CHAPTER V

CONCLUSION

5.1 Extraction, isolation and purification of MS, AS, MA and AA in *Centella asiatica* (L.) Urb. (CA)

Yellowish white powder of triterpenes glycosides 20 g (yield = 1 %w/w) was extracted from 2 kg of dried ground CA, which was contained the mix compounds of madecassoside (MS) 42.9 %w/w and asiaticoside (AS) 38.8 %w/w. The ratio of madecassoside and asiaticoside (MS/AS) in extract powder was average at 1.11.

A 100 mg of the mixture of MS and AS was dissolved and separated from the solution by fractional crystallization using drowning-out technique with the mixture of methanol and acetonitrile as ratio 1: 0.5. AS was separated first and then MS was separated last. After purification by recrystallization, AS and MS were obtained 23.5 mg (%R = 78) and 20 mg (%R = 67), respectively.

A 2.5 g of each separated MS and AS was performed alkaline hydrolysis for transformed the abundant of glycosides to their aglycones. After purification by recrystallization, madecassic acid (MA) and asiatic acid (AA) were obtained 0.82 g (%R = 63.1) and 1.14 g (%R = 89.6), respectively.

According to the results of accelerated stability study, the isolated MS, AS, MA and AA in solid form were remain unchanged under the accelerated condition (45 °C, 75% RH) within 4 months.

5.2 Development of extraction method

Heat reflux extraction, ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) were used to extract the interested compounds from the dried ground of CA. This study revealed that heat reflux extraction was showed the maximum percent contents of MS, AS, MA and AA as followed; 2.61 %w/w, 2.51 %, 0.28 %w/w and 0.15 %w/w, respectively. The maximum percent contents of MS, AS, MA and AA of UAE were showed as followed; 3.34 %w/w, 2.83 %w/w, 0.42 %w/w and 0.14 %w/w, respectively, and the maximum percent contents of MS, AS, MA and AA of MAE were showed as followed; 3.29 %w/w, 2.93 %w/w, 0.43 %w/w and 0.14 %w/w, respectively.

By comparing various extraction methods for MS, AS, MA and AA, ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) were more efficient by other time consuming techniques. UAE and MAE were also required 3 minutes to reach the exhausted extraction. However, the heat reflux extraction technique was required more than 60 minutes to reach the exhausted extraction.

However, the extraction temperature was found to be one of the important factors because of the characteristic of MS and AS, which can be degraded with high temperature during the long times of extraction. When compared with conventional extraction techniques such as heat reflux extraction, and the UAE and MAE system showed extraction efficiency in short time. The UAE can be carried out at lower temperature by controlled the cooling bath and the MAE can be extracted the interested compounds under the boiling point of solvents by using an opened MAE

(FMAE), which can avoid the degradation of thermally unstable compounds with economy in time.

These findings agree with previous studies in that the alternative extraction techniques such as UAE and MAE are powerful techniques; thus can be applied to large scale of samples.

5.3 Determination of MS, AS, MA and AA in various CA samples

The present study has revealed the presence of considerable content variation of active compounds in the 12 CA accessions collected from different parts of Thailand and cultivated at Thailand Institute of Scientific and Technological Research. The percentage contents of MS, AS, MA and AA in various CA samples were showed in Table 4.8. The average of percentage contents of them in dried ground plant of various CA samples were 4.22 (MS), 2.58 (AS), 0.18 (MA) and 0.11 (AA). The maximum contents of MS, AS, MA and AA that obtained from CA samples were 5.48 %w/w (Phitsanulok province), 3.47 %w/w (Rayong province), 0.91 %w/w (Chiang Mai province) and 0.39 %w/w (Trat province), respectively.

Information on chemical constituents of these accessions can now be further used as the criteria for plant varieties selection.

5.4 Determination of MS, AS, MA and AA of CA samples in annually study

Table 4.9 showed percentage contents of MS, AS, MA and AA in the dried ground of CA that collected every 2 month from the commercial crop at Nakhon Pathom province. The maximum contents of MS (1.92 %w/w) and AS (1.55 %w/w)

were obtained from CA, which collected on May. The maximum contents of MA (1.09 %w/w) and AA (0.96 %w/w) were obtained from CA, which collected on May and March, respectively.

This finding agrees with a previous study by Bungon Kongthong **(58)** in that the maximum contents of glycosides (MS and AS) in CA samples, which also collected from Nakhon Pathom province were around middle of year (May-July). However, the variation of aglycones (MA and AA) was also noted.

Table 4.10 showed percentage contents of MS, AS, MA and AA in the dried ground of CA that collected every 2 month from the commercial crop at Ubon Ratchathani province. The maximum contents of MS (1.77 %w/w) and AS (1.76 %w/w) were found in CA, which also collected on March. The maximum contents of MA (0.64 %w/w) and AA (0.47 %w/w) were found in CA, which collected on March and July, respectively.

Table 4.11 showed percentage contents of MS, AS, MA and AA in the dried ground of CA that collected every 2 month from the commercial crop at Nakhon Si Thammarat province. The maximum contents of MS (2.31 %w/w) and AS (1.99 %w/w) were found in CA, which also collected on May. The maximum contents of MA (1.35 %w/w) and AA (1.53 %w/w) were found in CA, which also collected on January.

Information on the chemical content profiles in this study may be utilized as criteria for further used in breeding programs for high biomass of triterpenes in commercial cultivars.

5.5 Determination of MS, AS, MA and AA in CA samples during the cultivation

The contents of MS, AS, MA and AA of CA3 that investigated during its growth period were found the maximum glycoside contents in samples, which collected on 20 days. The maximum glycosides of CA5 and CA6 were found in samples, which collected on 28 days. The statistical analysis of both glycosides revealed that the contents observed on 20 days and 28 days were not significantly difference (95% Confidence Interval, p-value>0.05)

Information obtained can be further used to optimize the collecting time of CA, which was given a high biomass of triterpenes.