CHAPTER 5

Conclusion

This study was conducted to investigate antibacterial and antioxidant activities of twenty two medicinal plant extracts in various tested model. The mode of action of selected plant extracts were investigated in terms of mRNA level and protein expression in different interested target. Additionally, the plant which had high activity was fractionated and elucidated phytochemical constituents. In addition, the rapid detection of methicillin resistant *S. aureus* (MRSA) based on molecular technique was performed. Moreover, the *mecA* sequencing was analyzed for the nucleotide mutation in different clinical MRSA isolates.

Twenty two medicinal plants that related in the use of Thai folklore were determined for the antibacterial activity against pathogenic bacteria including *E. coli* O157: H7, *Ps. aeruginosa*, *S. aureus, S. epidermidis, St. pyogenes, P. acnes* and ten isolates of MRSA. The ethanolic extracts could inhibit the growth of tested bacteria greater than aqueous extracts. Moreover, Gram positive bacteria were inhibited by medicinal plant extracts with high efficacy, while Gram negative bacteria were not susceptible to the medicinal plant extracts. It might be caused by the morphological different between these two groups. The susceptibility testing results showed that the ethanolic extracts of *C. fenestratum* and *S. venosa* gave the highest antibacterial effect, thus two plant extracts were used for further study. After that, *C. fenestratum* and *S. venosa* were extracted with other solvents including methanol and dichloromethane for comparing between extract solvents that gave higher antibacterial activity. Antibacterial activities of methanol and dichloromethane extracts of *C. fenestratum* were higher than ethanolic and aqueous extracts. The methanolic extract of *C. fenestratum* had the highest inhibitory effect. Methanolic, dichloromethane and ethanolic extracts of *S. venosa* could inhibit all tested bacteria while the aqueous extract

could not inhibit *E. coli* O157: H7, *Ps. aeruginosa, St. pyogenes* and MRSA isolate number 66.

Time-killing study of medicinal plants extracts revealed that the ethanolic extract of *H. cordata, P. palatiferum and S. venosa* could inhibit growth *E. coli* O157: H7 by 100% during 4 - 6 hours while the ethanolic extract of *P. palatiferum* and *S. venosa* could inhibit growth of *Ps. aeruginosa* by 100% after 6 hours. Moreover, the ethanolic extracts of *C. fenestratum , P. amarus, S. alata* and *S. venosa* completely inhibited the growth of *S. aureus, S. epidermidis, St. pyogenes* and *P. acnes* during 6-8, 4-10, 4-8 and 12-24 hours, respectively. Furthermore, these plant extracts also inhibited the growth of MRSA isolate number 64, 72 and 80 after 4-10 hours.

Moreover, the ethanolic extract of *C. fenestratum* and *S. venosa* were selected to study cell morphology alteration of *S. aureus, S. epidermidis*, MRSA isolate number 64 and 80 and *E. coli* O157: H7 after treating with plant extracts using scanning electron microscope (SEM). The ethanolic extract of *S. venosa* at 4MIC concentration could affect to the cell morphology of *S. aureus* and *E. coli* O157: H7 by showing membrane bleb or destruction. However, both extracts could not affect cell morphology of *S. epidermidis*, MRSA isolate number 64 and 80.

The mRNA expression of normal strain of *S. aureus* and MRSA isolate 80 were also conducted after treating with *C. fenestratum* and *S. venosa* extracts. Five genes including α-toxin gene (*hla*), methicillin resistance gene (*mecA, mecR1, mecI*) and nucleaseA gene (*nucA*) was studied by quantitative real time PCR (qPCR). Both *C. fenestratum* and *S. venosa* extracts showed strong decrease in the expression levels of *hla* gene in *S. aureus* by 12.76 and 7.38 fold, respectively. However, *S. venosa* extract significantly decreased *hla* expression level in MRSA by 2.65 fold while *C. fenestratum* extract reduced *hla* expression but the reduction was not statistical significance. For resistant gene expression, the ethanolic extracts of *C. fenestratum* and *S. venosa* induced a significant decrease in *mecR1* mRNA level by 1.97 and 1.79 folds, respectively. On the other hand, both extracts increased *mecI* transcription level but the induction was not statistical significance. However, the result showed that the *C. fenestratum* extract increased *mecA* transcription level by 7.52 fold while *S. venosa* extract did not affect the expression level. In this study, *C. fenestratum* and *S. venosa* extracts affected nuclease

