CHAPTER 4

Conclusion

From the results, it was found that natural compounds such as flavonoids can inhibit hepatic aromatase activity of Nile tilapia. Microsomes from fish liver were extracted and characterized by 10% and 12% gel SDS-PAGE. The major band of male microsome was revealed at 13.3, 20.9, 29.8, 44.4, 50.7, 58.5 and 110.5 kDa and female microsome was revealed at 19.3 and 67.2 kDa. The different sizes of microsome in male and female tilapia maybe originated from the different shape and morphology of endoplasmic reticulum (ER) fractions as visualized by SEM. Interestingly, it was also found that the yield of the obtained fish microsome was influenced by the age and body weight. Similarly to those using in freshly prepared and lyophilized form, it was found that lyophilized female microsome is proper for long term studying by the reason of to ensure that fish has never been masculinized or exposed to masculinizing agent.

At the optimal condition of AIs screening method by using Dibenzylfluorescene (DBF) in cooperated with fish microsomes at pH 8.0, the effect of flavonoids, hesperidine, morin, quercetin, quercitrin, and rutin, on antiaromatase activity were investigated. It was found that chrysin showed the highest antiaromatase activity with the IC_{50} value of 0.25 mg/mL, approximately 2 times higher than quercetin. The result also indicated the effects of structure activity relationship of the compounds that substitution of OH group to chrysin, such as in quercetin molecule, caused significant decrease in antiaromatase activity. It was also found that among 22 Thai plants extracts, *O. indicum* revealed the highest potential on tilapia microsome inhibiting activity closely to *A. conyzoides* and *C. odorata*. However, the major compound, coumarin, in *A. conyzoides* is moderately toxic to the liver and kidneys and may cause hepatic cancer and lung tumors in animals (Vassallo, 2004; Born, 2003) whereas *C. odorata* was also never been proven for oral route administration. Therefore, *O. indicum* was selected for further studied.

The ethyl acetate fraction of *O. indicum* obtained after maceration was the most active fraction. It possesses cytotoxic against HepG2 and MCF-7, hormone-sensitive cancer cells, within 24 hours. The chromatogram of HPLC indicated that the major compound in ethyl acetate fraction is chrysin. Unfortunately, chrysin is an insoluble flavone. Thus, to achieve more soluble properties, chrysin has to be entrapped into nanoparticles.

Polymeric micelle is one of the powerful nanotechnologies to enhance insoluble drug. Several advantages over other drug delivery systems such as polymeric micelle can compromised hydrophobic drug entrapment into nanosize range, can be designed and optimized for advance properties, and adjustable for drug releasing rate. In this thesis, the HPβCD entrapment has the most excellent properties for entrapment efficiency with cost effectiveness among pluronic F68, pluronic F127, PLGA and bilosome entrapment. The entrapment efficiency of chrysin loaded HPβCD is 100 % as well as pluronic F68 and pluronic F127 but greater than PLGA (nearly 57 %) and bilosome system (nearly 88 %). Moreover, the results from releasing studied in 10mM HEPES (pH 7.6, 7.8 and 8.0) indicated that chrysin loaded HPβCD released chrysin nearly 70 % within 8 hours, but possesses slow releasing in water whereas entrapment by using pluronic F68, pluronic F127, PLGA and bilosome possess releasing rate of chrysin in medium nearly 25, 25, 35 and 35 % within 8 hours, respectively. The results from toxicity testing in zebrafish embryo indicated that the effect of chrysin loaded HPβCD, chrysin loaded pluronic F68 (CS-P68), and chrysin loaded pluronic F127 (CS-P127) were obviously seen particularly at the low dose range of 1-100 ng/mL whereas the effect of chrysin loaded PLGA and chrysin loaded bilosome were not significantly different at concentration of 1-1,000 ng/mL. It was also found that the toxicity of all samples was seen in a dose dependent manner but was not time dependent toxicity. Interestingly, there is obviously no abnormal development achieved in zebrafish embryo treated with chrysin loaded HPβCD.

Therefore, Chrysin loaded HPβCD incorporated with tween 80 was selected for masculinization study in adult female zebrafish. After 60 days of treatment, all fish were terminated and removed gonad for investigating the masculinization effects. The results from histology examination by histological studies indicated that chrysin loaded

nanoparticles possess potential on inhibiting aromatase in gonad and displayed spermatogonia-like gonads in female zebrafish.

Regarding to the cost effectiveness in fish masculinization, chrysin loaded HPβCD is the potent candidate nanoparticle for mass production due to the soluble chrysin properties with the properties of targeting through blood brain barrier. However, more research in mass production and clinical trial in the different economical species of freshwater fish should be investigated.