CHAPTER VI
CONCLUSION

Phytochemical investigation of *Thunbergia laurifolia* Linn. (Acanthaceae) leaves, which is used in Thai traditional medicine of treatment liver disease, led to the isolation of caffeic acid and rosmarinic acid from the ethanolic extract of leaves. From the anti-hepatotoxic study, rosmarinic acid showed hepatoprotective activity against ethanol-induced toxicity in hepatoma HepG2 cell line. Effects of rosmarinic acid pretreatment against ethanol-induced HepG2 cells damage at 6, 12, and 24 h were investigated. Rosmarinic acid effectively protected the damage induced by ethanol at 6 and 12 h. The effect of rosmarinic acid post-treatment on ethanol-induced cells damage was clarified. Rosmarinic acid also increased %cell viability after treated with ethanol for 12 h. These results suggested that rosmarinic acid possessed anti-hepatotoxic activity on ethanol-induced toxicity in hepatoma HepG2 cell line. The anti-hepatotoxic study of rosmarinic acid, a potent anti-hepatotoxic substance contained in *T. laurifolia* leaves, may be useful for the treatment and prevention of the toxic effect of ethanol.

The chemical study on the whole plant of another Thai medicine plant for treatment liver disease, *Phyllanthus amarus* Schum.&Thonn. (Euphorbiaceae), led to the isolation and purification of a bioactive lignan compound, phyllanthin from hexane and ethyl acetate crude extracts. HepG2 cell line was selected as the model to investigate the anti-hepatotoxic effect of phyllanthin on ethanol induced toxicity. After treated with ethanol at 6, 12, and 24 h, %cell viability was decreased. In hepatoprotective study, HepG2 cells were pretreated with phyllanthin for 24 h. After 24 h, HepG2 cells were then treated with ethanol for various time periods (6, 12, and 24 h). Phyllanthin was showed potent protective effect after incubation with ethanol for 12 h. In addition, the effect of phyllanthin post-treatment on ethanol-induced toxicity was clarified. In the post-treatment with phyllanthin 24 h after treated with ethanol 12 h, phyllanthin increased %cell viability when compared to effect of ethanol alone. In this study, the determination of phyllanthin, anti-hepatotoxic substance, might be usefully applied for quality control of *P. amarus* plant materials and its commercial herbal drugs.

Phyllanthin is a bioactive compound in the whole plant *P. amarus*, and reports indicate that it exhibits anti-hepatotoxic activity. The phyllanthin contents in many *P. amarus* commercial herbal drugs are unknown. An analysis method is necessary to detect and quantitate phyllanthin in plants and commercial products to ensure their pharmacological activities. We developed a rapid TLC-image analysis method for simple quantitation of bioactive phyllanthin in *P. amarus* plant materials from different locations in Thailand that can be applied to determine phyllanthin contents in commercial herbal drugs which are claimed to include *P. amarus*. The phyllanthin contents in the plant materials and commercial herbal drugs were determined using the TLC-image analysis method, which showed no significant difference from the results obtained using the TLC-densitometric method. These results suggested that the proposed TLC-image analysis method could be used as an alternative method for quantitating phyllanthin in *P. amarus* plants and standardizing phyllanthin in commercial products. The validated TLC-image analysis method was effective for detecting and quantitating phyllanthin in *P. amarus* plants and could be developed to quantitate phyllanthin in *P. amarus* commercial herbal drugs.