CHAPTER V

CONCLUSION

Extraction, isolation and purification active constituents in *Centella asiatica* (CA)

Yellowish white powder of triterpenes glycosides 4.936 g (yield = 1.65%) was extracted from 300 gm of dried plant (which was from 3 kg of fresh plant). Asiaticoside (AS) and madecassoside (MS) were isolated and purified from the CA extract, they yielded 22% and 25% respectively.

A triterpenic acid 1.4 g (yield = 56%) was prepared from alkaline hydrolysis of 2.5 g of triterpene glycoside. Asiatic acid (AA) and madecassic acid (MA0 were obtained 20% and 39% respectively.

High-Performance Liquid Chromatography method (HPLC method)

Due to the presence of impurities in CA crude extract, the deposit of impurities on to the stationary phase may cause column failure or minimize the column efficiency. To avoid this problem, precondition step is considerably necessary to clean up sample by using the solid phase extraction technique before injecting the sample into the HPLC system. Mixture of acetonitrile : water (9 : 1) was used in washing out the impurities from the catridge and mixture of acetonitrile phosphate buffer pH 7.1 (45 : 55) was used to elute the analytes out of the cartridge. The optimize condition was comprised with a Hi-Q sil (C18, 15 x 0.46 cm) as

stationary phase, the mixture of acetonitrile and 10 mM, pH7.1 phosphate buffer (29:71) as mobile phase at the flow rate 1 ml/min and prednisolone as internal standard and detected by photodiode array detector at wavelength 210 nm.

Method validation was studied by adding known amount of pure compounds (MS, AS, MA and AA) to crude extract before analysis. The parameter determined base on the ICH guideline. The accuracy measured as percent recovery was found 100.2 for MS, 100.6 for AS, 99.68 for MA and 100.1 for AA; all of them falled in range of 97.00-103.0%. The intra-day and inter-day precision measured as %RSD were found not more than 2.0. The linearity was determined by injecting the solutions of concentration range from 0.06-0.40 for MS, 0.05-0.33 for AS, 0.008-0.048 for MA and 0.004-0.024 for AA mg/ml. The good linearity was measured as linear regression (r^2) $\geq$ 0.999 with insignificant intercept from origin. The data of HPLC method validation is presented in Table 5.2. The developed HPLC method is suitable for determine all four analytes from the CA plant samples.

Thin-Layer Chromatography method (TLC method)

TLC method combined with densitometer was used to determination of madecassoside and asiaticoside in *Centella asiatica* by using chloroform-methanol-water (30-15-2) as developing solvent. The developed TLC plate was sprayed with 0.2% Anthrone reagent and heated at 110 °C for 10 min. The plate was kept at room temperature for 30 min and detected with densitometer at wavelength of 525 nm..

Method validation was studied with 4 parameters (accuracy, precision, linearity and range). The accuracy presented as percent recovery was found in range

of 95.00-105.0%. The intra-plate precisions measured as %RSD were found less than 3. The linearity was determined by spotting the solutions, that amount was range of 1.08-21.60 mcg for madecassoside and 0.78-15.60 mcg for asiaticoside. The good linearity was measured as linear regression (r^2) ≥ 0.999 with insignificant intercept from origin. The data of TLC analytical method validation was presented in Table 5.3.

Determination of interested compound by HPLC method

Table 5.4 showed percentage content of madecassoside, asiaticsoside, madecassic acid and asiatic acid that were analyzed by HPLC. The interested compounds (MS, AS, MA and AA) were contained in leaves more than 7 to 27 times in stems. The average of percentage content of them in dried plant materials from two gardens were 0.994 (MS), 0.827 (AS), 0.135 (MA) and 0.097 (AA). The maximum content of glycosides (MS and AS) were around middle of year (May – July) and of aglycones (MA and AA) were in February. The minimum contents of glycosides were in February and of aglycones were in January.

The average of percentage content of madecassoside and asiaticoside in extract powder were 41.65 and 38.41. Ratio of madecassoside and asiaticoside (MS/AS) in extract powder was average at 1.10. They were stable not less than 3 months at 50 °C and 75% RH so their intensive shelf life was 2 years.

Determination of interested compound by TLC method

Table 5.5 showed percentage content of madecassoside and asiaticoside that were analyzed by TLC. The average of percentage content of madecassoside and asiaticoside in dried CA plant materials were 0.920 and 0.746. The maximum content of madecassoside and asiaticoside were around middle of year (May – July). The minimum content of them were in February.