Establishment of Simple Cell-based Screening Assay and the Identification of Potent Antiviral Activity of a Plant Extract against HSV-1

Ratika Rahmasari1, Takahiro Haruyama2, Muhareva Raekiansyah3, Farhana Mossadeque4, Marina Ika Irianti1, Ayun Erwina Arifianti1, Nobuyuki Kobayashi2,*

ABSTRACT

Backgrounds: Drug screening is a time-consuming and costly process confronted with low productivity and challenges in using animals, which limits the discovery of new drugs. The cell-based assay allows the minimization of using the animal models and can provide more relevant in vivo biological information than biochemical assay. Objective: We aimed to establish a simple cell-based screening assay for the discovery of lead extract against HSV-1. Materials and Methods: Assay setting up was performed by optimization of the cell, incubation time, virus titer, and determination of Z value. Results: We have successfully established reproducible methods, by setting up assay plate including determination: 1) Vero cells as a model for HSV-1 infection, 2) Incubation for 5 days as sufficient time for CPE endpoint at monolayer cells, 3) 100 TCID50/well HSV-1 as infection titer which caused high percentage of cell detachment, 4) determination of Z value of 100 TCID50/well infection > 0.5. In addition, the established system was tested using ACV as the most common anti-HSV drug. Furthermore, we demonstrated the current system to screen extracts from Acacia nilotica, Uncaria gambir and Aspalathus linearis against HSV-1. It was observed that the alkaline extract of Uncaria gambir exhibited the highest SI (12.5) compared to other extracts. Conclusion: We demonstrated current cell-based screening system was reproducible and able to identify lead extracts against HSV-1 infection. Key words: Simple cell-based screening, HSV-1, Natural product activity.

INTRODUCTION

The journey of drug discovery from screening to its commercialization is complex, lengthy, and costly which usually takes more than ten years to get one approved drug from millions of compounds. Current advances in chemistry, genomics, proteomics, and bioinformatics have led to tremendous progress in high-throughput screening (HTS) for lead identification and optimization. There are two well-known strategies of the HTS system for drug discovery screening, namely biochemical assays and cell-based assays. In biochemical assay, the specific binding or affinity of tested compounds to the target of interest is carried out in homogeneous reactions that allow miniaturization with low variations. However, biochemical assays have limitations because not all targets can be purified or prepared in a manner suitable for biochemical measurement and cannot precisely represent tissue-specific responses.1 Cell-based assays can distinguish between agonists and antagonists, identify allosteric modulators, and provide direct information on cell permeability, the stability inside cells, and acute cytotoxicity, thus provides more relevant in vivo biological information than biochemical assay. There are three major cell-based assays for HTS that are used for lead compounds screening: second messenger assay, reporter gene assay and cell proliferation assay.1-3 These three types of assays are using different approaches for targeting lead compounds. The second messenger and reporter gene assays allow the target identification which provides relevant in vivo biological information. However, these systems are costly and limit the identification of late phase of compounds activity. On the contrary, cell proliferation monitors changes in cell growth as a response to external stimuli without a focus on target identification1 and is cheap and easy to perform thus more reliable for HTS assays. Some research has been proved the use of cell proliferation in identifying potential compounds against virus infection.1,4 Herpes simplex virus (HSV) is one of the viruses which cause medical problems in human.5-8 There are two distinct serotypes of HSV, type 1 (HSV-1) and type 2 (HSV-2). The transmission of HSV-1 through contact with infected lesions or other parts of the body is much easier than HSV-2 which is transmitted through genital contact. It has been reported that the HSV-1 infection rate is two times higher than HSV-2.5-7 In immunocompromised individuals such as cancer, HIV or organ transplant patients, the clinical manifestation may be more complicated resulting in HSV encephalitis, damage of temporal lobes, pneumonitis, esophagitis, and hepatitis. The HSV-1 infection is also related to the establishment of latent infection in the trigeminal area, where it can be reactivated and cause recurrent
episodes of viral shedding at the skin through lesions or sores. Thus, the HSV-1 ability to establish lifetime infection and induce severe diseases in some patients represents socially significant problems for which no vaccines are available yet. The prolonged therapies with the available anti-herpes drugs, have resulted in some undesirable effects and also induced the emergence of drug-resistant strains. Resistance to acyclovir and related nucleoside analogs can occur following mutation in either HSV thymidine kinase or DNA polymerase. These conditions encourage the invention of new types of anti-herpes virus agents with different mechanisms of action and high efficacy on resistant mutant viral strains.

