CHAPTER 5

CONCLUSION

Herpes simplex viral infectious disease is an important health problem since the patients are increased and the infection is not entirely cured. Thus, the disease is easily transmission to other people. Furthermore, the problem in the viral treatment is from their rapid adaptation and development of drug-resistance as emergence of mutant viruses.

Hence, the purpose of this study was to demonstrate the inhibitory effect of aqueous and ethanolic extracts from twenty-three medicinal plant species against HSV infection by plaque reduction assay. The medicinal plants extracts were selected to fractionate and determine their anti-HSV activity. The fractions of the plant extracts, which showed highest activity against HSV infection, were selected for evaluation of their active compound.

The result showed that the highest percentage yield of recovered extract was obtained from ethanolic extract of *C. roxburghii*. The medicinal plant extracts were also investigated for their cytotoxicity on Vero cells. It was found that ethanolic extract of *A. paniculata* revealed strong cytotoxicity in Vero cells with CD_{50} value of 20.10 µg/ml while aqueous extract of *S. tuberosa* exhibit lowest cytotoxicity with CD_{50} value of 5,677.00 µg/ml. Furthermore, both types of HSV were treated with the highest non-toxic concentration of plant extracts to investigate the potential activity against HSV infection. The result on plaque reduction assay showed that *C. roxburghii, E. prostrata, G. glabra, G. pentaphyllum, H. cordata, P. amarus,*

P. indica, R. nasutus, S. leucantha, Sphenodesme sp., *S. tuberosa, T. crispa* and *Z. montanum* could inhibit plaque formation of HSV-1F or HSV-2G more than 70%. From our results, five plant species; *E. prostrata, H. cordata, R. nasutus, S. tuberosa* and *Sphenodesme* sp., which showed strong effective inhibition of standard HSV infection were selected to further evaluate anti-HSV infection on various stages of HSV multiplication cycles.

The result showed that ethanolic extract of *S. tuberosa* and *Sphenodesme* sp. extracts exerted the highest inhibitory effect against HSV-1F and HSV-2G infection with TI values of 41.30±0.25 and 10.83±0.10, respectively when treatment before viral attachment. Furthermore, ethanolic extract of *Sphenodesme* sp. revealed the highest anti-viral activity against HSV-1F and HSV-2G infection with TI values of 44.73±3.78 and 110.59±1.57, respectively when supplement the extract and virus at the same time. Moreover, HSV-1F and HSV-2G infection were inhibited by ethanolic extract of *Sphenodesme* sp. with TI values of 38.85±2.67 and 44.46±0.42, respectively when treatment after viral attachment. HSV-2G was inhibit by ethanolic extract better than HSV-1F when treatment before and during viral attachment except HSV-1F was inhibited better than HSV-2G when treatment after viral attachment.

Furthermore, direct inactivation of HSV-1F and HSV-2G particles by medicinal plant extracts was performed. The result showed that ethanolic extracts of *E. prostrata*, *H. cordata* and *Sphenodesme* sp. were completely inactivated both types of HSV.

The efficacy of plant extracts on viral multiplication cycle was also demonstrated. It was found that amount of HSV-1F and HSV-2G titer was the

highest reduction by 5.34±0.04 and 5.60±0.04 log PFU/ml compared to viral control after treatment with ethanolic extract of *Sphenodesme* sp. at 30 hours.

Inhibition viral DNA synthesis was also performed in this study. It was found that ethanolic extract of *Sphenodesme* sp. showed that highest inhibitory effect on HSV-1F and HSV-2G, which observed by lowest percentage of DNA remaining by 23.19±5.11 and 6.21±2.60, respectively. In addition, the efficacy on inhibition of HSV protein synthesis was evaluated in the presence or absence of medicinal plants. The result revealed that, all extracts could inhibit various viral proteins especially ethanolic extract of *E. prostrata* and *Sphenodesme* sp. completely inhibited both types of HSV protein synthesis. It was also notice that ethanolic extract of all plant extracts revealed anti-HSV activity more than aqueous extract.

This present study suggested that HSV-1F and HSV-2G infectivity on Vero cells was inhibited when treatment with medicinal plant extracts by directly inactivation of the virus particle and interfering or blocking the viral adsorption and viral entry across the cell membrane. Viral DNA replication and viral protein synthesis also inhibited after treatment with medicinal plant extracts.

Therefore, the ethanolic extract obtained from *E. prostrata* and *Sphenodesme* sp. showed the strongest anti-HSV activity on various stage of multiplication cycle of HSV infection. These extracts were selected to investigate their bioactive compounds that affected HSV infection. It was found that HSV-1F and HSV-2G were sensitive to K101 fraction isolated from *E. prostrata*, which was inhibited by 47.80±4.86 and 52.62±1.95%, respectively, while P107-03 isolated from *Sphenodesme* sp. showed the highest inhibition on both types of HSV by 100%.

Determination of phytochemical groups was also performed. Crude ethanolic extract of *E. prostata* and K101 fraction composed of tannin, flavonoid and phenolic compound, whereas crude ethanolic extract of *Sphenodesme* sp. and P107 fraction showed the presence of tannin and phenolic compound. Moreover, GC-MS investigation showed that, the major constituents in ethanolic extract of *Sphenodesme* sp. composed of 1,2-benzenediol, methoxy phenol, isoeugenol, phenyl propanol, propanamide, lidocaine and ester of palmitic acid.

Ethanolic extract of *Sphenodesme* sp. revealed the highest efficacy against HSV infection. Thus, gel contained ethanolic extract of *Sphenodesme* sp. at concentration of 4000 µg/ml was developed. Stability test of herbal gel product was also demonstrated in this study. The result showed that, the herbal gel was changed their physical properties when storage at 4, 25 and 45ºC for 7 months. However, the herbal gel also retained strong anti-HSV activity. Moreover, the herbal gel was also showed strong efficacy to inhibit HSV infection after heating-cooling at 4ºC for 48 hours and changed to 45ºC for 6 cycles. Furthermore, skin irritation test was carried out on healthy human volunteer. The result confirmed that herbal gel did not generate any irritation on applied skin area.

This herbal gel containing ethanolic extract of *Sphenodesme* sp. demonstrated strong antiviral activity against HSV infection and it also did not irritate to skin. Thus, this obtained result indicated the potential of this herbal gel for development as an alternative therapeutic anti-herpetic agent. However, further study should be confirmed their anti-viral activity *in vivo*.

In summary, these finding supported the application of traditional medicine as a valuable source for development of potential new antiviral agent candidates that are inexpensive and safe as new therapeutic antiviral agents.