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Anti-herpes simplex virus type-1 activity of *Eleusine indica* methanol extract

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ABSTRACT

Aims: The present study is aimed at determining the antiviral activity of *Eleusine indica* whole plant methanol extract.
Methodology and results: Whole dried plants were extracted with methanol and the solvent was evaporated using a rotary evaporator. The crude methanol extract was previously shown to have antiviral activity towards herpes simplex virus type 1 (HSV-1) with selective index (SI = CC50 / EC50) of 12.2. The extract was further studied for the possible mode of action including pretreatment, attachment, penetration or virucidal activity. The observations suggested that *E. indica* crude methanol extract protects cells from HSV-1 infection, inhibits virus from docking to the surface of the cells and penetrating into the cells, as well as modifying virus through the virucidal effect.
Conclusion, significance and impact of study: Methanol extract of *E. indica* is safe with antiviral potential as a prophylactic agent, inhibits viral attachment, penetration and virucidal effect.

Keywords: *Eleusine indica,* anti-HSV-1, mode of action

INTRODUCTION

Herpes simplex virus type 1 (HSV-1) is a member of the Herpesviridae family with large double-stranded DNA within the capsid covered by an envelope. It is able to infect humans and can be latent within the neuron cells. Herpes viruses take over the nucleus and cytoplasm of the infected cell resulting in fatality during the process (Shukla and Spear, 2001). HSV-1 infections can cause fatal symptoms in immunocompromised or immunosuppressed patients. However, it might also appear to be asymptomatic in healthy persons (Greenberg *et al.*, 1987).

Acyclovir (ACV) is an antiviral agent that acts as a specific inhibitor to herpes virus DNA polymerase (Gnann *et al.*, 1983). The ACV-resistant HSV-1 infection can be treated with other antiviral agent such as Foscarnet and Cidofovir (Chilukuri and Rosen, 2003; Morfin and Thouvenot, 2003) but the usage was limited due to its toxicity (Brady and Bernstein, 2004). To solve these implications of hazardous antiviral drugs, the search for new anti-HSV-1 agents from natural products is crucial as alternative agents that are less toxic to humans.

Eleusine indica or locally known as Sambau is a weed that grows annually especially in the tropic and subtropic regions. Local medicinal practitioners in Asian countries use the weed especially the root to cure illnesses related to kidney and urinary problems (Lans, 2006), influenza, hypertension and oligouria (Ali *et al.*, 1996). The weed has been shown to display antioxidant, anti-inflammatory (Sagnia *et al.*, 2014), antimicrobial (Al-Zubairi *et al.*, 2011) and antiviral activities (Ali *et al.*, 1996). The methanol extract contains secondary metabolites such as tannin, flavonoid, triterpenoids, alkaloids, steroid, quinones and phenols (Iberahim *et al.*, 2015). The aim of this study was to determine the antiviral mode of action of *E. indica* crude methanol extract against HSV-1 infections.

MATERIALS AND METHODS

Plant Material

The methanol crude extract of *E. indica* was prepared according to Iberahim *et al.* (2015). For antiviral treatment, a stock solution of 10 mg/mL was prepared by sonication of the extract in 1% DMSO and 99% of Dulbecco Modified Essential Medium (DMEM, Gibco. USA).

Viruses and cells

African green monkey kidney cells (Vero) were obtained from the stock available at the Virology Laboratory (UKM, Bangi). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% Fetal Bovine Serum (FBS, JR Scientific. USA), 100 U/L of penicillin/streptomycin (Gibco. USA), non-essential amino acid (Gibco, USA 100×) and 20 U/L of amphostate B (Sigma-Aldrich. USA). The cell culture was maintained in an incubator at 37 °C and humidified with 5% CO_2 atmosphere.

The clinical strain of HSV-1 used in this study obtained from the Virology Laboratory of UKM, Bangi

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were propagated in monolayer of Vero cells. The titer of the virus was estimated using a standard plaque assay as described in Blaho *et al.* (2005). The Multiplicity of Infection (MOI) of virus in the antiviral assay was 0.01. The viral stock was kept in a freezer at −80 °C until use.

