.415	615.6948	6.48668818	14.05589762	18.56969	81.43030883
EUD	10221	8028	5431	7893.333	2397.838	1384.392	17.53875412	28.528311	37.68965	62.31034552
11C	5016	5091	3119	4408.667	1117.513	645.1967	14.6347353	60.08088857	79.37476	20.62523676
11D	3995	4350	2438	3594.333	1017.023	587.1787	16.33623275	67.45442473	89.11618	10.88382479

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	9245	10600	7923							
cells+med. Only	9960	10310	9884	9653.667	962.853	393.0831	4.071852673			
virus+cells	2152	2583	2567							
virus+cells	2027	3159	2593	2513.5	399.5116	163.0999	6.488956366	73.96326094		
6C	3410	3004	2990	3134.667	238.5484	137.726	4.393640236	67.52874555	91.30039	8.699610187
2A	3455	3659	4035	3716.333	294.2199	169.8679	4.570847319	61.50340113	83.15399	16.846012
4A	3604	3347	3315	3422	158.4266	91.46766	2.672929947	64.55232899	87.27621	12.72379263
4B	6003	5774	5221	5666	402.0311	232.1128	4.09658948	41.3072753	55.84837	44.15163045
6D	2674	2723	2965	2787.333	155.8022	89.95246	3.227186914	71.12668761	96.16489	3.835111225
8D	2420	2154	2696	2423.333	271.0154	156.4708	6.456841797	74.89727565	101.2628	-1.262808991
11B	6003	5357	5380	5580	366.5092	211.6042	3.79218979	42.19812852	57.05282	42.94717677
13A	2983	4426	5098	4169	1080.668	623.9239	14.96579212	56.81433652	76.81427	23.18573329
13B	5450	4301	5050	4933.667	583.2669	336.7493	6.825538743	48.89333932	66.1049	33.89510049
13C	3925	4434	4638	4332.333	367.2116	212.0097	4.893660748	55.12240599	74.52674	25.4732616
13D	2761	2386	2617	2588	189.1745	109.22	4.220245809	73.19153344	98.95661	1.043393011
14A	3508	5361	3862	4243.667	983.6942	567.9361	13.3831465	56.04088257	75.76854	24.23146052
18A	9190	8124	7472	8262	867.2739	500.7208	6.060527884	14.41593868	19.49067	80.50932518
19C	5457	5618	5800	5625	171.6071	99.07741	1.761376192	41.73198439	56.42259	43.57741416
19D	2799	2744	3039	2860.667	156.8704	90.56919	3.166016819	70.36704534	95.13784	4.862164749
21D	1990	2368	2302	2220	201.901	116.5676	5.250791755	77.00355651	104.1105	-4.110548307
cp6	2711	2379	2207	2432.333	256.1978	147.9159	6.081234426	74.80404682	101.1368	-1.136761514
ct1	2774	3334	2201	2769.667	566.5124	327.0761	11.80922267	71.30969234	96.41232	3.587684695
mdz6	3104	2992	2526	2874	306.5355	176.9783	6.157910279	70.22892856	94.9511	5.048901753
5B	7067	8368	3890	6441.667	2303.563	1329.963	20.64625145	33.27233176	44.98494	55.01505567
5C	3321	3103	2034	2819.333	688.7977	397.6776	14.10537586	70.79520735	95.71672	4.283280035
15D	2789	2356	1704	2283	546.1712	315.3321	13.81218134	76.35095473	103.2282	-3.228215961
17B	6638	6419	4126	5727.667	1391.399	803.3248	14.0253418	40.6684852	54.98471	45.01528909

Sample ID	RFU1	RFU2	RFU3	RFU Mear	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	10018	11838	11283							
cells+med. Only	12643	11085	10946	11302.17	884.1288	360.9441	3.193583046			
virus+cells	2580	2962	2881							
virus+cells	2275	3082	2595	2729.167	299.4244	122.2395	4.479004443	75.85271261		
cp3	4720	5407	5970	5365.667	626.0242	361.4353	6.736073608	52.525327	69.24647	30.75352852
12C	3454	3543	3266	3421	141.4178	81.64762	2.386659364	69.73146742	91.93009	8.069909405
17D	3170	3670	3967	3602.333	402.7857	232.5484	6.455494822	68.12705528	89.81492	10.18507718
21B	4578	4809	4393	4593.333	208.4234	120.3333	2.619738752	59.358825	78.25538	21.7446246
sb1	3646	3824	3800	3756.667	96.58847	55.76538	1.48443784	66.7615354	88.0147	11.98530269
cp5	3690	3853	4079	3874	195.3484	112.7845	2.911317893	65.72338637	86.64606	13.35394067
3C	126	68	61	85	35.67913	20.59935	24.23453264	99.24793181	130.843	-30.84295657
1B	3689	3958	4036	3894.333	182.0504	105.1068	2.698968346	65.54347986	86.40888	13.59111941
2B	3873	3906	3940	3906.333	33.50124	19.34195	0.495143411	65.43730553	86.26891	13.73109374
9B	4717	3933	4850	4500	495.5189	286.088	6.357510707	60.18462537	79.34406	20.6559353
10A	3805	3661	3829	3765	90.86253	52.45951	1.393346831	66.68780322	87.91749	12.0825071
16B	3163	3575	3043	3260.333	279.0364	161.1018	4.941266604	71.15302376	93.80419	6.195808546
MAB	4450	3668	3600	3906	472.3431	272.7074	6.981756616	65.44025482	86.27279	13.72720557
FLD	2851	2918	2369	2712.667	299.5035	172.9184	6.374480719	75.99870231	100.1925	-0.192464715
MAD	3164	4243	4231	3879.333	619.5259	357.6834	9.220229636	65.67619778	86.58385	13.41615148
6B	3040	3311	2352	2901	494.3794	285.4301	9.839023952	74.33235515	97.99565	2.004354757
ct2	3569	3520	2757	3282	455.323	262.8808	8.00977559	70.9613201	93.55146	6.44853999
12B	3193	3808	2035	3012	900.2516	519.7605	17.25632542	73.35024258	96.70088	3.299117384
10B	3384	3757	2625	3255.333	576.8642	333.0527	10.23098654	71.19726306	93.86251	6.137485905

