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 ± 0.2 µg/ml. The highest antioxidant activity was found in CHCl$_3$: MeOH (1:1) supernate with the EC$_{50}$ of 2.1 ± 1.0 µg/ml, which is higher than that of BHT (EC$_{50}$ = 11.2 ± 2.4 µg/ml). In addition, the CHCl$_3$: MeOH (1:1) precipitate and MeOH fractions showed comparable antioxidant activity to BHT with the EC$_{50}$ of 11.1 ± 0.9 and 8.9 ± 0.1 µg/ml, respectively. The similar results were also found in the lipid peroxidation of liposome assay where CHCl$_3$: MeOH (1:1) supernate showed the lowest EC$_{50}$ of 1.1 ± 0.1 µg/ml. It is indicated from this result that the active components in 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 ± 0.4 %. The 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 ± 0.4, followed by the CHCl$_3$: MeOH (1:1) precipitate with the % inhibition values of 99.4 ± 0.1 and MeOH fractions possessed their % inhibition value of 91.9 ± 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: CHCl$_3$ (1:1) and CHCl$_3$ fractions had the lowest anti HIV-1 integrase activity in this test with the % inhibition values of 12.8 ± 1.5, -2.0 ± 0.2 and -14.1 ± 0.8, respectively. Three fractions of ethanolic extract (CHCl$_3$: MeOH (1:1) S, CHCl$_3$: MeOH (1:1) and P MeOH) showed high


antioxidant and anti HIV-1 integrase activities. Therefore, the supernatant of (CHCl₃: MeOH) fraction which had the highest percentage of yield was separated by column chromatography.

Five compounds (β-sitosterol, β-sitosterol-3-O-β-D-glucopyranoside, quercetin, astilbin and engeletin) were isolated from the CHCl₃: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 (EC₅₀ = 0.6 ± 0.1 and 0.3 ± 0.1 µg/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 β-sitosterol-3-O-β-D-glucopyranoside and β-sitosterol showed less antioxidant activity on both assay (EC₅₀ >100 µg/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 IC₅₀ value of 8.9 ± 1.2 µM which related with the previous report (Tewtrakul et al., 2001), followed by astilbin, β-sitosterol-3-O-β-D-glucopyranoside, β-sitosterol and engeletin (IC₅₀ = 50.3, 80.5, 80.8 and 174.3 µM, 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 quercetin 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.