Pretreatment Assay

The assay was conducted based on Medini *et al.* (2014) with slight modification in terms of incubation period. Confluent Vero cells in 24-well plate were treated with different concentrations of extract (0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mg/mL). The cells were further incubated at 37 °C for 24 h. The media was removed and washed with phosphate buffered saline (PBS) before being infected with 50 plaque forming unit (pfu) of HSV-1. Each well was overlaid with 1% (w/v) of methylcellulose (MCS) and incubated at 37°C for another 48 h before staining with crystal violet (0.4%, w/v). Plaque inhibition was calculated using the formula below:

Percentage of plaque = [(mean number of plaque $count_{control}$ – mean number of plaque $count_{test}$) / (mean number of plaque $count_{control}$)] × 100%

Attachment assay

The assay was performed as previously described by Saddi *et al.* (2007). Monolayer cells in 12-well plates were pre-chilled at 4 °C for an hour. Next, 100 pfu of virus was mixed with several concentrations of the crude extract (with 0.1, 0.2 and 0.3 mg/mL) and incubated at room temperature for 1 min. The virus-extract mixture was then added to the pre-chilled cells and further incubated at 4 °C for 2 h. After 2 h incubation, the virus-extract mixture was decanted and cells were washed twice using PBS. Finally, MCS was added to each well. The plaques were stained with crystal violet solution after 48 h of incubation. The percentage of plaques inhibition was calculated using the formula as mentioned in the pretreatment assay.

Penetration assay

The assay was done according to the procedure of Cheng *et al.* (2006) with slight modifications in incubation period. The confluent monolayers of Vero cells in the 12-well plate were incubated at 4 °C for an hour. Then, the cells were infected with 100 pfu of HSV-1 for each well and further incubated for 2 h at 4°C. Crude extract (0.1, 0.2 or 0.3 mg/mL) was added to infected cells and incubated at 37°C at several incubation periods (30, 60, 90 or 120 min). At each incubation period, cells were washed for one minute with PBS (pH 3), PBS (pH 7) and DMEM. Finally, MCS was added and further incubated for 48 hours before staining with crystal violet solution. The percentage of plaques inhibition was calculated using the formula as mentioned in the pretreatment assay.

Virucidal assay

The assay was performed as described by Cheng *et al.* (2006). *E. indica* extract (0.2 mg/ml) was added to 1 × 10^7 pfu/mL of HSV-1 and incubated for 30, 60, 90 and 120 minutes at 37 °C. As the control, the extract was replaced with DMEM+5% FBS and mixed with the same concentration of virus. For each incubation time, both the mixture of test and control were immediately placed on ice. These were then diluted to 1 × 10^5, 1 × 10^4 and 1 × 10^3 pfu/mL. The media in the 24-well which contain monolayer cells were removed and replaced with 200 µL of DMEM with 5% FBS. The test and control mixtures that have been diluted were added into each well, respectively. The plate was then incubated for 2 h at 37 °C. Finally, 700 µL of MCS were added to each well. The plaques formation was stained with crystal violet solution after 48 h of incubation. The percentage of plaques inhibition was calculated using the formula as mentioned in the pretreatment assay.

RESULTS AND DISCUSSION

Antiviral activity

In preliminary studies, the methanol extract of *E. indica* was shown to be non-cytotoxic with CC_{50} of 2.07 mg/mL and has anti-HSV-1 activity with EC_{50} value of 0.17 mg/mL as evaluated by plaque reduction assay in post treatment assay (Iberahim *et al.* 2015). The calculated selective index (SI) value of 12.2 for *E. indica* methanol extract was more than 10 which according to Dargan (1998) worth to be further studied as antiviral agent. The anti-HSV-1 activity can be related to the presence of active compounds in *E. indica* methanol extract such as flavonoid and phenolic groups, which was previously reported by Iberahim *et al.* (2015). Flavonoids have been proven able to inhibit the RNA synthesis of viruses in post treatment assays according to Hayashi *et al.* (1997) and Barnard *et al.* (1993). Polyphenols can act principally by binding to the protein coat that prevents absorption of the virus (Behravan *et al.*, 2011).