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: raw	IC50	% virus Inhib
cells+med. Only	11410	12088	12527							
cells+med. Only	12348	12715	13223	12385.17	611.8024	249.7673	2.016664671			
virus+cells	2247	3701	4078							
virus+cells	2520	3263	3508	3219.5	705.5559	288.042	8.946792418	74.00519439		
1A	2770	2741	2759	2756.667	14.64013	8.452482	0.306619645	77.7421916	105.0496	-5.049641779
19B	5109	5502	5450	5353.667	213.4768	123.2509	2.302176826	56.77355977	76.71564	23.28435829
ct3	3259	3422	3799	3493.333	276.9771	159.9128	4.57765716	71.7942162	97.0124	2.987598647
ct4	3528	3572	3421	3507	77.65951	44.83674	1.278492754	71.68386914	96.86329	3.136705822
mdz4	4112	3408	2845	3455	634.8063	366.5056	10.607976	72.10372623	97.43063	2.569371204
15B	3893	3775	3397	3688.333	259.1087	149.5965	4.055937487	70.21975212	94.8849	5.115103466
cp1	5451	4448	4636	4845	533.1632	307.8219	6.353393565	60.88062333	82.26534	17.73466196
cp2	7949	8521	5346	7272	1692.307	977.0539	13.43583478	41.2846012	55.78609	44.21391425
cp4	7027	7013	5193	6411	1054.842	609.0134	9.49950725	48.23646566	65.17984	34.8201622
lp3	9135	7676	5178	7329.667	2001.105	1155.339	15.76249949	40.81899046	55.15693	44.84307379
ANB	5987	5249	3723	4986.333	1154.63	666.6257	13.36905539	59.73947329	80.72335	19.27664836
EUB	5309	5619	3139	4689	1351.259	780.1496	16.63786646	62.14019459	83.96734	16.03265811
FLB	5075	6097	3088	4753.333	1530.073	883.3879	18.58459733	61.62075601	83.26545	16.73455286
RIB	4971	4125	4322	4472.667	442.6673	255.5741	5.714132094	63.88690773	86.3276	13.67240063

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	% Inhib: cells		% virus Inhib
cells+med. Only	11427	11896	11027							
cells+med. Only	12638	12217	12728	11988.833	673.6828	275.0299	2.2940503			
virus+cells	5211	4949	4981							
virus+cells	4993	4469	3834	4739.5	506.3373	206.7114	4.361459072	60.46737937		
ANC	6718	7145	6412	6758.3333	368.1607	212.5577	3.145120166	43.62809837	72.15146	27.8485378
PAC	9959	7855	10743	9519	1493.431	862.2328	9.058018285	20.60111493	34.0698	65.93020048
RIC	6201	6707	6428	6445.3333	253.4449	146.3265	2.270270472	46.23886116	76.4691	23.53089939
14B	14297	15508	15402	15069	670.6691	387.211	2.569586353	-25.69196335	-42.489	142.4889645
16C	11979	12014	11796	11929.667	117.0741	67.59273	0.566593657	0.493514798	0.816167	99.183833
18A	9824	13272	11057	11384.333	1747.151	1008.718	8.860580798	5.04219204	8.338698	91.66130219
5B	11169	11034	12584	11595.667	858.5793	495.701	4.2748811	3.279440591	5.423487	94.57651278
DEA	5492	5561	5393	5482	84.44525	48.75449	0.889355837	54.27411619	89.75768	10.24232113
EUA	5295	6532	7043	6290	898.7764	518.9088	8.249742305	47.53451128	78.61183	21.38817363
3B	11870	10692	8116	10226	1919.895	1108.452	10.83954404	14.70396063	24.31718	75.68282141
7B	14663	13433	13584	13893.333	670.8132	387.2942	2.787626018	-15.88561578	-26.2714	126.2713813
DEC	13013	13589	7946	11516	3105.096	1792.728	15.56727865	3.94394784	6.522439	93.47756116
AND	9854	10958	9010	9940.6667	976.8876	564.0063	5.673727156	17.08395312	28.25317	71.74682729
DED	12997	10895	10088	11326.667	1501.773	867.0491	7.654935983	5.523195196	9.134173	90.86582674
EUD	9113	9975	7934	9007.3333	1024.595	591.55	6.567426935	24.8689753	41.12792	58.87208019

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib-cells		% virus Inhib
cells+med. Only	10655	10754	11453							
cells+med. Only	10711	10949	12112	11105.67	572.7868	233.8392	2.105584658			
virus+cells	3685	3812	3960							
virus+cells	3280	3330	4525	3765.333	457.9702	186.9655	4.965444438	66.09538674		
14B	8575	8590	7015	8060	905.0276	522.5179	6.482852882	27.42443797	41.49221	58.50778802
16C	11100	10994	10560	10884.67	286.1212	165.1921	1.51765919	1.989975088	3.010762	96.98923755
8B	5942	6373	6154	6156.333	215.5095	124.4245	2.021080504	44.56583726	67.42655	32.57345261
7C	5044	4723	4623	4796.667	219.9553	126.9913	2.647489593	56.80883633	85.94978	14.05022479
16A	6303	5411	6588	6100.667	614.0328	354.512	5.811037499	45.06708287	68.18491	31.8150856
19A	6521	6289	5358	6056	615.5152	355.3679	5.868029797	45.46927995	68.79342	31.20657554
cp1	7231	7705	6208	7048	765.0941	441.7273	6.267413329	36.53690308	55.27905	44.72094819
cp2	5698	4723	5446	5289	506.1057	292.2003	5.524679028	52.37566408	79.24254	20.75745879
cp4	3981	4303	3713	3999	295.4116	170.556	4.264965083	63.99135576	96.81667	3.183325008
lp3	5968	5912	5180	5686.667	439.6787	253.8486	4.463926122	48.79490951	73.82499	26.17501476
ANB	6292	6927	5854	6357.667	539.5056	311.4837	4.899340302	42.75294894	64.68371	35.316289
EUB	5335	5377	4492	5068	499.2725	288.2551	5.687748527	54.36563916	82.2533	17.74669634
FLB	6080	7359	5417	6285.333	987.1486	569.9305	9.067625731	43.40426809	65.66913	34.33086599
RIB	7332	6664	7642	7212.667	499.8013	288.5604	4.000745146	35.05417655	53.03574	46.96426139
ANC	3157	4602	3672	3810.333	732.3649	422.8311	11.0969574	65.69018819	99.38695	0.613051178
PAC	2724	3939	2683	3115.333	713.6108	412.0034	13.22501726	71.94825464	108.8552	-8.855183688
3B	5690	5208	4814	5237.333	438.7361	253.3044	4.836514466	52.84089204	79.94641	20.05358521
7B	5692	4899	5111	5234	410.5594	237.0366	4.528784207	52.87090674	79.99183	20.00817402
DEC	4749	3442	4125	4105.333	653.7219	377.4265	9.193565761	63.0338866	95.36806	4.631942237
AND	4457	3522	3542	3840.333	534.1426	308.3874	8.030224355	65.42005583	98.97825	1.021751964
DED	5937	5034	4403	5124.667	771.0086	445.142	8.686263492	53.85538914	81.48131	18.51868671
EUD	7309	6394	5233	6312	1040.426	600.6904	9.51664189	43.16415043	65.30584	34.69415558
18A	2593	3802	3416	3270.333	617.5227	356.5269	10.9018526	70.55257076	106.7436	-6.743562963
5B	3316	4084	4748	4049.333	716.6291	413.746	10.21763329	63.53813369	96.13097	3.869034104
MAC	3834	3564	3965	3787.667	204.4758	118.0541	3.116803592	65.8942882	99.69574	0.304255029
RIC	2543	3719	3838	3366.667	715.7935	413.2636	12.27515553	69.68514572	105.4312	-5.431179329