In recent years, a large number of small molecules such as phenols, polyphenols, terpenes, flavonoid and sugar from natural products have shown promising antiviral activity as reported previously. *Acacia nilotica* is abundantly found in the pantropical and subtropical areas such as in Asia, Australia, America, and Africa. It is traditionally used as a source of nutrients, to treat colds, hemorrhoids, and to promote health. It contains a variety of chemical components such as gallic acid, ellagic acid, isoorcinit, leucocyanadin, kaempferol-7-glucoside, glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6,8-bis-C-glucopyranoside, m-catechol and their derivatives. *A. linearis* has been reported to exhibit antiplasmodial, molluscicidal, anti-fungal, anti-microbial activity and inhibitory activity against HCV and HIV. However, its anti-HSV activity has not been reported. *Uncaria gambir* is a plant species found in Indonesia, Singapore, and Malaysia. Research studies show that *U. gambir* possesses antimicrobial, anti-Nematoda, anti-diarrhea activity but its antiviral activity has not been confirmed. *U. gambir* contains abundant procyanidin and catechin which has been known to exhibit antiviral activity. *Aspalathus linearis* is a leguminous plant belonging to the Fabaceae family native to the western cape of South Africa. Mixtures of dried leaves and flowers from this plant have been used as traditional medicines and a common health beverage in Europe, Africa, and Japan. The reported biological activity of *A. linearis* includes antimutagenic, antiviral activity against rotavirus, human immunodeficiency virus, and influenza virus. However, its anti-HSV-1 activity has not been investigated. Since the occurrence of HSV-1 resistant, the discovery of new anti-HSV-1 drugs is urgently required. In this study, we have successfully established a simple screening system using cell-based assay and used it to screen for the anti-HSV-1 activity of extracts derived from *A. nilotica*, *U. gambir*, and *A. linearis*.

### MATERIALS AND METHODS

**Cell, virus and sample preparation**

Vero cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin. The HSV-1 strain F was kindly supplied by Professor Yasushi Kawaguchi, Institute of Medical Science, University of Tokyo, Japan. HSV-1 had been propagated several times in either HSV thymidine kinase or DNA polymerase. These conditions encourage the invention of new types of anti-herpes virus agents with different mechanisms of action and high efficacy on resistant mutant viral strains.

For ethanol, ethyl acetate and n-hexane extraction, 1 g of sample was extracted with 10 mL of 70% ethanol (or ethyl acetate or n-hexane, as appropriate) and shaken for 20 h at room temperature, followed by three separate centrifugation steps at 8740 ×g (Beckman J-21 centrifuge, JA-20 rotor) for 20 min. The collected supernatant was then evaporated, and the pellet diluted with 1% DMSO to become ethanol (or ethyl acetate or n-hexane) extract. All extracts were stored at -80°C to await further analysis. The samples were extracted with hot water. Acyclovir (ACV) or 9-(2-hydroxyethoxymethyl) guanine a control positive, was purchased as a powder from Sigma Aldrich company and dissolved in DMSO at a concentration of 225 µg/ml.

### Cytopathic effect optimization: incubation time and serum concentration

The 2.5 × 10^4 Vero cells per well were seeded into 96-well-plates and incubated overnight. The cells were washed by 100 µL DMEM, followed by the addition of 100 µL DMEM 10%FBS and 100 µl serial ten-fold dilution of HSV-1. The plate was incubated at 37°C for 3 or 5 days in 5% CO₂ incubator, then fixed by 70% ethanol for 5 minutes and stained by 0.5% crystal violet (CV). The development of cytopathic effect (CPE) in different incubation times was observed and compared. To optimize for appropriate serum concentrations, cells were incubated in the presence of 100 µL DMEM supplemented by 10% FBS or 2% FBS, and 100 µl serial ten-fold dilution of HSV-1. After fixation by 70% Ethanol and 0.5% CV staining, the cell density at mock (uninfected cell) were compared by analyzing the OD value measured at 560 nm. The virus infectivity was compared after calculating the virus titer by Reed-Muench method.

