

## CHAPTER 4

### CONCLUSIONS

The investigation of *Smilax corbularia* Kunth was performed based on the use of Thai traditional doctors. It has been reported that crude ethanolic extract of the rhizome of *Smilax corbularia* demonstrated antioxidant and anti HIV-1 integrase activities. Thus, the main objectives of this study were to investigate the antioxidant and anti HIV-1 integrase activities as well as identify the pure compounds from the most active fraction.

The antioxidant studies were performed by DPPH assay which is a total chemical antioxidant screening assay. The ethanolic extract exhibited the  $EC_{50}$  value of  $4.1 \pm 0.2 \mu\text{g/ml}$ . The highest antioxidant activity was found in  $\text{CHCl}_3$ : MeOH (1:1) supernate with the  $EC_{50}$  of  $2.1 \pm 1.0 \mu\text{g/ml}$ , which is higher than that of BHT ( $EC_{50} = 11.2 \pm 2.4 \mu\text{g/ml}$ ). In addition, the  $\text{CHCl}_3$ : MeOH (1:1) precipitate and MeOH fractions showed comparable antioxidant activity to BHT with the  $EC_{50}$  of  $11.1 \pm 0.9$  and  $8.9 \pm 0.1 \mu\text{g/ml}$ , respectively. The similar results were also found in the lipid peroxidation of liposome assay where  $\text{CHCl}_3$ : MeOH (1:1) supernate showed the lowest  $EC_{50}$  of  $1.1 \pm 0.1 \mu\text{g/ml}$ . It is indicated from this result that the active components in  $\text{CHCl}_3$ : MeOH (1:1) supernate may have suitable polarity for biological membrane which is mimic to the liposome membrane.

The anti HIV-1 integrase activity were tested by multiplate integration assay. The % inhibition value of the ethanolic extract was  $99.4 \pm 0.4 \%$ . The  $\text{CHCl}_3$ : MeOH (1:1) supernate fraction exhibited the highest anti HIV-1 integrase activity in this test with the % inhibition value of  $99.8 \pm 0.4$ , followed by the  $\text{CHCl}_3$ : MeOH (1:1) precipitate with the % inhibition values of  $99.4 \pm 0.1$  and MeOH fractions possessed their % inhibition value of  $91.9 \pm 1.1$ . Interestingly, crude ethanolic extract and three fractions of ethanolic extract possessed high anti HIV-1 integrase activity with their values of inhibition more than 90 %. The hexane, hexane:  $\text{CHCl}_3$  (1:1) and  $\text{CHCl}_3$  fractions had the lowest anti HIV-1 integrase activity in this test with the % inhibition values of  $12.8 \pm 1.5$ ,  $-2.0 \pm 0.2$  and  $-14.1 \pm 0.8$ , respectively. Three fractions of ethanolic extract ( $\text{CHCl}_3$ : MeOH (1:1) S,  $\text{CHCl}_3$ : MeOH (1:1) and P MeOH) showed high

antioxidant and anti HIV-1 integrase activities. Therefore, the supernatant of (CHCl<sub>3</sub>: MeOH) fraction which had the highest percentage of yield was separated by column chromatography.

Five compounds ( $\beta$ -sitosterol,  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside, quercetin, astilbin and engeletin) were isolated from the CHCl<sub>3</sub>:MeOH (1:1) supernate fraction which possessed the most active antioxidant and anti HIV-1 integrase activities. They were tested with two antioxidant assay and anti HIV-1 integrase activities. It was found that quercetin showed the highest antioxidant activity for DPPH and lipid peroxidation assay ( $EC_{50} = 0.6 \pm 0.1$  and  $0.3 \pm 0.1 \mu\text{g/ml}$ , respectively), followed by astilbin and engeletin but less effect than quercetin. This result related with the previous report which found that quercetin exhibited high antioxidant activity (Rao *et al.*, 2007). For  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside and  $\beta$ -sitosterol showed less antioxidant activity on both assay ( $EC_{50} > 100 \mu\text{g/ml}$ ) which showed the same result as previous report (Itharat *et al.*, 2007). Quercetin also possessed the most potent inhibitory activity against HIV-1 IN with an  $IC_{50}$  value of  $8.9 \pm 1.2 \mu\text{M}$  which related with the previous report (Tewtrakul *et al.*, 2001), followed by astilbin,  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside,  $\beta$ -sitosterol and engeletin ( $IC_{50} = 50.3, 80.5, 80.8$  and  $174.3 \mu\text{M}$ , respectively).

The results were concluded that the extract of *Smilax corbularia* Kunth possessed high anti HIV-1 integrase and antioxidant activities. The compounds which are responsible for these activities are quercetin and astilbin. This is related with previous data where quercein and astilbin possessed anti HIV-1 integrase activity (Tewtrakul *et al.*, 2001). From our data, *Smilax corbularia* can be used as antioxidant and anti-HIV-1 integrase in health promotion products. In addition, its active compounds, quercetin and astilbin can be use as markers for the quality control for antioxidant and HIV-1 integrase activity.