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	9051	8386	9032							
cells+med. Only	9738	8183	9030	8903.33333	554.5426	226.3911	2.542767405			
virus+cells	3701	3615	4914							
virus+cells	4564	3721	4751	4211	594.2784	242.6131	5.7614138	52.70310745		
cp1	5874	6796	5630	6100	614.9764	355.0568	5.820603327	31.48633471	59.74284	40.25715706
RIB	6815	6822	7343	6993.33333	302.8404	174.845	2.500166979	21.45263946	40.7047	59.2953044
14B 4	3763	5000	4406	4389.66667	618.6617	357.1845	8.136939366	50.6963684	96.19237	3.807629467
14B 8	2344	4245	4523	3704	1185.968	684.7192	18.48593855	58.39760389	110.8049	-10.80485899
14B 16	2902	3663	3830	3465	494.6706	285.5982	8.24237237	61.08199176	115.8983	-15.89827378
14B 32	2591	3080	3809	3160	612.9282	353.8743	11.19855289	64.50767503	122.3982	-22.39823826
16C 4	3879	4117	3437	3811	345.0623	199.2218	5.227547085	57.19580681	108.5245	-8.524543582
16C 8	3883	4328	4618	4276.33333	370.2139	213.7431	4.998279587	51.96929989	98.60766	1.392342118
16C 16	4170	4414	4308	4297.33333	122.3492	70.63836	1.643771931	51.73343317	98.16012	1.839880656
16C 32	3703	4052	4098	3951	216.0023	124.709	3.15639065	55.62336204	105.541	-5.540953328

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	12327	12327	12526							
cells+med. Only	12251	11827	12138	12232.67	235.6715	96.2125	0.786521034			
virus+cells	3388	2385	3437							
virus+cells	2731	2787	3712	3073.333	512.2783	209.1367	6.804883217	74.87601504		
14B ONLY	14377	14619	16539	15178.33	1184.568	683.9107	4.505834965	-24.08033135		
14B 1	4947	5179	4820	4982	182.0412	105.1015	2.109625416	59.2729849	79.16151	20.83848897
14B 2	4774	4894	4082	4583.333	438.2937	253.049	5.521068288	62.53201809	83.51408	16.48591601
14B 4	3645	3760	3984	3796.333	172.3959	99.5328	2.621813965	68.9656112	92.10641	7.893587597
14B 8	3983	4834	4764	4527	472.4161	272.7496	6.024952063	62.99253365	84.12912	15.87087852
14B 16	2760	4078	3794	3544	693.6541	400.4814	11.30026459	71.02839392	94.86134	5.13865638
14B 32	2856	2845	3151	2950.667	173.5809	100.217	3.396418441	75.87879448	101.3393	-1.339253221
16C extract only	12777	11836	11615	12076	617.0583	356.2588	2.950139128	1.280723745		
16C 1	12636	13437	13105	13059.33	402.4479	232.3534	1.779213476	-6.757861464	-9.0254	109.0254021
16C 2	3541	3042	2943	3175.333	320.522	185.0534	5.827843137	74.04218214	98.88638	1.113618167
16C4	3436	3878	2954	3422.667	462.1443	266.8191	7.795650265	72.02027358	96.18604	3.813960259
16C8	4187	3362	2469	3339.333	859.2243	496.0734	14.85546095	72.70150962	97.09586	2.904141495
16C16	4041	2451	2013	2835	1067.14	616.1136	21.73240303	76.8243501	102.6021	-2.602081665
16C32	3634	1745	2059	2479.333	1012.22	584.4058	23.57108535	79.7318655	106.4852	-6.485188151
7C ONLY	15633	15159	11180	13990.67	2445.619	1411.979	10.09229269	-14.37135539		
7C 1	2008	1706	1309	1674.333	350.5743	202.4042	12.08864192	86.31260559	115.274	-15.27403741
7C 2	2875	2430	1759	2354.667	561.801	324.3559	13.77502589	80.7509946	107.8463	-7.846277022
7C 4	3731	3686	2569	3328.667	658.2753	380.0554	11.41764687	72.78870783	97.21232	2.787684693
7C 8	4545	3689	1999	3411	1295.566	747.9955	21.92892242	72.11564663	96.31341	3.686585632
7C 16	3631	3600	2554	3261.667	613.0533	353.9465	10.8517061	73.3364216	97.94381	2.056190407
7C 32	3114	3438	1684	2745.333	933.3088	538.8461	19.62771098	77.55736007	103.581	-3.581046656

