ANTI-HERPES SIMPLEX VIRUS ACTIVITIES OF *SCOPARIA DULCIS* LINN.

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In this study, *Scoparia dulcis* Linn. was investigated for anti-HSV activities against HSV-1F and HSV-2G standard strains. Both ethanol and water extracts of *S. dulcis* were tested for toxicity on GMK cells and 50% cytotoxic doses were 39.7 and 2,000 µg/ml respectively. Inhibition of HSV plaques was determined by plaque inhibition assay. This results showed that ED_{50} of water extract of *S. dulcis* against HSV-1F and HSV-2G were 1,024.6 and 1,190.4 µg/ml while ethanol extract of *S. dulcis* showed lower ED_{50} against HSV-2G, which was 13.8 µg/ml and did not inhibit HSV-1F. Therefore, therapeutic index (TI) of the water extract of *S. dulcis* on both HSV-1F and HSV-2G were 2.0 and 1.7 whereas ethanol extract of *S. dulcis* showed higher TI (2.9) on HSV-2G. In addition, the 50% inhibition concentrations (IC_{50}) of ACV on HSV-1F and HSV-2G were 6.84 and 2.95 µg/ml respectively. Therefore, this preliminary study showed that *S. dulcis* extracts could be developed as a choice for a new antiherpetic drug.

Keywords: Herpes simplex virus, *Scoparia dulcis* L.

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Introduction: Herpes simplex viruses (HSV) have common biological activities but they are different in many aspects. HSVs are classified into 2 types. They can cause acute primary and recurrent infections. The infections vary from mucocutaneous lesions to life-threatening diseases. HSVs are latent at nerve ganglion after primary infection. In many instances, the infections are asymptomatic, but they may present as acute gingivostomatitis, eczema herpes, keratoconjunctivitis, genital herpes, neonatal herpes, meningitis and encephalitis (Collier and Oxford, 2000). They may also be implicated in certain cancer. In addition, HSV infection may be a risk for HIV infection (Severson and Tyring, 1999). The illness is more serious in patients with deteriorated cellular immunity. Although, most antiviral drugs have a very narrow spectrum of activity, the effective chemically synthesized antiviral drugs have been used to treat HSV infection. ACV is the drug of choice for treatment and prevention of HSV infection but it is expensive and resistant strain of virus may emerge after long term treatment. Therefore, in this present study, *S. dulcis* was preliminary tested for the efficiency of anti-HSV using plaque inhibition assay.

Methods: 1) Preperation of plant extracts: *S. dulcis* L. (Scrophulariaceae) was authenticated by Assistant Professor Paritat Trisonthi, Department of biology, Faculty of Science, Chiangmai University. Dried leaves of *S. dulcis* were milled and soaked in 95% ethanol for 24 hr. or soaked in distilled water at 45°C for 3 hr. The extracts were filtered, concentrated and lyophilized. 2) Viruses and cells: HSV-1F and HSV-2G strains were propagated in GMK cells, which maintained and cultured in Eagle's

minimal essential medium (MEM) (Sigma-Aldrich, Germany) supplemented with 10% calf serum (Starrate, Australia) and incubated at 37°C in 5% CO_2 incubator. 3) ACV was purchased as powder from Sigma-Aldrich and dissolved in distilled water before use. 4) Cell toxicity: Ethanol crude extract was suspended in dimethylsulfoxide (DMSO) while water extract was reconstituted with MEM. Then, the extract was diluted 2-fold by MEM and each dilution was added in quadruplicate wells. Cell suspension containing $1X10^5$ cells/ml was added in each well and incubated for 4 days at 37°C in 5% CO_2. After incubation, the cells were stained with 0.1% crystal violet in 1% ethanol for 5 mins and cytotoxicity dose at 50% (CD_{50}) was calculated. 5) Antiviral assay: non toxic concentrations of *S. dulcis* extracts were tested against both HSV-1F and HSV-2G compared with ACV at 50% inhibition concentration. The 50% effective dose (ED_{50}) and therapeutic index (TI) of extracts were calculated.

Results and Discussion: Plant extracts were used as a primary screening for anti-HSV-1F and HSV-2G activities by plaque inhibition assay. Both ethanol and water extract of *S. dulcis* were tested for toxicity on GMK cells and CD_{50} were 39.7 and 2,000 µg/ml respectively. ED_{50} of water extract of *S. dulcis* on HSV-1F and HSV-2G were 1,024.6 and 1,190.4 µg/ml while ethanol extract of *S. dulcis* showed lower ED_{50} against HSV-2G, which was 13.8 µg/ml and did not inhibit HSV-1F. Therefore, TI (CD_{50} /ED_{50}) of the water extract of *S. dulcis* both HSV-1F and HSV-2G were 2.0 and 1.7 whereas ethanol extract of *S. dulcis* showed higher TI (2.9) on HSV-2G. In addition, EC_{50} of ACV on HSV-1F and HSV-2G were 6.84 and 2.95 µg/ml respectively(Table 1).

Table 1. Activity of *S. dulcis* L. extracts against HSV-1F and HSV-2G.*

Test	CD_{50} (µg/ml)	ED_{50}(µg/ml)		TI[a]	
		HSV-1F	HSV-2G	HSV-1F	HSV-2G
ethanol extract	39.7	-	13.8	-	2.9
water extract	2,000	1,024.6	1,190.4	2.0	1.7

*= averaged from 3 experiments
[a]= CD_{50} /ED_{50}

This study showed that ethanol and water extracts of *S. dulcis* inhibited both HSV-1F and HSV-2G when using plaque inhibition assay while the extracts were added at the same time of virus adsorption. Therefore, the adsorption step of virus to host cell was interfered and extracellular virus was also affected by *S. dulcis*. However, it may also inhibit intracellular viruses. Thus, future study will be carried on to clarify mode of action of this plant. It will be useful in order to develop therapeutic potential drugs from *S. dulcis* extracts as a new antiherpetic drug.

References:

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