### Infectivity assay

The suitable virus titer for infecting Vero cells was determined by testing the serial five-fold dilution of a virus. The 2.5 × 10^4 Vero cells were seeded into each well of 96-well-plates and incubated overnight. The cells were washed by 100 µL of DMEM, followed by the addition of 100 µL of DMEM 4%FBS. The 100 µL of serial five-fold dilution of the virus in DMEM, were added and mixed for 30 seconds in plate mixer. Then the plate was incubated at 37°C for 5 days. The plate was fixed by 70% Ethanol for 5 minutes and stained by 0.5% CV. The percentage of infected cells was observed by analyzing the OD value at 560 nm. The experiments were performed in four replicates.

### Assay validation

The 2.5 × 10^4 Vero cells per well were seeded into 96-well-plates and incubated overnight. The supernatant was aspirated, followed by the addition of 100 µL serial two-fold dilution of ACV and 100 µL of 4, 20 and 100 TCID<sub>50</sub>/well HSV-1. The infected cells were cultured with DMEM containing 2%FBS and incubated at 37°C for 5 days. After incubation, the cells were fixed by 70% EtOH for 5 minutes and stained by 0.5% CV. The activity of ACV against HSV-1 indicated by cell attachment was analyzed from OD value measured at 560 nm. The control drug assay was used for measurement of assay quality and reproducibility using the most widely accepted statistical tools called Z factor. Z factor measures the separation of positive activity (in the present study, ACV at 11.2 µg/ml) and background of control (in the present study, 100 TCID<sub>50</sub>/well HSV-1) in the absence of test compound. It is determined at 11.2 µg/ml as follows:

\[
Z = \frac{\sigma_+ - \sigma_-}{\sigma_+ + \sigma_-}
\]

where \(Z\) is Z factor, \(\sigma_+\) is the standard deviation (SD) of positive active (ACV at 11.2µg/ml) in suppress infection, \(\sigma_-\) is SD of control (100 TCID<sub>50</sub>/well HSV-1) in causing cell detachment, \(\mu_+\) is the average of % relative OD value of positive active in suppressing infection and \(\mu_-\) is the average of % relative OD value of control. Z ≥ 0.5 indicates excellent assay, 0 ≤ Z ≤ 0.5 indicates acceptable assay, Z < 0.5 indicates unacceptable assay.
incubated overnight in 5% CO$_2$ incubator. The cells were washed by 100 µL serial 10-fold dilution of Dimethyl sulfoxide (DMSO) and 100 µL of 100 TCID$_{50}$/well HSV-1. The infected cells were cultured with DMEM contained 2% FBS and incubated at 37°C for 5 days. After incubation, the cells were fixed by 70% ethanol for 5 minutes and stained by 0.5% CV. The effect of DMSO against cell and HSV-1 was analyzed from OD value measured at 560 nm.

**TCID$_{50}$ assay**

The 2.5 × 10$^4$ Vero cells per well were seeded into 96-well plate and incubated overnight in 5% CO$_2$ incubator. The supernatant was aspirated, followed by the addition of 100 µL serial ten-fold dilution of Dimethyl sulfoxide (DMSO) and 100 µL of 100 TCID$_{50}$/well HSV-1. The infected cells were cultured with DMEM contained 2% FBS and incubated at 37°C for 5 days. After incubation, the cells were fixed by 70% ethanol for 5 minutes and stained by 0.5% crystal violet. The virus titer was determined by Reed-Muench statistical method.

**Cellular toxicity and antiviral activity of the extract**

The cellular toxicity was represented as CC$_{50}$ which means the addition of extract concentration results in 50% of cell death. The antiviral activity was represented as IC$_{50}$ which is extract concentration causing 50% of cell survival after infection by HSV-1. The CC$_{50}$ and IC$_{50}$ values were calculated from OD value linear regression analysis using Microsoft Excel software. The SI was then determined by the ratio of CC$_{50}$/IC$_{50}$ values.

