CHAPTER V
CONCLUSION

This research focused on the microemulsion of the selected biological active essential oils from Thai herbs to use as an active nanocarrier for drug. The most suitable microemulsion formulations were selected as a vehicle for loading of water insoluble drug like clotrimazole. Many factors which might affect the physicochemical properties of the preparations were investigated. Stability and drug release property of the suitable formulations were also studied.

In the step of oil selection, 29 oil samples were of plants commonly grown in the northern part of Thailand. The essential oil samples were obtained by hydrodistillation of the fresh plant while the fixed oil was a commercial product obtained directly from the manufacturer. All oil samples subjected to the biological test of antioxidant and antityrosinase activities. The antioxidant activity of the samples was investigated by using FRAP and ABTS free radical scavenging assays. *O. gratissimum* essential oil showed the highest antioxidant activity when investigated by both methods. Tyrosinase inhibition activity was determined by using the modified dopachrome method which L-DOPA was used as a substrate. The essential oil of *H. cordata* showed the highest activity of 74.86% tyrosinase inhibition followed closely by the oil of *C. citratus*. Comparison with *H. cordata*, the essential oil of *C. citratus* (lemongrass oil) was selected for further study due to the high activity of tyrosinase inhibition similar to *H. cordata* oil, but higher antioxidant activity, higher yield of extracted oil and better characteristics of odor. *S. indicum* oil or sesame oil was also used for further study as a fixed oil model. Physicochemical characteristics of both selected oils such as outer appearance, density, refractive index, solubility and surface tension as well as chemical composition by GC-MS were comparatively investigated. The results indicated that both oils were clear liquids with lower density and higher refractive index than water. The solubility and surface tension results indicated that the oils are moderate polar liquid that complete immiscible with water. The GC-MS study demonstrated that the oil of different plant species is composed of different compounds.

The preliminary study for both oils prior to construct the phase diagram by using Tween 20 and Tween 80 with ethanol in the ratios of 1:1, 1:2, 1:3 and 1:9 indicated different outer appearance of each system. The results from the preliminary study indicated that Tween 20 was the better suitable major surfactant for both oils than Tween 80.

Pseudoternary phase diagram was constructed to study the effect of the oils, surfactants and cosurfactant type, electrolytes and pH on the microemulsion area of lemongrass oil and sesame oils. The phase diagram was constructed by titration method. Four surfactants; Tween 20, Tween 85, Brij 97 and Triton X 114 were comparatively studied paralleled with the study of surfactant and cosurfactant type and ratio. Pseudoternary phase diagrams of lemongrass oil showed that Tween 20 gave the widest microemulsion while that of sesame oil, Tween 85 showed the most suitable. Cosurfactants used in the experiment were moderate carbon chain (C2-C8) of alcohol. The results indicated that among five cosurfactants used, ethanol caused the widest area of microemulsions of both oils. It was also found that the greater the number of carbon in cosurfactant molecule, the less area of microemulsion in the phase diagrams obtained. The result also revealed that the ratio of 2:1 surfactant and ethanol gave the largest area of microemulsion on the phase diagram of lemongrass oil and s sesame oil.

After adding sodium chloride and calcium chloride to the aqueous system, the results revealed that both types of electrolyte had no significant affected on microemulsion area. pH also played a minor effect to the microemulsions of both oils. The results revealed that pH 4.0 - 6.0 did not affect the area of the microemulsions but the microemulsion area was decreased at pH 8.0.

On microemulsion base preparation, lemongrass oil could give the microemulsion from single surfactant and with combination with cosurfactant. However, sesame oil gave the microemulsion only when the formulas contained the mixture of surfactant and cosurfactant. All the developed microemulsion bases of lemongrass oil and sesame oil were o/w microemulsion due to the conductivity of the emulsion system which was greater than 50 μS/cm. The microemulsion of lemongrass oil showed smaller droplet size than that of sesame oil microemulsion. The microemulsion bases of both oils were undertaken for a stability test for 5 months. The results revealed that the microemulsion bases of both oils showed high stability in all period of study. There was no phase separation occurred with all microemulsions of lemongrass oil and sesame oil.

In case of the drug loaded microemulsion, 1% clotrimazole was loaded in to the microemulsion bases of lemongrass oil and sesame oil. The physical appearance of drug loaded microemulsions was similar to microemulsion bases. However, the drug loaded microemulsions showed larger, internal droplet size than those without drug.

In the stability test of microemulsions, all microemulsion bases and clotrimazole loaded microemulsions were kept in various conditions; i.e. 4ºC, room temperature (30ºC), 45ºC for 5 months and heating-cooling cycle condition for 6 cycles. Microemulsions which composed of surfactant and cosurfactant showed better stability of outer appearance as well as the quantity of drug that more than 80% of drug still remained as molecular dispersion in the preparation particularly when kept at4ºC and room temperature.

The drug release property of the developed microemulsions in comparison with Defungal® (the commercial product) was done within 10 hr. The results demonstrated that the microemulsions with smallest droplet size could give the highest drug release. Drug release from sesame oil microemulsions was slower than that of lemongrass oil microemulsions which was due to the bigger droplet size of sesame oil microemulsions.

In conclusion, microemulsions of lemongrass oil and sesame oil were the suitable nano-vehicles for clotrimazole due to their high stability, small size and good appearance. The lemongrass oil microemulsion formulations composed of 10/33/17/40 of oil, Tween 20, ethanol, water and the sesame oil microemulsion composed of 10/40/20/30 of oil, Tween 85, ethanol, water were the best formulations that should be further developed as the drug nanodelivery for clinical use. Both were different in quantity of surfactant systems the sesame oil microemulsion required a higher amount of surfactant than lemongrass oil microemulsion.