***Eleusine indica* extract can affect virus infection to pretreated cells**

The pretreatment assay was conducted to investigate the effect of the extract to the cells before being infected with HSV-1. During the 24 h of cell-extract incubation period, the extract can have enough time to be absorbed and affect the cells. The activity of the extract in the pretreated cells towards HSV-1 infection was concentration-dependent as shown in Figure 1. One possible explanation is that the extract was able to interfere with the glycoprotein receptor on the cell membrane or block the HSV-1 from binding to the cell surface (Nizet and Esko, 2009).

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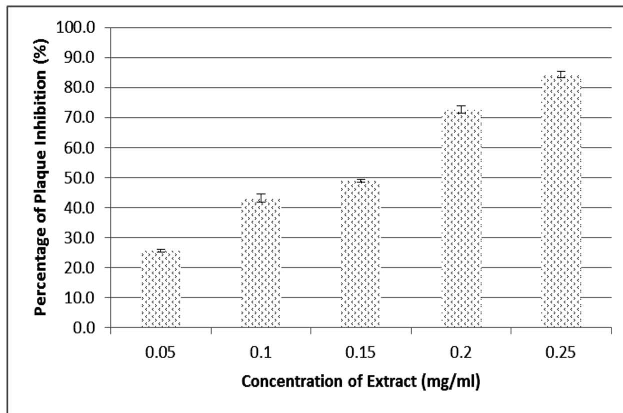

Figure 1: Pretreatment assay where cells were pretreated with *E. indica* extract and later infected with HSV-1. Plaque inhibition percentage increased as extract concentration increased.

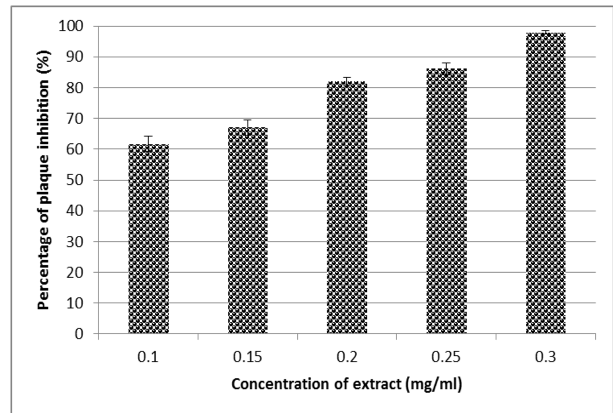

Figure 2: Effect of *E. indica* methanol extract on the virus attachment activity. Plaque inhibition percentage increased as extract concentration increased.

Eleusine indica prevents attachment and penetration of virus to the cell

The antiviral activity during attachment and penetration were conducted to explain the inhibitory effects of the extract during the early events of viral infection. For both assays, the percentage of plaque inhibition increases with increase in the extract concentration. Inhibition towards virus attachment can affect via binding of virion to the cell membrane molecules, adsorption onto the cell or interruption to viral glycoproteins that prevents penetration into the cell membrane (Kratz *et al.*, 2008). However, plaque inhibition percentages at different concentrations in the attachment assay (Figure 2) were higher compared to the penetration assay (Figure 3). Treatment with the extract directly affects the virus ability to attach to host cells. The effect seen in the attachment assay can be related to the virucidal activity revealed in the next section.

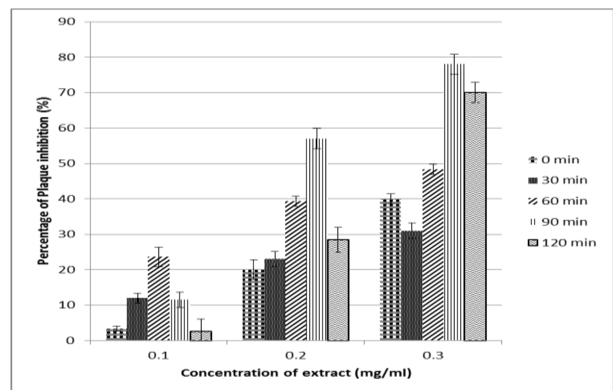

Figure 3: Effect of *E. indica* extract during virus penetration into the cell.