**Statistical analysis**

Data are represented as the mean ± standard error of the mean from two independent experiments (each in triplicate or six replicate). The comparison of test samples and controls was performed using the student’s t-test. A p-value < 0.05 was considered as statistically significant.

**RESULT**

**Screening optimization**

The determination of CPE induced by HSV-1 infection in infected cells is important criteria for setting up the assay plate. For this purpose, Vero cells were subjected to the infectivity test by different dilution of HSV-1 which was done at 90 mm sterile dish. The CPE endpoint indicated by cell detachment was observed from day 2 post-infection at 90 mm dish with a hundred-times dilution of HSV-1 infection, as shown in Figure 1, respectively. Meanwhile, at the same condition multi giant cells were observed clearly at ten thousand-times dilutions of HSV-1.

In order to develop simple-efficient screening assay for screening large amounts of compounds against HSV-1, 96 well-plate assay approach was established. In this assay, the attached cells which were alive were stained by crystal violet. The appearance of plaques similar to that observed in plaque assay as reported previously$^{36}$ was able to be observed at day 3 post-infection starting from the infection of more than a thousand-times dilution of HSV-1, as shown in Figure 2A. It was observed that at day 5 post-infection, almost all infected cells were detached as shown in Figure 2B, respectively.

As described in the methodology section, the Vero cells were maintained in DMEM supplemented with 10% FBS. However, in order to know the best medium condition in which CPE produced more effectively indicated by cell detachment, the HSV-1 infection was performed in the presence of different FBS concentrations, which are 2 and 10 %. The cell density of mock cultured in both 2 and 10 % of FBS were shown in Table 1, respectively.

The virus titer was further tested at the cells supplemented with 2% and 10% FBS. The TCID$_{50}$ assay shown that the titer of HSV-1 at cell supplemented by DMEM 2% FBS was two-fold higher than DMEM 10% FBS.

In order to know the virus titer required to cause complete CPE as indicated by cell detachment, infection of five-fold serial dilution of HSV-1 to cell supplemented by DMEM 2% FBS and the infected cells were incubated for 5 days. Cell detachment was observed from the infection of 4 TCID$_{50}$/well (data not shown). The analysis of OD value at 560 nm showed no significant differences in infected cells (p<0.05) as shown in Figure 3A. The CPE appearance was also observed as shown in Figure 3B, separately.

**Control drug assay**

To know the sensitivity and reproducibility of the current plate assay in terms of incubation time, serum concentration and virus titer, ACV, the most common anti-HSV-1 drug, was tested in this system. For this purpose, the ACV activity was tested in the monolayer cells infected with HSV-1 at 4, 20 and 100 TCID$_{50}$/well. The cells were cultured in DMEM supplemented by 2% FBS and ACV were observed on day 5.

It was shown that in the absence of ACV, all virus titers employed in the experiment demonstrated clear detachment, which was similar
Figure 2: The CPE at Vero cells induced by HSV-1 at different dilutions were observed at day 3 and 5 post infection as indicated by plaque forming and cell detachment. A) The appearance of CV stained cells at day 3 post infection. The plaques were observed starting from 10⁴ virus dilution. B) The appearance of stained cells at day 5 post infection, almost all cells died at day 5 post infection as shown by unstained cell. M refers to mock as untreated cells.

Table 1: The comparison of mock (untreated) cell density.

<table>
<thead>
<tr>
<th>Medium addition</th>
<th>% Cell density of mock (Average ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM10% FBS</td>
<td>100 ± 0.2</td>
</tr>
<tr>
<td>DMEM2% FBS</td>
<td>90 ± 0.4</td>
</tr>
</tbody>
</table>

Experiment was performed in two independent experiments; each were tested in 6 wells.

Table 2: The % Relative OD value of each control for measuring Z factor.

