

## CHAPTER V

### CONCLUSIONS



Six sesquiterpenes; germacrone (**1**), zederone (**2**), dehydrocurdione (**3**), curcumenol (**4**) zedoarondiol (**5**) and isocurcumenol (**6**) were isolated from the hexane extract of *C. aeruginosa* rhizomes. They showed inhibitory activity against the conversion of testosterone to DHT. Of the 6 active components identified, **1** was the most active. Our studies demonstrated that **1** also showed anti-androgenic effect on testosterone-induced growth suppression of human prostate cancer cells (LNCaP) and on the hamster flank gland model. One of the possible mechanisms was inhibition of  $5\alpha$ -reductase activity. No binding activity on androgen receptor of **1** was found. Compound **1** might be potentially useful for the treatment of the androgen-dependent disorders.

Two cell models; human foreskin fibroblast cell (HF) which represented the normal cell and LNCaP cells which represented the cancer cell were used to assess the cytotoxic effect of tested compounds. Compounds **2-4** together with crude extract of *C. aeruginosa* (0.0001–10  $\mu\text{g/mL}$ ) showed no cytotoxic effect on HF cells while compounds **1** and **5** tended to increase cell viability at the concentration of 10 and 1  $\mu\text{g/mL}$ , respectively. In LNCaP cell, *C. aeruginosa* extract and compounds **3-6** showed no cytotoxic effect on LNCaP cell at 0.00001-10  $\mu\text{g/mL}$  except **1** at the highest concentration (10  $\mu\text{g/mL}$  or 45  $\mu\text{M}$ ) decreased cell viability. Interestingly, **2** dose-independently reduced LNCaP cell viability by 20-30%. Such an effect could perhaps be explained by growth inhibition rather than cell killing.

HPLC and GC-FID were developed for quantitative analysis of the anti-androgenic compounds from *C. aeruginosa*. The gradient elution system of  $\text{CH}_3\text{CN}$  and 0.5 mM phosphate buffer pH 3.0 could be applied to determine **1** and **2** in crude extract. Moreover, the simple, rapid, sensitive, accurate and precise GC-FID (system 1 and system 2) for determination of marker compounds (compounds **1**, **2**, **4** and **6**) in crude extract and compound **1** which was studied as pure compound were developed and validated. The proposed method was highly sensitive and gave the good

resolution. It could be used for the routine analysis of marker compounds in *C. aeruginosa* extract for quality control purpose.

The effect of temperature, pH and light on stability of *C. aeruginosa* extract was studied comparing to the pure compound, **1**. The results indicated that the level of **1** in crude extract and as pure compound could maintain and showed the relative content more than 75% (compared to base line) for 14 days. At pH 5.5 which is the common pH for cosmetic products was selected as the pH of the sample solution for the study the effect of temperature and light. The relative concentrations of solid form of **1** in the crude extract and as pure compound were found to be unstable at high temperature and decreased more rapidly than that of **1** in solution form. Our results revealed that **1** was sensitive to temperature. One of the degradation products might be elemenone. Moreover, oxygen might be one of factors causing the degradation of **1**. We found that keeping solid form of **1** in nitrogen atmosphere could retard the degradation process. In contrast, dissolving **1** in both PEG-40 and MeOH in our system could prolong its stability. Our studies demonstrated that **1** was also slightly sensitive to light. The content of **1** in *C. aeruginosa* extract are protected when kept at low temperature and in dark or away from direct expose of light.

The quality control of *C. aeruginosa* extract was done using TLC and GC-FID method on the basis of four-marker compounds isolated, i.e. compounds **1**, **2**, **4** and **6**. The results showed slightly variation of these compounds in different sources or locations of *C. aeruginosa*. The contents of the main anti-androgenic compound, **1**, in all of the hexane and CH<sub>2</sub>Cl<sub>2</sub> extract were ranged from 0.78 to 2.31 %w/w and 0.51 to 2.22 %w/w, respectively while the level of **1** in the dried powder rhizomes from three different sources were ranged from 0.22-0.39 mg/g (dried weight). These methods could be used for evaluating of different samples or batches of *C. aeruginosa* for the further study on anti-androgenic activity.

In conclusion, our studies provide the information of chemical components in *C. aeruginosa* rhizome and their anti-androgenic activity. Compound **1** could be used as a marker of the extracts. The quality controls method using TLC, HPLC and GC were proposed. Both the extract and **1** showed very high potential to be developed for therapeutic application. However, one must concern on the sensitivity of **1** to temperature and light.