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	10628	10863	10964							
cells+med. Only	11343	10950	11238	10997.667	258.9244	105.7055	0.961162618			
virus+cells	3587	3590	3747							
virus+cells	3522	3364	3773	3597.1667	150.691	61.51933	1.710216239	67.29154669		
16A ONLY	14492	13818	14197	14169	337.8713	195.0701	1.376738463	-28.83641985		
16A 1	5093	6030	6298	5807	632.695	365.2866	6.290453684	47.19789046	70.1394	29.860595
16A 2	5919	6223	6408	6183.3333	246.9015	142.5486	2.305368634	43.77595247	65.05416	34.94583699
16A 4	5672	5465	5073	5403.3333	304.2241	175.6439	3.250658139	50.86836602	75.59399	24.4060086
16A 8	4642	4681	5091	4804.6667	248.7375	143.6087	2.988940967	56.31194496	83.68353	16.3164651
16A 16	4141	4830	4644	4538.3333	356.4468	205.7947	4.534586832	58.73367078	87.28239	12.71760917
MAC ONLY	10272	12313	13044	11876.333	1436.664	829.4585	6.98412971	-7.989573546		
MAC 1	4474	6016	5214	5234.6667	771.2077	445.257	8.505928034	52.40202467	77.87312	22.12688332
MAC 2	4136	4646	5446	4742.6667	660.3282	381.2407	8.038529619	56.87570091	84.52132	15.47868387
MAC 4	3241	3209	5546	3998.6667	1340.125	773.7218	19.34949517	63.64077229	94.57469	5.425309101
MAC 8	3908	4104	4726	4246	427.0878	246.5793	5.807330754	61.39181038	91.23257	8.767425624
MAC 16	3964	4316	4079	4119.6667	179.4891	103.6281	2.515448036	62.5405389	92.93967	7.060333761
RIC ONLY	9562	10739	9393	9898	733.2128	423.3206	4.276829698	9.999090716		
RIC 1	3504	4281	3709	3831.3333	402.6864	232.4911	6.068151183	65.16230716	96.8358	3.164200617
RIC 2	4373	5234	3172	4259.6667	1035.661	597.9393	14.03723292	61.2675416	91.0479	8.952097831
RIC 4	4658	3687	2646	3663.6667	1006.203	580.9315	15.85656078	66.68687297	99.10141	0.898587933
RIC 8	3805	4262	4503	4190	354.5264	204.6859	4.885105926	61.90100931	91.98928	8.010719996
RIC 16	3660	3388	4210	3752.6667	418.7617	241.7722	6.442676165	65.8776104	97.89879	2.101209378

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	13783	14894	15264							
cells+med. Only	15075	14694	16351	15010.167	834.9686	340.8745	2.270957492			
virus+cells	5756	4293	5117							
virus+cells	5482	4634	5323	5100.8333	546.7502	223.2098	4.37594829	66.01747704		
cp 1 only	11893	13895	13760	13182.667	1118.922	646.0099	4.90044925	12.17508133		
cp1 1	7879	9225	9397	8833.6667	831.2264	479.9088	5.432724663	41.14877694	62.33013	37.66987352
cp1 2	7095	6927	6923	6981.6667	98.16992	56.67843	0.811818049	53.48708098	81.01958	18.9804225
cp1 4	5812	6594	6555	6320.3333	440.6612	254.4159	4.025355552	57.89298364	87.69342	12.30657966
cp1 8	6769	6176	6432	6459	297.4206	171.7159	2.658551642	56.96916534	86.29407	13.7059338
cp1 16	4942	5705	5097	5248	403.2902	232.8397	4.436732393	65.03703046	98.51487	1.485131862
7B ONLY	14569	14863	14871	14767.667	172.0969	99.36018	0.672822441	1.615571668		
7B 2	8917	9330	8928	9058.3333	235.3345	135.8704	1.499949713	39.65201364	60.0629	39.93709634
7B 4	7193	8063	6571	7275.6667	749.4273	432.6821	5.946974958	51.52840852	78.05268	21.94732239
7B 8	6034	7819	6128	6660.3333	1004.535	579.9685	8.707799696	55.62785223	84.26231	15.73768837
7B 16	5914	6202	5242	5786	492.6337	284.4222	4.915696878	61.45279311	93.08564	6.914356835
EUB ONLY	14779	14392	13410	14193.667	705.7211	407.4483	2.870634503	5.439646462		
EUB 1	7975	8703	7190	7956	756.6789	436.8688	5.491060618	46.99592498	71.1871	28.81290366
EUB 2	7058	7412	5940	6803.3333	768.3341	443.5979	6.520302538	54.67516461	82.81923	17.18077234
EUB 4	6200	6429	4145	5591.3333	1257.784	726.1819	12.98763334	62.74969188	95.05012	4.949878902
EUB 8	5642	5567	3539	4916	1193.106	688.8403	14.01221175	67.24886466	101.8652	-1.86524489
EUB 16	4334	3617	3013	3654.6667	661.305	381.8046	10.44704395	75.65205805	114.594	-14.5939855

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as % of mean	%Inhib: cells		% virus Inhib
cells+med. Only	11581	12121	12345							
cells+med. Only	11743	8915	9224	10988.1667	1513.66	617.949	5.623769916			
virus+cells	4472	4171	4639							
virus+cells	4710	4686	5621	4716.5	486.302	198.532	4.209309261	57.07655205		
5B ONLY	13095	14842	10411	12782.6667	2231.95	1288.62	10.08097468	-16.33120478		
5B 1	8992	8541	14491	10674.6667	3312.73	1912.6	17.91721491	2.853069211	4.99867	95.00132873
5B 2	10707	10999	9529	10411.6667	778.23	449.311	4.315457765	5.24655311	9.19213	90.80786606
5B 4	9122	9384	7272	8592.66667	1151.21	664.651	7.735091376	21.80072502	38.1956	61.80441137
5B 8	10290	9216	8684	9396.66667	818.101	472.331	5.026578893	14.48376284	25.376	74.62397024
5B 16	5557	5159	5162	5292.66667	228.924	132.17	2.497219498	51.8330325	90.8132	9.186819027
5C ONLY	12338	14343	15004	13895	1388.31	801.544	5.768576602	-26.4542159		
5C 1	7914	6416	6071	6800.33333	979.769	565.67	8.318263022	38.11221162	66.7739	33.22614935
5C 2	8421	6466	6512	7133	1115.68	644.137	9.030378385	35.08471234	61.4696	38.53042785
5C 4	7353	7575	6542	7156.66667	543.767	313.944	4.386733703	34.86932913	61.0922	38.90778634
5C 8	7474	4943	4859	5758.66667	1486.12	858.009	14.89944528	47.59210666	83.3829	16.61706086
5C 16	6706	4625	4713	5348	1176.89	679.475	12.7052177	51.32946048	89.9309	10.06909381
14B+virus	15253	13157	11782	13397.3333	1747.94	1009.17	7.532628375	-21.92510125	-38.413	138.4134999
14B alone	10776	13405	14056	12745.6667	1736.56	1002.6	7.866229047	-15.99447891	-28.023	128.0228541
19A ONLY	13844	15097	11249	13396.6667	1962.61	1133.12	8.458194376	-21.91903411		
19A 1	10226	11051	8950	10075.6667	1058.54	611.147	6.065569403	8.304388054	14.5496	85.45043848
19A 2	9235	8925	8220	8793.33333	520.152	300.31	3.415201194	19.97451804	34.996	65.00398618
19A 4	9318	8629	9617	9188	506.667	292.524	3.183762223	16.38277541	28.7032	71.29683763
19A 8	8336	7312	4072	6573.33333	2225.9	1285.13	19.55059606	40.17807035	70.3933	29.60669678
19A 16	4640	4336	5272	4749.33333	477.482	275.675	5.804488903	56.77774576	99.4765	0.523518469