<table>
<thead>
<tr>
<th>Control</th>
<th>% Relative OD value (experiment)</th>
<th>SD (σ)</th>
<th>Average (µ)</th>
<th>Z factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.2 µg/ml of ACV (s)</td>
<td>94.9</td>
<td>7.0</td>
<td>102.9</td>
<td>0.68</td>
</tr>
<tr>
<td>100 TCID₅₀/well (c)</td>
<td>7.1</td>
<td>9.0</td>
<td>6.1</td>
<td></td>
</tr>
</tbody>
</table>

*Z factor was calculated based from three independent experiments.

to the previous result, shown in Figure 4. In the current system, infection at 4 and 20 TCID₅₀/well can partially suppress (IC₅₀) by ACV at a concentration of less than 0.2 µg/mL which in line with previous reports. However, infection at 100 TCID₅₀/well was suppressed by 21-fold higher ACV concentration than 4 and 20 TCID₅₀/well. The Z factor, calculated from OD value of ACV to suppress 100 TCID₅₀/well and control infection, were found 0.68, as shown by Table 2, which in line with the previous report.

Since the samples were provided in dried forms, there was a need to be diluted in DMSO. In order to know the effect of DMSO into the cell and 100 TCID₅₀/well HSV-1 infections, the serial ten-fold dilution of DMSO was performed in the absence and presence of HSV-1. It was shown DMSO was cell-cytotoxic at a concentration of more than 0.125 %. However, no activity was observed against HSV-1 as shown in Figure 5.

First screening

Based on the optimization result, the anti-HSV-1 activity of samples was tested using established cell-based screening assay: performed at Vero cell supplemented by DMEM2% FBS which infected by 100 TCID₅₀/well of HSV-1 for 5 days at 37°C in 5% CO₂ incubator. In addition, 100 µl of tested samples were diluted at two-fold dilution in DMEM2%FBS with the final highest DMSO concentration of 0.1%. The anti-HSV-1 activity was observed for hot water, water, alkaline, methanol and ethanol extract of U. gambir and A. linearis, with the selective index (SI) value was observed at the alkaline extract of U. gambir with SI of 12.5 (Table 3). Meanwhile, no activity was remarked for A. nilotica.
Figure 4: The ACV activity against different HSV-1 titers. ACV partially inhibited 4 and 20 TCID₅₀/well at a concentration of less than 0.2 µg/ml, while a higher concentration of ACV (4.2µg/ml) needed for partially inhibited 100 TCID₅₀/well of HSV-1. IC₅₀ was calculated using Reed-Muench method from two independent experiments each in duplicate.

Figure 5: The effect of DMSO at cell and 100 TCID₅₀/well infection. The cytotoxic effect of DMSO was shown at a concentration more than 0.125 %, while no activity against HSV-1 was observed at a concentration of less than 1.25%. The error bar was calculated from two independent experiments each in duplicates.

Table 3: Anti-HSV type 1 activity of extracts using an established cell-based screening system.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Hot Water</th>
<th>water</th>
<th>alkaline</th>
<th>Ethanol</th>
<th>methanol</th>
<th>Ethyl Acetate</th>
<th>n-Hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ NA</td>
<td></td>
<td></td>
<td>IC₅₀ NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>CC₅₀ 0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.07</td>
<td>&gt;1</td>
</tr>
<tr>
<td></td>
<td>SI 4.2</td>
<td>2.9</td>
<td>12.5</td>
<td>2.9</td>
<td>5.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Uncaria gambir</td>
<td>CC₅₀ 0.09</td>
<td>0.06</td>
<td>0.13</td>
<td>0.06</td>
<td>0.12</td>
<td>0.83</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>SI 4.2</td>
<td>2.9</td>
<td>12.5</td>
<td>2.9</td>
<td>5.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aspalathus linearis</td>
<td>CC₅₀ 0.27</td>
<td>0.18</td>
<td>0.11</td>
<td>0.1</td>
<td>0.08</td>
<td>&gt;1</td>
<td>&gt;1</td>
</tr>
<tr>
<td></td>
<td>SI 2.3</td>
<td>1.7</td>
<td>2.6</td>
<td>1.9</td>
<td>2.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

IC₅₀ is 50% inhibitory concentration of extract. CC₅₀ is 50% cytotoxic concentration of extract. SI is the selectivity index which is the ratio of CC₅₀ to IC₅₀. NA means no activity. All data are represented as the mean of two independent experiments.
DISCUSSION

Vero cells are extensively used for virus propagation including HSV. In the present study, we performed the Vero cells sensitivity against HSV-1 infection. It was observed that Vero cells were sensitive against HSV-1 infection as indicated by the appearance of a multi giant, rounded, and cell detachment (Figure 1) which are typical CPE for HSV-1-infected cells. This demonstrates that the cells are susceptible to HSV-1 because specific receptors are expressed on the cell surface. The appearance of CPE in the infected cell are important criteria in setting up the assay system, since the effectiveness of infection will determine the activity of tested samples. Since Vero cells are susceptible to HSV-1 infection and induce CPE, the cells become a suitable in vitro model for drug screening against HSV-1.

