CHAPTER V

DISCUSSION

These three plant extracts exhibited anti-HSV activity at different magnitudes of potency. *I maxima* showed the smallest EC_{50} value. The most active extracts of these three medicinal plants were F4/*G. pentaphylla*, F3/ *I. maxima* and F4/*W. edulis* with the EC_{50} against HSV-1 (HSV-2) of 11.36 ±+0.51(9.66±+0.49), 9.14 ±0.49(10.11±0.21) and 15.05±0.46 (18.87± 0.86) µg/ml, respectively. The results of antiviral activity against HSV of extracts from *G. pentaphylla, I. maxima* and *W. edulis* showed that in prophylactic assay, Cells were pre-treated one hour before infection, these extracts were less active in reducing the percent of plaque forming compared to the inactivation assay. The same results were obtained in plaque reduction assay or post-treatment activity, after virus had invaded into cells, the three plant extracts were less active in inhibiting virus replication in the cells . The extracts inhibited virus replication at the higher concentration than that of inactivation assay but were less than the corresponding cytotoxic concentration.. In contrast, inactivation of all three plant extracts could reduce some number of plaque forming but less than activity of ACV. It was suggested that medicinal plant extracts have antiviral activity when virus was directly exposed to the extracts. Results of inactivation activity indicated virus was interacted by the active compounds from three plant extracts, the reaction of the active compounds and virus is not understood. They may bind to the viral membrane then virus can not entry into the cells or they demolished virus by property of antiviral drugs (Sydiki et al., 1991, Yayawasu et al., 1992).

The selective index for each extract with antiviral activity was calculated to determine which extract had the best activity. The highest selective index of 80.93 against HSV-1 and 95.17 against HSV-2 for F4/*G. pentaphylla* against HSV-2 were observed indicating the extracts with the best activity for each medicinal plant tested F3/ *I.maxima* exhibited lowest EC_{50} compared to other two plant extracts but the lower selective index was observed. The selective index of more than 10 could be acceptable

for the good candidate of plant extracts in further investigation(Abou-karam and Sheir 1990). Active compound and their antiviral properties should be further investigated. This could result in reducing the toxic materials in the crude extracts.

The inhibition effect of the extract against HSV adsorbed on the cell membrane was evaluated to identify the target steps in virus replication for the antiherpes simplex virus activity of the extracts. The most active fraction of each plant, F4/*G. pentaphylla*, F3/ *I. maxima* and F4/ *W. edulis* were selected for preliminary mechanism study of antiviral activity in post binding assay , penetration inhibition assay and virus yield inhibition assay. The effect of these three plant extracts on post binding assay, the magnitude in percent inhibition of plaque forming was concentration dependent(r=0.95-0.99) in both against HSV-1 and HSV-2. It was showed that the extract added to cell cultures after the initial viral binding period at 4 °C, was able to inhibit the infectivity of HSV-1 and HSV-2 stably attached to Vero cells with the maximum of inhibition in plaque forming were 55, 70, and 55% at 1600 µg/ml for F4/ *G. pentaphylla*, F3 / *I. maxima*, and F4/ *W. edulis*, respectively. in penetration inhibition assay, the inhibitory effect was more pronounced when the 1-min treatment period with extract was made 15, 30 min and 60 min after the temperature shift at 37°C. The effect of these plant extracts also was concentration dependent(r=0.88-0.97) as indicated by higher inhibition in plaque forming of virus compared to the lower concentration. The maximum inhibition in plaque forming were exhibited at o min of penetration of HSV-1 and HSV-2 into the cells were 60% at 1600 µg/ml for all 3 plant extracts tested In virus yield inhibition assay, it was found that the percent inhibition of plaque forming was maximum at 400 ug/ml at 72 h incubation. The magnitude of inhibition in virus yield was concentration dependent (r=0.81-0.97) and incubation-time dependent(r=0.87-0.99) for all 3 plant extracts. There were no differences in the effect in virus yield inhibition assay of plant extracts against HSV-1 and HSV-2.

