

## CHAPTER V

### CONCLUSION

In this study, the sponge *Xestospongia* sp. was collected in every month at Sichang Island, Cholburi Province during August 2005 to July 2006. The crude EtOAc extracts of semi-dried weight sponges were determined by a newly developed high performance liquid chromatography (HPLC) method. The suitable HPLC condition consisted of a RP-18e column (125x4 mm, 5  $\mu$ m) as stationary phase, an isocratic solution of the mixture of methanol/water (7:3) as mobile phase at flow rate 1.0 mL/min, a photodiode array at 270 nm and acenaphthene as the internal standard. The retention times of renieramycin M reference and internal standard acenaphthene are 8.9 and 13.7 min, respectively. Annual variation of renieramycin M quantity (%w/w, semi-dried weight sponge) was significantly different with the mean of  $0.13 \pm 0.02$ . The highest content of renieramycin M in the sponge was found in April (0.20% w/w) and in October (0.20% w/w) and the lowest in January (0.08% w/w) and in July (0.09% w/w). During the collection of the sponge *Xestospongia* sp. samples, seawater sampling was also analyzed. The environmental parameters measured in this study period were salinity, pH, water temperature, chlorophyll, total dissolved solids (TDS) and total suspended solids (TSS). Data on relationships between renieramycin M and environmental parameters were also investigated. The positive relationships were found between renieramycin M annual content and both total dissolved solids, TDS and chlorophyll-a.

Scanning electron microscope (SEM) images of sponge tissues revealed that the sponge *Xestospongia* sp. contained a number of associated bacteria in cocci and bacilli forms (approximately  $<1 \mu$ m in diameter). Choanocytes and archaeocytes were major cell types in this sponge *Xestospongia* sp. in sizes ranging from 2-3  $\mu$ m and 6-10  $\mu$ m, respectively. The sponge *Xestospongia* sp. tissue was fractionated into the single cell type fractions by centrifugation method. HPLC analyses of renieramycin M amounts revealed that choanocytes and archaeocytes were associated with the renieramycins production rather than bacteria.