As reported previously, optimization of incubation time, serum concentration, and HSV-1 titer are critical for the complete development of CPE. We demonstrated that prolonged incubation time until 5 days was unable to make cell detachment at infected–monolayer cell even by lower virus titer infection, compared to 3 days incubation (Figure 2B). Thus, by employing lower virus titers, the usage of the virus could be minimized. Therefore, the 5 days incubation was selected as the standard incubation time.

As it was reported previously, culture condition will affect CPE, including the medium. The effect of medium condition to induce CPE was investigated by performing. HSV-1 infection in different serum concentrations (2 and 10 % of FBS) for 5 days. The similar cell density of untreated cells (mock) on both serum concentrations (Table 1), indicated the similar ability of both serum concentrations in maintaining cells condition. On the other hand, we found that the virus titer tested at lower serum concentrations was higher. We suggest that the addition of medium supplemented by 2% FBS could maintain monolayer without inducing cell growth, thus reduce the second layer form cause the virus efficiently infected the cells. We concluded that the addition of 2% FBS is suitable for HSV-1 infection.

We demonstrated that the detachment of the complete cells occurred from 4 TCID₅₀/ well and found a high percentage of the infected cell until 100 TCID₅₀/ well HSV-1 (Figure 3). The decreased percentage of infected cells at infection more than 100 TCID₅₀/ well may indicate the defective interfering particles (DIPs), which is not suitable for further evaluation. Furthermore, the ACV was employed to check the infectivity of those titers in the presence of the antiviral drugs and to measure the quality of the assay. The ACV activity shifted to 21-fold higher concentration at 100 TCID₅₀/ well (Figure 4) compared with the lower tested titer and previous report. This result indicated that 100 TCID₅₀/ well is a high-enough titer for selecting good compounds. Taken together, 100 TCID₅₀/ well of HSV-1 is suggested as the best titer for selecting the good candidates. The quality assay (Z factor) of the present system was more than 0.5 indicate the good system for screening. Since no cytotoxicity or activity against HSV-1 was seen at DMSO concentration below 1% (Figure 3.8), the sample was tested at two-fold dilution with 1% (w/v) as the highest sample concentration, contained 0.1% final DMSO concentration. The two-fold concentrations tested suggest the selective criteria in screening extracts, including the selection of candidates with selective index more than 10, and identification of cytotoxicity of a compound into the cell.

In this study, we have established an easy and efficient cell-based screening system based on cell proliferation assay without limiting the compound’s target. This system allows a selective, rapid estimation of anti-HSV-1 activity from natural products and assesses cytotoxicity in parallel with antiviral activity. A current established cell-based screening system would be useful tools for primary screening against HSV-1 and drug discovery. By using the current established cell-based screening system, approximately 21 samples were tested. Some extracts from U. gambir and A. linearis exhibited anti-HSV-1 activity (Table 3), with higher SI value was obtained from the alkaline extract of U. gambir. On the other hand, the active constituent of the alkaline extract of U. gambir, which is responsible for inhibiting HSV-1 infection, is presently unknown. Since HSV-1 activity could only be observed in ethanol, methanol and alkaline extracts (Table 3), the phenolic content present in the alkaline extract could be promising candidates as the active constituents. However, further evaluation for selected candidates and target identification is required. In conclusion, this simple cell-based screening assay result is potential to be adopted in drug discovery, particularly in exploring anti-HSV-1 drug screening.

ACKNOWLEDGMENT

We thank Dr. Juliann Makau from Nagasaki University for useful discussions and help in revising this manuscript.

CONFLICTS OF INTEREST

None.