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	14329	13769	13524							
cells+med. Only	12442	12325	12773	13193.667	803.201	327.905	2.485323036			
virus+cells	5909	7158	6353							
virus+cells	4758	6373	6779	6221.6667	832.775	339.979	5.46443589	52.84353604		
15D ONLY	11071	12105	7811	10329	2241.1	1293.9	12.52686657	21.71243779		
15D 1	4763	6680	6581	6008	1079.34	623.156	10.37209852	54.46299992	103.06	-3.06463951
15D 2	7204	6690	5241	6378.3333	1017.94	587.706	9.214096732	51.65609762	97.753	2.247083572
15D 4	4886	7871	6154	6303.6667	1498.12	864.939	13.72119821	52.22202572	98.824	1.176133104
15D 8	5827	7225	6241	6431	718.106	414.599	6.446876086	51.2569162	96.998	3.002486135
15D 16	5516	7086	5613	6071.6667	879.776	507.939	8.365727402	53.98044516	102.15	-2.15146299
18A only	12698	12354	13110	12720.667	378.509	218.532	1.717932596	3.585053435		
18A 1	12486	10384	11967	11612.333	1094.96	632.177	5.444011404	11.98554862	22.681	77.31879901
18A 2	14145	11817	13127	13029.667	1167.05	673.796	5.171241661	1.243020641	2.3523	97.64773379
18A 4	10215	9396	10122	9911	448.421	258.896	2.612205952	24.88062454	47.084	52.91642762
18A 8	9656	8761	9471	9296	472.467	272.779	2.934368645	29.54195195	55.905	44.09542934
18A 16	7621	6923	7615	7386.3333	401.27	231.673	3.136510782	44.01606832	83.295	16.7049149
cp4 ONLY	12603	12940	13125	12889.333	264.663	152.803	1.18550017	2.306662287		
cp4 1	16842	13342	9438	13207.333	3703.84	2138.41	16.19108859	-0.10358505	-0.196	100.1960222
cp4 2	11254	8553	8154	9320.3333	1686.45	973.67	10.44673034	29.35752002	55.556	44.44444444
cp4 4	11308	10219	6768	9431.6667	2370.19	1368.43	14.50891218	28.51368081	53.959	46.04130809
cp4 8	8412	8007	5357	7258.6667	1659.29	957.994	13.19793547	44.9837043	85.126	14.87378084
cp4 16	7297	6474	5246	6339	1032.14	595.908	9.400662418	51.95422046	98.317	1.682922165

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	11916	11882	13307							
cells+med. Only	11321	13317	13096	12473.167	869.7879	355.0894	2.846826528			
virus+cells	4277	4562	5147							
virus+cells	4367	4565	4297	4535.8333	324.8387	132.6148	2.923714898	63.63527038		
PAC only	12003	12443	12070	12172	237.0717	136.8734	1.124494092	2.414516495		
PAC 1	4541	6294	5038	5291	903.4705	521.619	9.858608092	57.58094042	90.48589	9.514110533
PAC 2	5093	5169	5072	5111.3333	51.03267	29.46373	0.576439129	59.02136587	92.74945	7.250545943
PAC 4	4800	5036	4053	4629.6667	513.1592	296.2726	6.399436634	62.88298882	98.81782	1.182177054
PAC 8	4208	5360	4478	4682	602.4849	347.8448	7.429406087	62.46342148	98.15849	1.841508483
PAC 16	3894	4371	3721	3995.3333	336.6398	194.3591	4.864651943	67.96857254	106.8096	-6.809591802
lp3 only	10929	10424	11196	10849.667	392.0667	226.3598	2.086329915	13.01594089		
lp3 1	6078	4882	5693	5551	610.5137	352.4803	6.349851549	55.49646575	87.21023	12.78976986
lp3 2	5092	3841	5404	4779	827.1753	477.5699	9.993092492	61.68575208	96.93642	3.063581388
lp3 4	5484	4640	5213	5112.3333	430.9111	248.7866	4.866400422	59.01334866	92.73686	7.263144633
lp3 8	4776	4213	4653	4547.3333	296.0006	170.896	3.758158733	63.54307246	99.85512	0.144884932
lp3 16	4637	3969	4647	4417.6667	388.5889	224.3519	5.078515946	64.58263739	101.4887	-1.488745171
8B ONLY	5919	11832	8772	8841	2957.104	1707.285	19.31099068	29.11984393		
8B 1	5639	6017	5299	5651.6667	359.1676	207.3655	3.669103247	54.68939991	85.94196	14.05803796
8B 2	6231	5922	5014	5722.3333	632.5918	365.227	6.382484224	54.12285039	85.05165	14.94834537
8B 4	6003	5805	5089	5632.3333	480.8423	277.6144	4.928941958	54.84439931	86.18554	13.8144633
8B 8	5072	4550	4322	4648	384.4841	221.982	4.775860182	62.73600663	98.58685	1.413153032
8B 16	4787	4556	3736	4359.6667	552.3227	318.8836	7.314404228	65.04763559	102.2195	-2.219469175

