CHAPTER VI

DISCUSSION

HSV-2 isolation

Result of HSV-2 isolates cultured positive in this study was 31.9%, and was similar to the result from Punnarugsa et al. (89). It was studied at Bangrak Hospital in 118 patients with suspected HSV infection and found 41.53% positive for HSV isolation. The decreased percent of HSV isolation in our study may be related to the stage of lesions which the specimens were collected. In our study there were less vesicle lesion specimens (21/119) than the study from Punnarugsa et al. (38/118)) and some specimens were transported from Chonburi to cell culture laboratory in Bangkok.

The significant difference between HSV isolation in genital herpes appearance can be explained by the studies from Gonik et al. (91). They indicated the vesicle lesion specimens gave highest prevalence of HSV isolation because these lesions had large quantities of viral particles.

For the prior of history of genital herpes studies from Chang et al. (92) pointed a frequency of recurrence as high as 60% and HSV-2 infection appears to recur more frequently than does HSV-1 infection (3,6,27,93). Almost of the patients attending at sexually transmitted diseases (STD) clinics were recurrently infected. Many times of frequency of recurrence gave the more period of viral excretion that may persist for 3 weeks.

High prevalence of HSV isolation from patients with HIV infection in our study indicated that they are at increased risk for severe HSV infection, at greatest risk of recurrence and long period for cure. These patients may develop progressive disease (94). Then, there are large quantities of viral particles in the lesion.

There were no statistical significant difference of HSV isolation between place of specimen collection, sex, age and prior history of antiviral drug uptake in our study. Because Bangkok and Chonburi are the same big metropolis which there are many people, sex shops and prostitutes. The prevalence of HSV isolation in Bangkok was 35.44% and Chonburi was 25% . Percent of HSV isolation from Chonburi less than from Bangkok, it may be caused by time of specimen transportation. For sex, it was significant difference for HSV isolation, because patients attending STD clinics are the risk group to infect by HSV. Studies by Rawls et al. (95, 96) reported infected women had have higher rates of infection than men, particularly prostitutes and others with multiple sex partners. They estimated that the risk of susceptible females for contracting HSV from infected males is 80% after single contact (96,97). So, we can culture frequently positive for HSV isolation both male and female in STD clinics. In this study the age of patients was 14-62 years, it is the age of sexual activity. Then, viral isolation had no significance in the groups of age.

The prior history of antiviral drug uptake data was suggested by clinicians that the patients obtained insufficient dose of drugs because they have to buy the expensive drugs by themselves. Thus, virus was not fully eliminated and the symptoms still appeared when they visited the doctors in STD clinics.

So, the frequency of HSV isolation from the clinical specimens depend on many factors such as lesion appearance, technique of collection, specimen transportation, frequency of recurrence and HIV infection.

The development of ELISA technique for HSV-2 identification

ELISA technique for HSV-2 identification in this study was modified from the studies by Punnarugsa et al. (89) and Gerand et al. (90). The optimal concentration of anti-HSV-2 coated plate, was 1: 4000 which was the economic titer for using from other studies (89, 90). The appropriate viral concentration and conjugate were $1x10^4$ PFU/ml and 1: 200 (Figure 13,14). Rabbit anti-HSV-2 serum dilution 1:4000, conjugate dilution

1:200 and the viral concentration $1x10^4$ PFU/ml were chosen and performed in this study. The successful development of this method, 38 HSV isolates were identified as HSV-2. This result was similar to the study of Puttawatana (97) in laboratory diagnosis of genital herpes at Siriraj Hospital, it was found 97% was HSV-2 and 3% was HSV-1 (97). All of 38 HSV-2 isolates were used for clinical HSV-2 represents to study antiviral activity of ACV and medicinal plant extracts in this study.

Antiviral activity of acyclovir against HSV strain 186

The treatment which gave the least ED_{50} was used to test antiviral activity against HSV-2 strain 186; the laboratory strain which was obtained from the penile lesion. Table 8 showed the lowest ED_{50} of ACV in inactivation treatment. There are significant difference between treatments because pre-treatment and inactivation gave significantly different ED_{50} of ACV from post-treatment (Table 9). It indicated that in post-treatment; the study of antiviral activity of growth inhibition of viral replication, ACV had decreased antiviral activity or could not inhibit viral replication after virus had invaded into cells (19, 74, 79, 80).

Antiviral activity of acyclovir against HSV-2 isolates

Results of antiviral activity of ACV against 38 HSV-2 isolates by inactivation gave range of ED_{50} 0.38 - 0.87 μg/ml and showed the mean ± SD of 0.585 ± 0.1 μg/ml . It is similar to the report by Matungkasombat et al.(99) that studied in patients with herpes infection, the ED_{50} of ACV was 0.10 - 0.68 μg/ml. By the studies from Safrine et al.(100) indicated acyclovir resistance as ED_{50} of ≥ 2 μg/ml, their studies found 60% resistance in the immunocompromised patients. So, there are no ACV resistant strain in our study. This might be explained that: one, the studies from Safrine et al.(100) was done in the immunocompromised patients but in our study was done in STD patients. The second, between June 1997 though February 1998, there are no ACV resistant strain in HSV infected patients attending STD clinics at Bangrak Hospital and Venereal Disease Center 3 Chonburi.