REFERENCES

Rahmasari, et al.: Establishment of Simple Cell-based Screening Assay and the Identification of Potent Antiviral Activity of a Plant Extract against HSV-1

44. Boyd MR, Bacon TH, Sutton D, Cole M. Anti-herpes virus activity of 9-{(4-}

Rahmasari, et al.: Establishment of Simple Cell-based Screening Assay and the Identification of Potent Antiviral Activity of a Plant Extract against HSV-1

PHARMACOGNOSY JOURNAL, Vol 12, Issue 2, Mar-Apr, 2020

GRAPHICAL ABSTRACT

Establishment of anti HSV Screening assay

1. Infectivity
   - Vero cell susceptibility
   - Incubation time
   - Serum concentration
   - Virus titer

2. Z value

3. Acyclovir assay

Sample Screening assay

Identified potent extract

ABOUT AUTHORS

Ratika Rahmasari has completed his PhD from School of Biomedical Science, Nagasaki University, Japan. Currently she is working as researcher and lecturer in Laboratory of Microbiology and Biotechnology, Faculty of Pharmacy, University Indonesia. Her research interests are how to control virus, bacteria and yeast infections on the molecular level by utilizing natural resources. She has published several papers in natural products and virus research.

Takahiro Haruyama has completed his PhD at the age of 38 years from Tsukuba University and worked as assistant professor in Laboratory of Molecular Biology of Infectious Agents, Graduate School of Biomedical Sciences, Nagasaki University. And then, he is working as Executive Researcher in private company, AVSS Co. Ltd. in Japan. His research interests are how to control virus infections on the molecular level. He has published more than 15 papers in international journals.
Muhareva Raekiansyah is a virologist graduated from Institute of Tropical Medicine, Nagasaki University, Japan. After graduation he was working as “the Postdoctoral Research Fellow” at Institute of Tropical Medicine (NEKKEN), Nagasaki University, Japan from 2013 until March 2018. Currently, he is working as support scientist at PT. Sciencewerke Indonesia based in Jakarta. He has published more than 10 papers related to infectious disease.

Farhana Mosaddeque is working as “the Postdoctoral Research Fellow” at Institute of Tropical Medicine (NEKKEN), Nagasaki University, Japan since July 2018. Prior to her appointment at NEKKEN, she obtained her “Doctor of Philosophy in Medical Sciences in Infection Research” on June 2018 and “Master in Pharmaceutical Science” on March 2014 from Nagasaki University, Japan. Her research expertise areas are - infectious diseases, virology, parasitology, drug discovery, high/mid/low throughput screening, assay design and development, product development, herbal medicine, molecular biology and cell biology. She has more than 6 years’ experience on drug discovery on different settings against various viruses and parasites.

Marina Ika Irianti is a master graduate and currently working as an academic staff in the University Indonesia. She finished her bachelor’s degree in the Faculty of Pharmacy, University Indonesia and continued her master’s degree in the Medical Pharmaceutical Sciences, University of Groningen. Despite her background in pharmaceutical sciences, Marina has a big interest in Microbiology, Molecular Biology and Biochemistry. She has published several papers in the pharmaceutical research and teaching courses in microbiology, biotechnology, immunology and virology.

Ayun Erwina Arifianti is a master graduate and currently working as an academic staff in the University Indonesia. She finished her bachelor’s degree in the Faculty of Pharmacy, University Indonesia and continued her master’s degree in the Pharmaceutical Sciences, Faculty of Pharmacy, University of Indonesia. Despite her background in pharmaceutical sciences, she has a big interest in Microbiology, Biochemistry and Natural products. She has published several papers in the pharmaceutical research and teaching courses in pharmaceutic.

Nobuyuki Kobayashi is CEO of AVSS Co., Ltd. since 2006. He is focusing on R&D for therapeutics against virus infectious disease including AIDS. He obtained PhD in Faculty of Pharmaceutical Science in University of Tokyo (1976). His carrier had been started as Postdoctoral fellow in The Wharton School of the University of Pennsylvania (1976-1981). And then, he had worked as Assistant Professor in Institute for Virus Research of Kyoto University (1981-1985), as Associate Professor in Yamaguchi University Medical School (1985-1996), as Professor in Nagasaki University (1996-2017). He has published more than 150 papers in international journals. He has been consistently engaged in science to date.