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	15092	15308	14801							
cells+med. Only	15140	15252	15815	15234.6667	334.6184	136.6074	0.896687722			
virus+cells	6184	6354	6760							
virus+cells	5549	7211	6476	6422.33333	559.1203	228.2599	3.554158263	57.84395239		
7C ONLY	11836	11783	12073	11897.3333	154.4226	89.15592	0.749377326	21.90617889		
7C 1	5314	6049	6905	6089.33333	796.2665	459.7247	7.549671711	60.0297567	103.7788	-3.778794871
7C 2	3385	3144	4073	3534	482.0902	278.3349	7.875917661	76.80290565	132.776	-32.77603359
7C 4	4962	5513	5844	5439.66667	445.5495	257.2381	4.728931463	64.29415368	111.151	-11.15103832
7C 8	7530	6749	7450	7243	429.6824	248.0773	3.425062445	52.45711535	90.68729	9.312705678
7C 16	7116	6516	7400	7010.66667	451.3151	260.5669	3.716720759	53.98214598	93.32375	6.676249196
19A (CHEM) ONLY	8591	8405	8180	8392	205.8082	118.8234	1.415912755	44.9151059		
19A (CHEM) + virus	7099	7451	8152	7567.33333	536.0525	309.4901	4.089816964	50.32819884	87.00685	12.99315353
ANC ONLY	14575	14351	13734	14220	435.5353	251.4564	1.768329285	6.660248556		
ANC 1	7544	8806	8331	8227	637.3955	368.0005	4.473081961	45.99816209	79.52113	20.4788743
ANC 2	6908	6360	7751	7006.33333	700.6942	404.546	5.77400409	54.01058988	93.37292	6.627075689
ANC 4	7901	8242	7081	7741.33333	596.7414	344.5288	4.450510138	49.18606687	85.03234	14.96765896
ANC 8	6441	6492	5878	6270.33333	340.7262	196.7184	3.137287036	58.84167688	101.7249	-1.724855316
ANC 16	7011	6327	7302	6880	500.5267	288.9792	4.200279622	54.83983896	94.80652	5.193478836
FLB ONLY	15654	15175	12466	14431.6667	1719.082	992.5127	6.877325504	5.270873447		
FLB 1	7898	8040	5345	7094.33333	1516.63	875.6267	12.34262129	53.43295992	92.37432	7.625676136
FLB 2	7842	7630	5098	6856.66667	1526.734	881.4604	12.85552364	54.99299842	95.0713	4.928698415
FLB 4	7653	7024	4368	6348.33333	1743.617	1006.678	15.8573551	58.32968668	100.8397	-0.839732194
FLB 8	6772	6703	4466	5980.33333	1311.905	757.4286	12.66532442	60.74523018	105.0157	-5.015697696
FLB 16	6636	6064	4583	5761	1059.509	611.7077	10.61808226	62.18492911	107.5046	-7.504633657

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	11687	11137	12876							
cells+med. Only	10816	12422	13087	12004.17	935.0421	381.7293	3.179973735			
virus+cells	3305	4931	5454							
virus+cells	3966	4606	5039	4550.167	786.8564	321.2328	7.059802344	62.09510587		
EUD ONLY	9512	9576	9477	9521.667	50.20292	28.98467	0.30440753	20.68031933		
EUD 2	8903	9704	10910	9839	1010.288	583.2898	5.928344424	18.03679278	29.047	70.95295591
EUD 4	8955	10011	10083	9683	631.4935	364.5929	3.765288895	19.33634155	31.1399	68.86011985
EUD 8	8419	8577	8554	8516.667	85.36002	49.28263	0.578661036	29.05241236	46.787	53.21303998
EUD 16	6029	5064	5256	5449.667	510.8193	294.9216	5.411737357	54.60187435	87.9327	12.06734639
AND ONLY	9150	10807	10744	10233.67	939.0114	542.1385	5.297597419	14.74904547		
AND 2	6796	8277	7651	7574.667	743.4449	429.2281	5.66662716	36.89968761	59.4245	40.57552992
AND 4	9409	10182	10721	10104	659.4687	380.7444	3.768254614	15.82922596	25.4919	74.50809409
AND 8	9888	11498	12949	11445	1531.188	884.0319	7.724175292	4.658104825	7.50157	92.49843484
AND 16	6523	6612	6377	6504	118.6465	68.50061	1.053207384	45.81881291	73.7881	26.21187729
DED ONLY	9698	9296	7952	8982	914.3719	527.9129	5.87745348	25.17598056		
DED 2	9064	9247	6434	8248.333	1573.921	908.7035	11.0168139	31.28774731	50.3868	49.61318308
DED 4	9448	9880	5992	8440	2131.005	1230.337	14.57744715	29.69107949	47.8155	52.18450944
DED 8	13014	12655	9193	11620.67	2110.07	1218.249	10.483472	3.194724054	5.14489	94.85511135
DED 16	8525	10307	6521	8451	1894.084	1093.55	12.93989092	29.59944464	47.6679	52.33208121
DEC ONLY	12830	12575	12817	12740.67	143.6187	82.9183	0.650816021	-6.13536966		
DEC (2)	7944	7714	8174	7944	230	132.7906	1.671583106	33.82297813	54.4696	45.53036401
DEC (4)	5799	7489	7017	6768.333	872.0099	503.4552	7.438392114	43.61679972	70.2419	29.75807173
DEC (8)	7556	9017	6033	7535.333	1492.107	861.4686	11.43238846	37.22735161	59.9522	40.04784903
DEC (16)	5200	4657	3280	4379	989.7288	571.4202	13.04910164	63.52099965	102.296	-2.296306234