Eleusine indica act as a virucidal agent

The virucidal assay was performed to support the observation from the attachment assay, where *E. indica* was proven to prevent viral attachment. The extract was effective towards all virus dilutions used in the assay. The extract completely diminished HSV-1 infectivity at 37 °C for 60 min (Figure 4). When higher titer of virus (1×10^5 pfu) was treated with the extract, virucidal activity was reduced and longer time was needed for viral infectivity to diminish. Presence of active compound in the extract such as flavonoid (Iberahim *et al.*, 2015) may contribute to the virucidal activity (Hayashi *et al.* 1997). Virucidal capabilities include the ability to modify the whole virus structure or mask the virus envelope protein (Dargan, 1998), which can avoid the cell-to-cell infection (Kratz *et al.*, 2008). The activity can be proven by electron microscopic observation to conclude the structural modification during the virucidal activity.

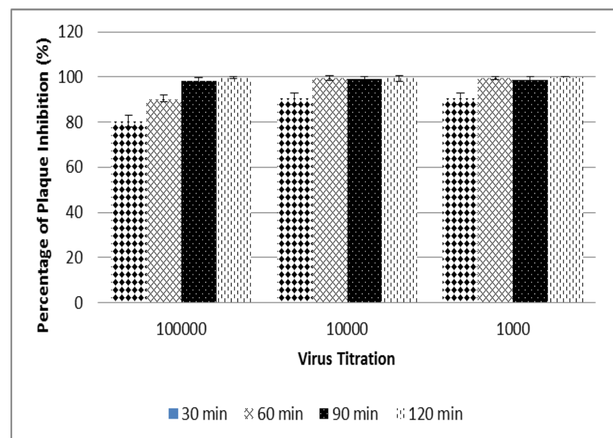

Figure 4: Effect of *E. indica* extract treatment upon high to diluted titered virus infection to cell.

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CONCLUSION

Crude methanol extract of *E. indica* has antiviral activity towards HSV-1 with prophylactic effect, inhibits attachment and penetration of virus into cell and virucidal activity. Future investigation should focus on the molecular component involvement either solely or synergistically in the antiviral activity. It is thus suggested that *E. indica* may be further investigated for the development of cutaneous anti-HSV-1 cream.

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REFERENCES

Al-Zubairi, A. S., Abdul, A. B., Abdelwahab, S. I., Peng, C. Y., Mohan, S. and Elhassan, M. M. (2011). *Eleusine indica* possesses antioxidant, antibacterial and cytotoxic properties. *Evidance Based Complementary Alternative Medicine* **Article ID 965370.**

Ali, A. M., Mackeen, M. M., El-Sharkawy, S. H., Hamid, J. A., Ismail, N. H., Ahmad, F. B. H. and Lajis, N. H. (1996). Antiviral and cytotoxic activities of some plants used in Malaysian indigenous medicine. *Pertanika Journal of Tropical Agricultural Science* **19 (2/3), 129-136.**

Barnard, D. L., Smee, D. F., Huffman, J. F., Meyerson, L. R. and Sidwell, R. W. (1993). Anti-herpesvirus activity and mode of action of SP-303, a novel plant flavonoid. *Chemotherapy* **39, 203-211.**

Behravan, J., Ramezani, M., Nobandegani, E. and Gharaee, M. (2011). Antiviral and antimicrobial activity of *Thymus transcaspicus* essential oil. *Pharmacology Online* **1, 1190-1199.**

Blaho, J. A., Morton, E. R. and Yedowitz, J. C. (2005). Herpes simplex virus: Propagation, quantification and storage. *Current Protocol in Microbiology.* New York. **pp. 1-23.**