However, as the clinical problem of resistance to antiviral agents becomes more frequent (7-10,73), clinicians may need to rely more often on the results of susceptibility testing to guide therapy (100).

Antiviral activity of medicinal plant extracts against HSV-2 strain 186 and HSV-2 isolates

Table 12 showed the 20 from 35 fraction - extracts gave active activity (ED_{50} were less than cytotoxic concentration). The propensity of active extracts were showed in methanol fraction (F1), chloroform fraction (F2), hexane fraction (F4) and aqueous - methanol fraction (F5) by the scheme of extraction (scheme 1).

The lowest ED_{50} (μg/ml) of antiviral activity against HSV-2 strain 186 of *C. odollam, C. excavata, C. amboinicus, P. nodiflora* and *T. peruviana* showed in Table 12, there were 4.25 (F5), 9.25 (F2), 3.25 (F2), 16.25 (F4) and 3.25 (F2), respectively. While the lowest ED_{50} (μg/ml) of *C. odollam, C. excavata, C. amboinicus, P. nodiflora* and *T. peruviana* against HSV-2 isolates showed in Table 13, there were 4.99 ± 0.91 (F5), 8.97 ± 0.76 (F2), 3.57 ± 0.77 (F2), 20.90 ± 3.14 (F4) and 2.13 ± 5.0 (F2), respectively. These extracts are the interesting fractions of each plant for further study.

Results of comparison of antiviral activity of medicinal plant extracts against HSV-2 isolates in Table 14 and 15 showed significantly difference in ED_{50} both between groups of fraction in the same plant and between groups of plants in the same fraction. For the same plant, for example, *C. odollam*, there were 3 different subsets for ED_{50} (Table 14) and in the same fraction extract but different plant, including, Fraction 2, there were 5 subsets difference for ED_{50} by statistical analysis (Table 15). The reason of the difference may relate to extraction , the active compounds may be dispersed in various fractions in each plant.

Antiviral activity of inhibiting viral entry into cell, growth inhibition of viral replication in the cell and direct viral demolishment of medicinal plant extracts against HSV-2 strain 186 and HSV-2 isolates.

Antiviral activity methods modified from the studies of Jayawasu et al. (98) was used to study the medicinal plant extracts effects to the stage of viral infection and direct viral demolishment.

Results of antiviral HSV-2 activity of ACV and F1 extracts from *C. odollam, C. excavata, C. amboinicus, P. nodiflora* and *T. peruviana* by plaque reduction assay in pre-treatment, post-treatment and inactivation for prolong exposure of extracts and ACV up to 3 hours (Figure 15) showed pre-treatment activity could not reduce the percent of plaque forming, it indicated medicinal plants extracts can not inhibit virus invading into cells. HSV-2 can pass the mechanism of adsorption and penetration into pretreated cells. The same results were obtained in post-treatment activity, after virus had invaded into cells the 5 plant extracts could not inhibit viral replication, viral DNA synthesis and protein synthesis in the cells (98). In contrast, inactivation activity of all 5 plant extracts can reduce some number of plaque forming but less than activity of ACV. It point that medicinal plant extracts have antiviral activity when virus was directly exposed to medicinal plant extracts. In the study when the more inactivated times were performed the more percent decrease in plaque forming. Results of inactivation activity indicated virus was interacted by the active compounds from 5 medicinal plant extracts , the reaction of the active compounds and virus is not understood. They may bind to viral membrane then virus can not entry into the cells or they demolished virus by property of antiviral drugs (98,102).

The antiviral activity of ACV in all treatments were effective. Inactivation activity (T3 in Figure 15) 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 (72-79).

This study could successfully detect active plant extracts from *C. odollam*, *C. excavata*, *C. amboinicus*, *P. nodiflora* and *T. peruviana* against HSV-2 at the concentration of 2.13 µg/ml (F2 of *C. amboinicus* and *T. peruviana*) to 35.0 µg/ml (F2 of *P. nodiflora*). Although the ED_{50} of extracts from all 5 plants is higher than that of ACV but ED_{50} of some extracts in this study are higher than that of pure aloe emodin from *Aloe barbadensis* leaves (7.2 µg/ml) studied by Sydiskis et al. (102). Thus, these extracts should be studied further in purification and chemical characterization of active principles and analysis of their antiviral properties.

A report by Berghe et al. (103) indicated constituents of medicinal plants with antiviral properties were obtained from a whole range of substance class: alkaloids, lignans, phenols quinones, flavanoid, phenilic glycosides, sesquiterpenes, triterpene saponin etc. Once identification has been achieved, the selectivity and toxicity of these compounds must be investigated before a successful candidate is found for application as a therapeutic agent.

This study supports the contention that traditional medicine remains a valuable resource in the potential discovery of natural product pharmaceuticals. New strategies are being used to identify plant sources of medicinal substances. This work is aided by realization that societies used locally grown plants as sources of medicine without contact with ' western medicine' (104). Due to the emergence of drug resistant strains of HSV strains, ethnobotany may provide new, effective pharmaceutical alternatives to existing drugs Thai medicinal plants may be important in the development of novel drug in treatment of HSV infection.