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	12400	13983	11826							
cells+med. Only	13138	15171	13345	13310.5	1181.306	482.266	3.623199381			
virus+cells	4427	5205	4731							
virus+cells	4842	4695	5198	4849.6667	304.7318	124.4062	2.5652533	63.56510524		
DMSO 1	12363	12608	11095	12022	812.0979	468.8649	3.90005774	9.680327561		
DMSO 2	10033	8792	9227	9350.6667	629.6748	363.5429	3.887882211	29.74969636		
3C ONLY	7062	9501	5264	7275.6667	2126.566	1227.773	16.87506348	45.33889285		
3C 2	5155	4460	3002	4205.6667	1098.802	634.3938	15.08426158	68.40339081	107.6115	-7.61154339
3C 4	4916	5274	4344	4844.6667	469.0856	270.8267	5.590203341	63.60266957	100.0591	-0.05909583
3C 8	5143	4798	3629	4523.3333	793.4925	458.1231	10.12799786	66.01680378	103.857	-3.85698808
3C 16	5068	5821	5091	5326.6667	428.2597	247.2558	4.641849085	59.98146826	94.36226	5.637742539
ACV 2	11306	12046	9922	11091.333	1078.149	622.4696	5.612216445	16.67230132	26.2287	73.77129912
ACV 4	10478	11017	11056	10850.333	323.0392	186.5068	1.71890366	18.48290197	29.07712	70.92287994
ACV 8	7461	8630	5535	7208.6667	1562.853	902.3138	12.51706863	45.84225486	72.11859	27.88141436
8B ONLY	13263	14820	13588	13890.333	821.3503	474.2068	3.413934077	-4.35621001		
8B 1	8564	8976	8758	8766	206.1165	119.0014	1.357533659	34.14221855	53.7122	46.28779671
8B 2	8910	8444	7222	8192	871.7591	503.3104	6.143925473	38.45460351	60.49641	39.503595
8B 4	9785	9270	8620	9225	583.8022	337.0584	3.653749084	30.69381316	48.28721	51.71279425
8B 8	10715	7491	9446	9217.3333	1624.118	937.6852	10.17306327	30.75141179	48.37782	51.62218064
8B 16	5324	6287	6332	5981	569.4234	328.7567	5.49668532	55.06554975	86.62858	13.37141732
cp2 ONLY	11196	7514	10057	9589	1885.086	1088.355	11.35003519	27.95913001		
cp2 1	6109	5680	4568	5452.3333	795.3266	459.182	8.421752195	59.03735146	92.87698	7.123017827
cp2 2	7783	7048	4908	6579.6667	1493.623	862.3434	13.10618649	50.56784744	79.55284	20.44715848
cp2 4	6316	6244	3832	5464	1413.812	816.2647	14.93895796	58.94970136	92.73909	7.260908106
cp2 8	6715	6550	4380	5881.6667	1303.096	752.3426	12.79131716	55.81182776	87.80262	12.19738008
cp2 16	4692	4266	3387	4115	665.475	384.2122	9.336869525	69.0845573	108.6831	-8.68314784

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	10562	11720	11983							
cells+med. Only	10507	12625	13182	11763.167	1079.518	440.7112	3.746535365			
virus+cells	4170	4612	5391							
virus+cells	4205	3769	4982	4521.5	594.3503	242.6425	5.366416036	61.56222106		
DMSO 1	11008	13065	12871	12314.667	1135.756	655.7292	5.324782336	-4.68836339		
DMSO 2	8672	10257	8850	9259.6667	868.2893	501.3071	5.413878138	21.28253446		
14B ONLY	11361	11700	11973	11678	306.5926	177.0113	1.515767247	0.724011391		
14B 1	5431	5764	5571	5588.6667	167.2015	96.53382	1.727313911	52.49011746	85.26352	14.73647871
14B 2	4730	5903	5834	5489	658.218	380.0224	6.923344284	53.33739498	86.63982	13.36018412
14B 4	4474	4249	5914	4879	903.3687	521.5602	10.68989874	58.52307344	95.06329	4.936708861
14B 8	5735	5031	5488	5418	357.182	206.2191	3.806185661	53.94097394	87.62025	12.37974684
14B 16	4815	4153	4739	4569	362.2651	209.1539	4.577672376	61.15841823	99.34407	0.655926352
AND ONLY	11235	10446	10892	10857.667	395.6189	228.4107	2.103681201	7.697757123		
AND 2	6128	6849	5724	6233.6667	569.895	329.029	5.278258536	47.00690007	76.35673	23.64326812
AND 4	4827	4843	4791	4820.3333	26.63331	15.37675	0.318997651	59.02180535	95.87342	4.126582278
AND 8	5443	4604	5241	5096	437.8915	252.8168	4.961083163	56.67833208	92.06674	7.933256617
AND 16	5611	5348	4013	4990.6667	856.8351	494.694	9.912382254	57.57378257	93.52129	6.478711162
DED ONLY	8663	8676		8669.5	9.192388	5.307228	0.06121723	26.29960753		
DED 2	5771	5145	4107	5007.6667	840.4578	485.2385	9.689913011	57.42926366	93.28654	6.713463751
DED 4	6276	5298	3407	4993.6667	1458.511	842.0717	16.86279389	57.54827923	93.47986	6.52013809
DED 8	5623	5526	4444	5197.6667	654.494	377.8723	7.270036416	55.81405234	90.66283	9.33716916
DED 16	4822	5364	2431	4205.6667	1560.616	901.0221	21.42400112	64.24715567	104.3613	-4.361334868
ACV 2	4187	3881	3467	3845	361.3475	208.6241	5.425853415	67.31322348	109.3418	-9.341772152
ACV 4	4333	4731	4285	4449.6667	244.821	141.3475	3.176585892	62.17288429	100.9919	-0.991944764
ACV 8	3820	6702	3331	4617.6667	1821.569	1051.683	22.775214	60.74469743	98.67204	1.327963176

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	12765	13237	13777							
cells+med. Only	12035	13279	13168	13043.5	590.0948	240.9052	1.846936809			
virus+cells	3576	3799	4394							
virus+cells	2769	3250	3410	3533	546.4138	223.0725	6.31396786	72.91371181		
3B	5469	4754	5726	5316.3333	503.6629	290.7899	5.469745821	59.24151238	81.2488	18.75120481
7B	5572	6898	6684	6384.6667	711.8773	411.0026	6.437337918	51.05097047	70.0156	29.9844032
DEC	3195	4737	3947	3959.6667	771.078	445.1821	11.24291885	69.64260615	95.51373	4.486269562
5B 1	6795	5130	5722	5882.3333	844.0002	487.2837	8.283851213	54.90218627	75.29748	24.70252177
5B 1	8090	7925	9636	8550.3333	943.8275	544.9191	6.373073831	34.4475537	47.24427	52.75572613
19A 8	3925	4706	4221							
19A 8	3793	3861	4887	4232.1667	464.4207	189.5989	4.479949941	67.55344297	92.64848	7.351523754
ACV 2	3762	6555	3706	4674.3333	1628.946	940.4723	20.11992329	64.16350417	87.99923	12.00077108
ACV 4	5004	7276	4319	5533	1547.851	893.6522	16.15131473	57.58040403	78.97061	21.02938857
ACV 8	6351	5113	3570	5011.3333	1393.285	804.4133	16.05188199	61.57984181	84.45578	15.54422305