A gene (*nucA*) by significant increase the expression level of 2.21 and 2.40 folds, respectively. In addition, both extracts could significantly increase *nucA* transcription level by 7.57 and 3.59 folds in MRSA. Moreover, the Western blotting results showed that the ethanolic extract of *C. fenestratum* could inhibit the expression of PBP2a protein by 100 % when treating at concentration of 3.13 mg/ml while the ethanolic extract of *S. venosa* at concentration of 0.39 mg/ml inhibited PBP2a expression by 59.62 %. Therefore, it was concluded that both ethanolic extracts of *C. fenestratum* and *S. venosa* inhibited the growth of MRSA by interfering some virulence gene such as α-toxin gene and nuclease A gene. Moreover, both plant extracts also affected to resistance gene and decreased the expression of PBP2a protein. The reduction of PBP2a expression correlated with the alteration of the membrane and resulted in cell dead and lysis.

In addition, the ethanolic extracts of *C. fenestratum* and *S. venosa* were selected to fractionate to obtain partial purified fractions. The result of partition method showed that the chloroform fraction of *C. fenestratum* gave the highest antibacterial activity against all Gram positive bacteria. After fractionation using column chromatography, CF01 fraction had the highest antibacterial activity. For phytochemical screening, alkaloids and phenolics were found as major bioactive compounds in CF01 fraction. From GC/MS result, berberine was a major volatile compound in this plant. For *S. venosa* fractionation, the chloroform fraction also gave the highest antibacterial activity. Alkaloids and phenolics were found as major bioactive compounds in *S. venosa.*

Antioxidant activity was also investigated in this study using various models including ABTS, DPPH, FRAP, total phenolic content and oxidative protein damage protection. From the study suggested that the different methods that determined antioxidant activities were based on the different reaction mechanism that resulting in different results. The ethanolic extract of *S. alata* gave the highest antioxidant activity in ABTS assay with TEAC value of 214.128 mg TE/g extracts while the ethanolic extract of *Hiptage* sp. showed the highest DPPH radical scavenging ability with GAE value of 77.913 mg GAE/ g extract. The aqueous extract of *Stemona* sp. had the highest ferric reducing activity with EC value of 433.900 mg $FeSO_4$/ g extract. Moreover, the

ethanolic extract of *Hipatge* sp. showed the highest total phenolic content with GAE value of 132.454 mg GAE/g extract. However, the correlations of these three models were analyzed using Pearson's correlation coefficients. It was concluded that the total phenolic content had high correlation with other antioxidant testing models. Moreover, ABTS and DPPH models gave strong correlation with each other. By contrast, FRAP method did not correlated with other antioxidant models.

Five aqueous extracts; *D. scandens* , *P. amarus* , *R. nasatus*, *S. alata*, *Hiptage* sp. and five ethanolic extracts; *E. prostrata*, *H. cordata*, *P. amarus*, *P. palatiferum*, *Hiptage* sp. were used to study the oxidative protein damage ability. The result showed that almost plant extracts had the protective activity against oxidative damage protein in dose dependent manner. Aqueous extract of *P. amarus* at 20 mg/ml showed significantly highest percentage of protection against BSA protein damage, which was 87.06 % protection compared to Cu^{2+} and H_2O_2 treated protein, solvent control and glutathione. However, almost plant extracts gave the high ability to protect oxidative protein damage as well as glutathione at the same concentration (10 mg/ml). After that, the plants that showed high antioxidant activity, *E. prostrata* and *Hiptage* sp. were selected to fractionate to obtain bioactive compound. After fractionation by partition method, the *n*-butanol fraction gave the highest antioxidant activity. Moreover, the EP07-07 fraction from column chromatography method also gave the highest antioxidant activity. The phytochemical screening showed that flavonoids and phenolics were found as major bioactive compounds in *E. prostrate* fraction. Fractionation of *Hiptage* sp. demonstrated that the ethyl acetate fraction had the highest antioxidant activity. After fractionation with column chromatography, SP05 fraction gave the highest antioxidant activity. However, after further isolation, it was found that the activity was decreased. For phytochemical screening, tannins and phenolics were found as major bioactive compounds in *Hiptage* sp. fraction. Therefore, the biological activities of the plants observed in this study will be useful for development of the potential plant extracts as new therapeutic agents for protecting and curing diseases, and health supplement products.