The different steps of HSV entry into cells are targets of choice for topical preparation or microbicides to prevent infection of susceptible cells. The attachment and penetration of HSV in target cells involve a three–step virus-cell interaction (McClain et al., 1994). These three stages correspond to (1) the initial treatment to cell surface heparan sulfate,

which involves gC and gB (and possibly gD) and is resistant to PBS wash, (2) the stable attachment to heparan sulfate or onother unknown component which involves gD (and possibly gH) and is resistant to heparan wash. , and (3) the fusion followed by virus penetration which involves gH, gB, gL and gK (and possibly others) and is resistant to low pH citrate buffer wash. It was reported that sodium lauryl sulfate and n-laurylsarcosine ,anionic surfactants , inhibited infectivities of herpes simplex virus on Vero cells by preventing binding of virus to the cells and and inhibition of the HSV-induced cytopathic effect (Piret et al., 2002).

The inhibition of both viral infectivity and binding to cell surface suggested that these plant extracts could act in a concentration- and time-dependent manner, by partial abrogating the attachment step of virus to target cells. In addition, the plant extracts decreased the rate of penetration of the virus into cells probably by affecting the initial steps involve in the fusion process between the viral envelope and the cell surface..

This study could successfully detect active plant extracts from *G. pentaphylla* (F4), *I. maxima* (F3), and *W. edulis* (F4).against HSV-1(and HSV-2) in inactivation assay at the concentration of 11.36±0.51 (9.66±0.20), 9.14±0.49 (10.11±0.21), and 15.05±0.46 (18.87±0.86) µg/ml, respectively. These extracts should be studied further in purification and chemical characterization of active principles and analysis of their antiviral properties.

The antiviral activity of ACV in all treatments were effective. Inactivation activity was the most active treatment. The mechanism of ACV is specific inhibition of viral DNA polymerase as the chain terminator for their incorporation into the DNA which does not allow further chain elongation (Corey et al., 1983, Mertz et al., 1984, Peacock et al., 1988, Safrin et al., 1991, De Clerque, 1993) ACV, as control, could inhibit binding of virus to cells, virus penetration, and virus yield inhiition assay in some extent at 0.25-4 µg/ml, tested concentration.

The antiviral activities of various medicinal plants have been reported. The extract of *Clinacanthus nutans* has been traditionally used in Thailand for the topical treatment of herpes simplex virus and varicalla zoster virus infections (Sangkiporn et al., 1993, Sangkiporn et al., 1995). The anti-HSV activities in vitro of some Thai medicinal plant extracts, *Aglaia edulis, Centella asiatica, Glyptopetalum sclerocarpum, Maclura cochinchinensis , Mangifera indica and Pachyrrhizus erosus*, have been reported (Saifah et al, 1999, Sotanaphun et al., 1999, Yoosook et al., 2000, Phrutivorapongkul et al. ,2002). .A report by Berghe et al. 1986, Hayashi, K. et al.1996 and Jassim, S.A.A. and Naji, M.A. 2003 indicated constituents of medicinal plants with antiviral properties were obtained from a whole range of substance class: alkaloids, , coumarins, flavonoids, furyl compounds, glycosides, lignans, polyenes, polyphenolics, proteins and peptides, sesquiterpenes, saponin, sulphides, and triterpenes.

Plants were reported to possess activity or used in traditional systems of medicine for prevention and treatment of sexually transmitted diseases (STDs) including herpes simplex viruses, herbal formulations for vaginal application, and topical microbicides from herbal origin (Vermani, and Garg 2002). A concentrated extract of *Melissa officinalis* (lemon balm) is one of the most widely used topical preparations in the treatment and prevention of herpes. Mellissa cream had been reported to interrupt the infection, promote healing of the symptoms, and prevent the recurrence of herpes (Vermani and Garg 2002). Viracea, a proprietary formula of Destiny BioMediX Cooperation, is a topical microbicide consisting of benzalkonium chloride and phytochemicals derived from *Echinacea purpurea.* Viracea has been reported to possess antiviral activity against acyclovir resistant as well as susceptible strains of HSV-1 and HSV-2 (Thompson, 1998). The sodium lauryl sulfates and lauroylsarcosine were used in topical vaginal formulations to prevent the transmission of HSV, HIV-1 and possible other pathogens causing STDs (Piret et al., 2002.)

There was no report in antiviral activity of these 3 medicinal plants. So this investigation successfully showed the anti-herpes simplex virus activity which could be applied further for antiviral drug development. Amides with antifungal activity and alkaloids with antitumor activity were isolated fom *G. pentaphylla* (Greger et al.1993

and Quader et al, 1999). Mosquito larvicidal potential was reported for *G. pentaphylla* (Latba, C and Joseph, A 1999) This study supports the contention that traditional medicine remains a valuable resource in the potential discovery of natural product pharmaceuticals.