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	16115	15796	15973							
cells+med. Only	16391	16646	18024	16490.833	809.5479	330.4965	2.004122718			
virus+cells	8124	7749	7229							
virus+cells	7276	7704	8915	7832.8333	625.1667	255.2232	3.258376808	52.50189499		
MAC	8750	9417	9551	9239.3333	429.0388	247.7057	2.680990994	43.97291425	83.75491	16.24509125
cp4	12971	13299	12625	12965	337.0401	194.5902	1.500888296	21.38056496	40.72342	59.27658428
lp3	10894	12051	12530	11825	841.0892	485.6031	4.106579801	28.29349639	53.89043	46.10957111
ANB	11251	10867	10888	11002	215.8958	124.6475	1.132953127	33.28414776	63.39609	36.6039116
EUD	9170	10062	11568	10634.333	876.6218	357.8794	3.365320233	35.51366921	67.64264	32.35735736
16A	9059	9359	9779	9832.8333	930.4733	379.8641	3.863221537	40.37394512	76.89998	23.1000231
ACV 2	14588	11884	11429	11016.333	2087.527	852.2293	7.736052215	33.19723078	63.23054	36.76946177
ACV 4	16166	15312	11666	14381.333	2390.001	1379.868	9.594852964	12.79195513	24.36475	75.63525064
ACV 8	16043	15913	12040	14665.333	2274.534	1313.203	8.95447084	11.06978624	21.08455	78.91545392

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	12857	12889	13742							
cells+med. Only	13198	13493	14593	13462	651.5477	265.9932	1.975881986			
virus+cells	3011	4339	5112							
virus+cells	3656	3767	4359	4040.6667	724.04	295.5881	7.315329403	69.98464815		
3B	5140	5520	6336	5665.3333	611.1017	352.8198	6.227696267	57.91610954	82.75545	17.24455137
8B	5927	5600	6043	5856.6667	229.7223	132.6302	2.264602667	56.49482494	80.7246	19.27540334
7C	4412	5534	6520	5488.6667	1054.731	608.9492	11.09466512	59.2284455	84.63063	15.36937447
5B1	5191	5349	4833	5124.3333	264.381	152.6405	2.978737986	61.9348289	88.49774	11.50226436
5B1	6699	5867	6198	6254.6667	418.8846	241.8432	3.866603399	53.53835488	76.50014	23.49985848
DED ONLY	12713	12739	9681	11711	1758.08	1015.028	8.667302109	13.00698262	18.58548	81.41452024
DED 2	5115	4294	3589	4332.6667	763.7345	440.9423	10.17715723	67.81557966	96.90065	3.099348995
DED 4	3812	4814	3320	3982	761.3698	439.5771	11.0391027	70.42044273	100.6227	-0.62270025
DED 8	6005	5140	4212	5119	896.6844	517.701	10.11332306	61.97444659	88.55434	11.44565525
DED 16	3509	4365	3051	3641.6667	666.9703	385.0755	10.57415455	72.94854653	104.2351	-4.23506935

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	14451	15333	14500							
cells+med. Only	14703	14560	15183	14788.33333	376.5027	153.7066	1.039377266			
virus+cells	4939	7951	8782							
virus+cells	5947	7135	6969	6953.833333	1374.253	561.0363	8.068015164	52.97757241		
14B ONLY	15535	15954	14966	15485	495.8941	286.3046	1.84891583	-4.710920771		
14B 1	6888	6386	7938	7070.666667	791.9604	457.2386	6.466696747	52.18753522	98.509	1.491267258
14B 2	8210	8217	6875	7767.333333	772.7913	446.1712	5.744201047	47.47661445	89.616	10.3835599
14B 4	7071	7573	6880	7174.666667	357.9418	206.6578	2.880381837	51.48427815	97.181	2.818729125
14B 8	8054	7749	6710	7504.333333	704.6136	406.8089	5.42098594	49.25504339	92.973	7.026613058
14B 16	6964	6603	6396	6654.333333	287.4584	165.9642	2.494076836	55.00281754	103.82	-3.8228349
EUB	8971	8462	9147	8860	355.7345	205.3834	2.318096855	40.08790713	75.67	24.33041887
FLB	6969	7428	8029	7475.333333	531.5829	306.9095	4.105629697	49.45114392	93.344	6.656455422
RIB	5524	6130	6004	6680.666667	954.844	389.8134	5.834948076	54.82474924	103.49	-3.48671474
PAC	7433	5672	6404	6194.5	684.2221	279.3325	4.509363273	58.11225065	109.69	-9.69217351
DEC	6581	6579	4852	6253.5	886.5012	361.9126	5.787360383	57.7132875	108.94	-8.93909418

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
	13030	14880	14148							
cells+med. Only	13512	14638	15160	14228	826.5698	337.4457	2.37170157			
	4272	6598	6888							
virus+cells	4010	3788	4069	4937.5	1410	575.6301	11.65833063	65.2973011		
DED	4821	6583	6384	5929.333333	964.9883	557.1362	9.39627101	58.32630494	89.32422	10.67577992
8B	5872	7121	7226	6739.666667	753.2532	434.8909	6.452706642	52.63096242	80.60205	19.39795131
ACV 2	7040	7613	7213	7288.666667	293.8985	169.6824	2.3280305	48.77237372	74.69279	25.30721346
ACV 4	10524	8536	11966	10342	1722.228	994.3286	9.614470936	27.31234186	41.82767	58.17232657
ACV 8	11929	11927	10900	11585.33333	593.5169	342.6672	2.957766887	18.57370443	28.44483	71.55517285
ACV 12	12188	12021	12104	12104.33333	83.5005	48.20904	0.398279147	14.92596758	22.85848	77.1415245
5B 1 ONLY	14271	15019	11512	13600.66667	1847.098	1066.423	7.840959261	4.409146284		
5B 2 ONLY	16929	15920	12577	15142	2277.924	1315.16	8.685511775	-6.42395277		

Sample ID	RFU1	RFU2	RFU3	RFU Mean	StDEV	SEM	SEM as %of mean	%Inhib: cells		% virus Inhib
cells+med. Only	10425	10094	10369							
cells+med. Only	10994	10803	11555	10706.66667	525.3999	214.4936	2.003365027			
virus+cells	2274	2589	2818							
virus+cells	2027	2436	2601	2457.5	278.3198	113.6236	4.623543212	77.04701121		
EUB	4497	4101	4324	4307.333333	198.5254	114.6187	2.661012804	59.76961395	77.57551	22.42448732
PAC	3252	3421	3366	3346.333333	86.19938	49.76724	1.487216934	68.74533001	89.22517	10.77482574
DED	5976	6124	4742	5614	758.7911	438.0883	7.803496102	47.56537983	61.73553	38.26447116