Brady, R. C. and Bernstein, C. I. (2004). Treatment of herpes simplex virus infections. *Antiviral Research* **61, 73-81.**

Cheng, H. Y., Yang, C. M., Lin, T. C., Shieh, D. E. and Lin, C. C. (2006). *ent*-Epiafzelechin-(4α→8)-epiafzelechin extracted from *Cassia javanica* inhibits herpes simplex virus type 2 replication. *Medical Microbiology* **55(2), 201-206.**

Chilukuri, S. and Rosen, T. (2003). Management acyclovir-resistant herpes simplex virus. *Dermatology and Clinical Research* **21, 311-320.**

Dargan, D. J. (1998). Anti-HSV of antiviral agents. *In*: Methods in Molecular Medicine. Herpes Simplex Virus Protocols. Brown, S. M. and MacLean, A. R. (eds). Humana Press, Clifton, New Jersey. **pp. 387-405.**

Gnann, J. W., Barton, N. H. and Whitley, R. J. (1983). Acyclovir: Mechanism of action, pharmacokinetics, safety and clinical applications. *Human Pharmacological and Drug Therapy* **3 (5), 275-283.**

Greenberg, M. S., Friedman, H., Cohen, S. G., Oh, S. H., Laster, L. and Starr, S. (1987). A comparative study of herpes simplex infections in renal transplant and leukemic patients. *Journal Infectious Disease* **156, 280-287.**

Hayashi, K., Hayashi, T., Otsuka, H. and Takeda, Y. (1997). Antiviral activity of 5,6,7-trimethoxyflavone and its potentiation of the antiherpes activity of acyclovir. *Antimicrobial Chemotherapy* **39, 821-824.**

Iberahim, R., Yaacob, W. A. and Ibrahim, N. (2015). Phytochemistry, cytotoxicity and antiviral activity of *Eleusine indica* (Sambau). *AIP Conference Proceedings.* **1678(1), 030013-1-030013-4.**

Kratz, J. M., Andrighetti-Frohner, C. R., Kolling, D. J., Leal, P. C., Cirne-Santos, C. C., Yunes, R. A., Nunes, R. J., Trybala, E., Bergstrom, T., Frugulhetti, I. C., Barardi, C. R. M. and Simoes, C. M. O. (2008). Anti-HSV-1 and anti-HIV-1 activity of gallic acid and pentyl gallate. *Memórias do Instituto Oswaldo Cruz* **103(5), 437-442.**

Lans, C. A. (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *Ethnobiology and Ethnomedicine.* **2 (45), 1-11.**

Medini, F., Legault, J., Pichette, A., Abdelly, C. and Ksouri, R. (2014). Antiviral efficacy of *Limonium densiflorum* against HSV-1 and influenza viruses. *South Africa Journal of Botany* **92, 65-72.**

Morfin, F. and Thouvenot, D. (2003). Herpes simplex virus resistance to antiviral drugs. *Clinical Virology* **26, 29-37.**

Nizet, V. and Esko, J. D. (2009). Bacterial and viral infections. *In*: Essential of Glycobiology. Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W. and Etzler, M. E. (eds.). Cold Spring Harbor Laboratory Press. La Jolla, California.

Saddi, M., Sanna, A., Cottiglia, F., Chisu, L., Casu, L., Bonsignore, L. and De Logu, A. (2007). Antiherpes virus activity of *Artemisia arborescens* essential oil and inhibition of lateral diffusion in Vero cells. *Annals of Clinical Microbiology and Antimicrobials* **6, 10-17.**

Sagnia, B., Fedell, D., Casetti, R., Montesano, C. and Faldoni, G. (2014). Antioxidant and anti-inflammatory activities of extracts from *Cassia alata*, *Eleusine indica*, *Eremomastax speciosa*, *Carica papaya* and *Polyscias fulva* medicinal plants collected in Cameroon. *PLoS One* **9, 1-10.**

Shukla, D. and Spear, P. G. (2001). Herpesviruses and heparan sulfate: An intimate relationship in aid of viral entry. *Clinical Investigation* **108(4), 503-510.**

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