CHAPTER V

DISCUSSION

An effective antiviral agent should be non-toxic to the cell culture at the antiviral concentration. Therefore, cytotoxicity test of crude extracts was primarily investigated. The concentrations of all crude extracts and ACV used in determining antiviral activity were lower than their CD_{50} and the incubated cultures did not show the CPE.

Acyclovir-triphosphate, an active form of acyclovir, has significantly high affinity to viral DNA polymerase than to intracellular α-DNA polymerase. In addition, acyclovir is a single purify compound. For these reasons, ACV exhibited high CD_{50} (141.59 μg/ml) and much lower ED_{50} (0.48-0.72 μg/ml) resulting in very high SI (196.65-294.98 μg/ml).

The exploration of Thai medicinal plants claimed to possess anti-HSV activities and/or recommended for uses in primary health care showed that some medicinal plants exhibited the activities while many plant extracts could inactivate HSV by the action similar to antiseptics (Chutinan Kantasuk, 1992). However, the criterion for selection of anti-HSV agents should be based on the inhibition at any of the replication steps of the viruses. Since HSV, in particular, can infect neighboring cells directly, i.e. they do not have to be released primarily from the previously infected cells, so anti-HSV agents of choice should be those which are not only inactivate directly the infectious viruses but also, and particularly, inhibit viral multiplication within the infected cells. However, most of the extracts in this study exhibited antiviral activity when they were directly exposed to the virus in the inactivation treatment. Several active and toxic compounds may be included in several crude extracts resulting in interfering action of anti-HSV in the pre- and post-treatments. Some compounds may interrupt an active compound to penetrate cells. In addition, a small amount of the active actively anti-HSV agent probably is in the mixture extract. Purification of these natural products would be elucidating these concerns.

The activity of *B. lupulina* extract was much higher than *C. nutans* extract in this study. The result was concordant with those reported by other investigators (Yoosook et al., 1999; Jayavasu et al., 1992). Yoosook et al. (1999) reported that the *B. lupulina* extract exhibited antiviral activity against all five HSV-2 isolates (ED_{50} were 442.1-987.7 μg/ml) but not the standard strain [HSV-2 (G)] while that of *C. nutans* did not show any activity against these viruses as determined by plaque inhibition assay. When the activities were verified by yield reduction assay, anti-HSV-2 activities of *B. lupulina* extract were observed against HSV-2 (G) as well. However, Jayavasu et al. (1991) and Chutinan Kuntasuk (1992) reported that the activity of *C. nutans* extract was much higher than *B. lupulina* extract. Chutinan Kuntasuk (1992) reported that the ED_{50} of *C. nutans* water extract were 160.43 and 162.46 μg/ml against HSV-1 and HSV-2, respectively, as determined by plaque reduction assay (inactivation). While that of *B. lupulina* water and alcohol extracts were more than 500 μg/ml against both type of HSV. In this study, ED_{50} of both plant extracts were lower. ED_{50} of *B. lupulina* active extract were ranging from 0.02 to 200.46 μg/ml and ED_{50} of *C. nutans* active extract were ranging from 28.10 to 202.72 μg/ml.

Clinacanthus siamensis, *Andrographis paniculata* and *Thunbergia laurifolia* are members of the same plant family of *B. lupulina* and *C. nutans* (Acanthaceae). Crude extracts of these plants showed anti-HSV-2 activity. Anti-HSV-1 activity of *Thunbergia laurifolia* water extract previously reported by Kongyingyoes, et al. (1990).

Interestingly, F3 extract of *Nephelium lappaceum* showed good results in all the three treatments. If this is any actively anti-HSV compound in pericarps of *N. lappaceum*, it would be useful to develop a natural medicine from inexpensive fruits. However, further study should be confirm this result. Nawawi, et al. (1999) indicated that the pericarp of *N. lappaceum* was a candidate for therapeutic application for anti-HSV-1 (EC_{50} = 62 μg/ml for water extract and 70 μg/ml for methanol extract). Regarding to the chemical constituents of this plant, it has not been reported hithero, except for that of the same genus (*Naphelium*) which contains large amounts of polyphenol and other fatty acids. The essential oils (eugenol derivatives) from *M. Fragrance* (Hattori et al., 1995)

and saturated fatty alcohol (n-docosanol) were reported to be active against HSV-1. *N. lappaceum* possessing potent activity *in vivo* also contained essential oils and fatty acids.

Grape seed (*Vitis vinifera*) has not been reported to show antiviral activity, except for that of grape juice (Konowalchuk and Speirs, 1976) and leaves of grape (Girre et al., 1990.). Girre et al. (1990) repoerted that the lowest concentration of the cold aqueous extract of freeze-powdered leaves for inhibition of the cytopathogenic effect of HSV-1 was 50 mg/ml. And Konowalchuk and Speirs (1976) found that 1,000-fold reduction in poliovirus infectivity occurred after incubation with grape juice, pH 7.0, for 24 h at 4^{0}C. *Cissus quadrangularis* is in the same family with *Vitis vinifera*. Selvaraj and Narayanasamy (1991) reported that the stem extract of *Cissus quadangularis* were inhibited rice tungro virus infection.

Many investigators reported anti-hepatitis B virus activity of *Phyllanthus amarus* (Venkateswaran, et al., 1987a, 1987b; Thyagarajan, et al., 1988; Brook, 1988; Blumberg, et al., 1989; Blumberg, et al., 1990a; Blumberg, et al., 1990b; Mehrotra, et al., 1990; Venkateswaran, et al., 1990; Mehrotra, et al., 1991; Munshi, et al., 1993; Unander, et al. 1995; Wang, et al., 1995; Lee, et al., 1996; Ott, et al., 1997) but not anti-HSV-2. *Bridelia ovata* is in the same family (Euphorbiaceae) with *Phyllanthus amarus*. This plant also showed anti-HSV-2 activity. It has not been reported any anti-HSV activity of *Orthosiphon aristatus* but this plant exhibited anti-HSV-2 acitivity in this study.

In addition, *Costus speciosus* had been reported some antiviral activities but not anti-HSV activity (นันทวัน บุณยะประภัศรและอรนุช โชคชัยเจริญพร, 2539).

It had been reported antiviral activity of *Citrus reticulata* (Yu and Xu, 1989; Song and Kim, 1989) but not anti-HSV activity.

Almost fractions of *Cocos nucifera* extracts had not showed anti-HSV-2 activity. However, Narayanasamy and Ramiah (1983) reported that coconut extract gave the best result against plant virus.

Several investigators reported that *Momordica charantia* showed anti-HIV activity (Lee-Huang et al., 1990; Leung et al.,1997; Zheng et al., 1999; Putnam and Tainer, 2000; Jiratchariyakul et al.,2001).

Glatthaar-Saalmuller, et al. (2001) and Vogler, et al. (1999) reported anti-HSV-1 activity of *Eleutherococcus senticosus* and anti-HSV-2 activity of *Panax ginseng*, respectively. These plants are in same family (Araliaceae) with *Schefflera leucantha*. But *S. leucantha* had not been reported anti-HSV activity.

This study supported that several medicinal plants remained a valuable resource for developing a natural medicine. Due to the emergence of drug resistant strains of HSV, a natural product may provide a new, effective pharmaceutical in treatment of HSV infection.