Anti-Herpes Simplex Virus Type-1 Activity of *Kyllinga nemoralis* Roots Aqueous Extract

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Abstract

*Human Herpesvirus 1* known as herpes simplex virus type 1 (HSV-1) is belonged to the *Herpesviridae* family, *Alphaherpesvirinae* subfamily, and *Simplexvirus* genus. *Kyllinga nemoralis*, known as whitehead spike sedge, white kyllinga, white globose spike, or poverty grass, is a monocotyledonous flowering graminoid similar to grasses and rushes. Acyclovir (ACV) did not eradicate HSV-1 infection as the drug only focuses on inhibiting the production of new viral genomes by interfering with viral DNA synthesis. This study aimed to identify the cytotoxic concentration (CC\textsubscript{50}) of *K. nemoralis* roots aqueous extract and evaluate its antiviral activities against HSV-1.

Cytotoxicity assay involved the incubation of Vero cells with 10 concentrations of extract ranging between 0.002 mg/mL and 1.000 mg/mL. MTT solution was added and absorbance was measured at 540 nm \[1\]. The CC\textsubscript{50} of extract was determined from the graph of cell viability (%) against extract concentrations. Antiviral assays of post-treatment, pre-treatment and virucidal tests determined the mode of antiviral activity. The 50 % effective concentration (EC\textsubscript{50}) of extract was derived from the graph of plaque inhibition (%) against concentrations. Selectivity index (SI) was evaluated as the ratio of CC\textsubscript{50} to EC\textsubscript{50} \[2\].

1. Post-treatment assay: Cells were inoculated with HSV-1 for 2 hours. Extract with (2% DMEM and 1% MCS) was added into cells and incubated for 48 hours.
2. Pre-treatment assay: Cells were treated with extract in 5% DMEM for 24 hours and inoculated with HSV-1 for 1 hour 30 minutes. Then, cells were overlaid with (2% DMEM and 1% MCS) and incubated for 48 hours.
3. Virucidal assay: HSV-1 was incubated with extract for 30 minutes. Then, virus-extract suspension was inoculated into cells and overlaid with 2% DMEM and 1% MCS for 48 hours.

After 48 hours incubation, cells were stained with crystal violet solution and incubated at room temperature for 30 minutes. The plaques were counted and percentage of plaque inhibition (%) was calculated.
Table 1: Antiviral activity of *K. nemoralis* roots aqueous extract against HSV-1

<table>
<thead>
<tr>
<th>Assay</th>
<th>CC₅₀ (mg/mL)</th>
<th>EC₅₀ (mg/mL)</th>
<th>Selectivity Index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-treatment</td>
<td>0.72</td>
<td>0.062</td>
<td>11.61</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>0.02</td>
<td>36.00</td>
<td></td>
</tr>
<tr>
<td>Virucidal</td>
<td>0.222</td>
<td>3.24</td>
<td></td>
</tr>
</tbody>
</table>

The CC₅₀ revealed aqueous extract concentration was able to kill at 50% of cell population. Post-treatment assay showed that 0.062 mg/mL of the extract inhibited intracellular viral replication. Pre-treatment study indicated that 0.02 mg/mL of extract produced protective effects towards cells. Finally, virucidal assay demonstrated that 0.222 mg/mL of extract initiated extracellular interaction with virus in inhibiting infection [3] as shown in Table 1.

![Figure 1: Viral plaque inhibition of different concentrations of *K. nemoralis* roots aqueous extract.](image)

The cytotoxicity study showed that CC₅₀, 0.72 mg/mL was toxic on cells. Therefore, lower concentrations between 0.016 mg/mL to 0.5 mg/mL, that were least or non-toxic to the cells were utilized in the subsequent antiviral screenings [4]. The extract showed good inhibition of intracellular virus replication with an EC₅₀ of 0.062 mg/mL and SI more than 10 which is 11.61. Similarly, in pre-treatment investigation, the extract produced excellent antiviral activity with an EC₅₀ of 0.02 mg/mL and corresponding SI of 36.00. This finding highlights that the extract is most effective when administered as pre-treatment. Antiviral activity exhibited by roots extract probably results from the inhibition of host cell glycoprotein receptors or due to prevention of binding between HSV-1 virions and host cells [5]. Virucidal assay revealed a weak extracellular interaction between HSV-1 and extract. It was supported by the low SI, 3.24 as shown in Figure 1.1. The finding suggests that 30 minutes of virus-extract exposure could be insufficient for roots extract to show an effective inhibition of virus [2]. In conclusion, the present study revealed that *K. nemoralis* roots aqueous extract inhibits in-vitro productive HSV-1 infection through three different modes that are effective prevention of intracellular viral replication, excellent interaction with host cell surface to inhibit HSV-1 infection and moderate direct destruction the virus particles.

**Keywords**
Antiviral activity, Effective concentration, Selectivity index

**References**


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