CHAPTER 5

CONCLUSION

This research project is focused on development of microemulsion for delivery the TD alkaloidal extract via transdermal route. The results from this study suggested that alkaloids which are the major constituents of TD crude extract possessed as high AChE inhibitory activity as galantamine, an AChEI drug in the market, with the IC_{50} value of 131.17±20.06 ng/mL. Moreover, the most obvious finding to emerge from this study is the higher selectivity of TD alkaloidal extract on AChE inhibition when compared with galantamine.

The fingerprint analyses of TD alkaloidal extract were investigated by TLC and HPLC. The TLC plates sprayed with dragendorff's reagent accompanied with the plates sprayed with Ellman's reagent assists in the understanding of anti-AChE of the alkaloids. The HPLC fingerprint of TD alkaloidal extract showed 4 prominent peaks at the retention time of 8.298, 10.744, 12.178 and 15.642 min. Since the reference material or marker of TD alkaloidal extract is not commercially available, the most prominent peak at 12.178 min was purified for the quantification of the TD alkaloidal extract. The pure alkaloid was revealed to be 3'-*R/S*-hydroxyvoacamine, a 3'-hydroxy derivative of dimeric indole alkaloid which normally occurs as a mixture of the *3'R* and *3'S* isomers.

MWM was undertaken to evaluate the *in vivo* anti-ChE of TD alkaloidal extract. In this study, scopolamine which blocks the cholinergic signalling was used for inducing cognitive deficits in mice. The findings suggest the same potential in

cognitive enhancing properties of transdermal TD alkaloidal extract in PG (1,000 and 500 mg/kg) as oral galantamine (1 mg/kg). Therefore, the subsequent development of microemulsion for delivery the TD alkaloidal extract via transdermal route would be a worthwhile study.

The preformulation study was the following focus in this study. The TD alkaloidal extract is practically insoluble in water but dissolve well in polar solvents such as ethanol and methanol. The calculated log *P* value of TD alkaloidal extract was 3.03±0.07 which was in an acceptable range of drug candidates for the passive transdermal delivery. The evidence of weight losing from TGA suggested the decomposition of TD alkaloidal extract after exposure to high temperature. DSC assists the perception of the weight losing by disclosure a Tg at 48.67°C, an endothermic peak of water evaporated at around 100°C and several exothermic peaks of decompositions after 150.86°C. After that, the stability of TD alkaloidal extract in various storage conditions was investigated and the findings recommend keeping the TD alkaloial extract away from light, oxygen and high temperature. The cytotoxicity test of TD alkaloial extract revealed its safety on PBMC because the IC_{50} against the cell was 6.04±2.35 µg/mL which is 46 times higher than that of AChE.

After the physicochemical properties of TD alkaloidal extract as well as its stability and cytotoxicity were determined, the formulations were then developed. Blank microemulsions were firstly formulated and evaluated before the incorporation of TD alkloidal extract. Essential oils were used as an oil phase because of the advantages of skin penetration enhancement. Essential oil from *C. citratus* stem and

Z. cassumunar rhizome were selected for the formulations in accordance with the commercial availability of raw material, high % yield, and low cost.

On preparation of blank microemulsion by using *C. citratus* essential oil, the microemulsions with average droplet sizes ranging from ~9 to 90 nm were readily obtained using different non-ionic surfactants of which Tween 20 was selected for more in depth studies. In the system comprising Tween 20 as surfactant, ethanol was found to be a more suitable co-surfactant than hexanol to form a large microemulsion region in the pseudoternary phase diagram. The phase behavior of the water/*C.citratus* oil/Tween 20/ethanol mixture was hardly influenced by both the pH and the ionic strength of the aqueous phase. In the system containing 10% *C. citratus* oil, phase inversion occurred at a water volume fraction >0.5.

On preparation of blank microemulsion by using *Z. cassumunar* essential oil, the most suitable surfactant was Triton X-114 because of the largest micoremulsion region existed in the phase diagram. Alkyl chain length of mono-ol alcohol and number of hydroxyl group in the co-surfactant molecule exhibited remarkable effect on the microemulsion formation. Microemulsion of *Z. cassumunar* oil with average droplet sizes of 21.87±0.14 nm was obtained using Triton X-114 and PG in the ratio of 2:1. The phase behavior of the *Z. cassumunar* oil/Triton X-114/PG/ water mixtures were hardly influenced by both pH and ionic strength of the aqueous phase. The microemulsions on dilution line I which the oil/Smix was 1:9 changed from bicontinuous to O/W microemulsion after 60% of water was added. And the microemulsions on dilution line II which the oil/Smix was 2:8 changed to O/W microemulsion when the water amount over 50%.

After the phase diagram construction and the characterization of blank microemulsions, the formulation of *Z. cassumunar* oil/Triton X-114/ethanol/water was selected for the incorporation of TD alkaloidal extract because of the smaller internal droplet size which tends to possess more effective transdermal delivery. The droplet size of microemulsion containing higher amount of TD alkaloidal extract which was larger supports the idea that TD alkaloidal extract was entrapped inside the internal droplet of microemulsion. The 5 mg/mL of TD alkaloidal extract was then incorporated into 20 formulations along dilution line A and B in the phase diagram and their microstructure evaluations were consequently undertaken. Three formulations of microemulsion (A4, A6 and B4), Liquid crystaline system (B6) and liquid crystal in microemulsion (A8) were in-depth investigated for their stability, *in vitro* anticholinesterase activity and *in vitro* permeation.

The formulations along dilution line A which had higher ratio of Smix to oil possess higher anti-AChE since the greater amount of surfactant caused higher solubility which leads to more attachment of the alkaloids with the enzyme. The anti-AChE of each formulation was then followed up for 180 days after the storage in 4°C, 30°C and 45°C. The most obvious finding to emerge from this study is that the decrease of inhibitory activity was related well with the depletion of the TD alkaloidal extract measured from the amount of 3'-*R/S*-hydroxyvoacamine by HPLC. Moreover, the TD alkaloidal extract encapsulated in microemulsions, liquid crystalline system and liquid crystal in microemulsion possess higher anti-AChE after storage in various temperatures for 180 days and high temperature (45°C) had no effect on their anti-AChE.

The next investigation was focused on skin permeation of the TD alkaloidal extract. Both microemulsions and liquid crystalline systems show an enhancing effect on skin permeation. B6 which was liquid crystal showed the greatest Q_{24} (0.54±0.28 µg/cm^2) when B4 which was microemulsion came in the second place (0.37±0.20 µg/cm^2). These results were in a good agreement with the results from skin retention which indicate that B4 and B6 possess higher skin retention of the TD alkaloidal extract than that of A4, A6 and A8. It also means that higher amount of the alkaloidal extract from B4 and B6 can partition into the skin which brings about to the high Q_{24}.

In conclusion, both microemulsion and liquid crystalline system of *Z. cassumunar* oil/Triton X-114/ethanol/water is possible to incorporate TD alkalidal extract which possess anti-AChE for the transdermal purpose. If the findings from this thesis are to be moved forward, more convenient preparations for transdermal drug delivery system such as transdermal patch are needed to be developed and evaluated.