CHAPTER 5

DISCUSSION AND CONCLUSIONS

Recently, many medicinal plants and their formulations were used for pharmaceutical and cosmetic fields. The herbal medicinal pharmaceutical products have become more widely used in industrials. Thus, evaluation of their pharmacological activities is important. *T. divaricata* has been mostly used as Thai folk medicine for treatment of fever or other purposes. Several parts of *T. divaricata* such as leaves, root, bark, stem and flower have been previously reported for various biological activities [2-4, 9]. However, the important problem of *T. divaricata* extract usage was its unstable property from chemical degradation as well as non-suitable physical appearances for applications. Loading of *T. divaricata* extract into SLN can protect the extract against chemical degradation. Therefore, the researchers are interested in pharmacological activity of *T. divaricata* extract, especially anti-inflammatory activity, and development of topical application of *T. divaricata* extract loaded into SLN in field of nanocream.

The *in vitro* anti-inflammatory activity of the extracts from *T. divaricata* was done by enzymatic assay via COX-2. The results indicated that the extract from maceration with ethanol possessed the highest inhibition activity against COX-2 when compared with the extract from several fractionations. Moreover, it was also found that the extract from maceration with ethanol exhibited higher inhibitory activity than that of extract from electrocoagulation. Therefore, the extract from maceration with ethanol, which was termed TDE, was selected for the further studies. The yield of this extract was 5.02% w/w and its appearances were semi-solid mass, herbal odor and intense brown color. The physicochemical properties of TDE related to pre-formulation study, such as thermal behavior by DSC and crystalline characteristic determined by PXRD as well as the solubility property were also investigated. The results from DSC and PXRD revealed that the compounds existing in the crude

extract showed no identical endothermic peak as an irregular pattern and were in amorphous form. From these results, the TDE could be degraded when exposed to the heat at 125 °C or more and had no crystalline in its internal structure. The solubility study showed that the TDE could be well dissolved in many intermediate polar solvents. The primary active compounds screening study of TDE exhibited that the chemical constituents existing in the TDE were alkaloids and glycoside such as cardiac and sterol/triterpene. Thus, this study could confirm that the active compounds which are alkaloids from *T. divaricata* had been related to biological activities such as anti-inflammation that are relevant with the previous study [2-4]. The TDE fingerprint by using HPLC indicated that the chromatogram showed the clearly identical peaks of two markers, vobasine and 19**,** 20dehydroervatamine, in the area ratio of 1:4. Retention time of vobasine and 19**,** 20 dehydroervatamine were 12.51 ± 0.05 min and 13.95 ± 0.11 min, respectively. The yields of these two markers, which were calculated base on TDE, were 0.64 and 2.79% w/w. Therefore, The HPLC condition was suitable to indentify the TDE and used for the quantitative analysis in the further studies.

According to development of nanocream base, Oil-in-Water (O/W) type of cream base was interested because it could increase the skin moisture and water-washable. The formulations were prepared using the high pressure homogenizer and factors influencing their characteristics such as particle size of droplets, size distribution, zeta potential, viscosity and rheological behavior were studied. It was found that the formulation 2A had better characteristics than that of 1A because the particle size and size distribution was more stable after 90 days. These results were from the higher amount of surfactant in formulation 2A. However, the viscosity of these two formulations was changed in the same way while they also represented the pseudoplastic flow with thixotropy property. When consider the number of homogenization cycles, both formulations (2AC3 and 2AC6) were rather stable and showed the similar results. Thus, the formulation 2AC3 was chosen for producing the nanocream base for the further used.

The study of TDE loaded SLN was aim to investigate the factors such as the number of homogenization cycles and the amounts of surfactant and lipid which influence on their physicochemical properties. The results showed that the particle

size and size distribution were increased while the zeta potential becomes more negative. This could be the effect from aggregation of nanoparticles when the number of homogenization cycles increased [65]. The amount of surfactant also showed in the similar effect with the number of homogenization cycles. When the amount of surfactant increased, it could be excess and form the liquid crystal [66], so the particle size could be increased. Moreover, the particle size remained in the range of 200 nm when the amount of lipid increased. Both of 3 and 6 homogenization cycles showed a good size distribution and their zeta potential were negative in range of 26 to 30 mV. Effect of entrapment efficiency (EE) indicated that EE of TDE in the SLN increased when the lipid component increased [67]. Because it showed a semi-polar property, so the increase of lipid may help the loading of TDE into SLN. The increase of amount of TDE affected the increase of particle size and size distribution, whereas, their zeta potential were more negative. Only a concentration of 7.5% w/w of lipid exhibited good characteristics and showed a high EE when the amount of TDE increased because of its solubility property. Thus, the suitable amounts of TDE and lipid had involved on good characteristics and EE value of nanoparticles.

According to the results from above studies, the TDE loaded SLN consisting of 7.5% w/w lipid, 10.0% w/w surfactant and 0.50 to 2.50% w/w TDE was produced by a hot high pressure homogenization. Then they were incorporated into nanocream base in a weight ratio of 1:1. The *in vivo* anti-inflammation of TDE loaded SLN nanocream were studied using the EPP-induced mouse ear edema method. The results indicated that all doses of TDE solutions could reduce edema formation and also be more effective when their concentration increased as well as TDE loaded SLN nanocream. They also found that the TDE loaded SLN nanocream could better reduce the edema formation than that of TDE at the same concentration. The percentage of ear edema inhibition of those formulations was higher than that of TDE solutions after that they were gradually increased at all determination times. This result indicated that SLN could enhance solubility and penetration of TDE into skin. Furthermore, the nanocream components also enhanced the moisture of skin.

Topical application of EPP induced the release of inflammatory mediators such as histamine, serotonin, and prostaglandins (PGs), which synergistically increase the vascular permeability and promote vasodilatation as well as edema formation [41].

The reference anti-inflammatory drug, indomethacin, acting by inhibit of COX enzymes, showed marked inhibitory activity on edema formulation in this model. Moreover, the *in vitro* results obtained suggest that TDE possessed an anti-inflammatory activity in acute phase of inflammation via inhibition release of inflammation mediator especially PGs. However, the mechanism of action of TDE with other models should be studied.

The characteristics study of 0.25% and 0.50% w/w TDE loaded SLN nanocream showed that their particle size were in range of nanometer (less than 200 nm). The particle size was increased when the TDE concentration increased. The SLN nanocream base, which was not loaded with TDE, had a better size distribution than that of TDE loaded SLN nanocream. In the case of rheological behavior, all formulations showed pseudoplastic flow with thixotropy property. However, the SLN nanocream base had higher viscosity than that of TDE loaded SLN nanocream and its viscosity was also increased and associated with an increase of TDE concentration. It could be the effect of the amount of water increased when TDE loaded SLN was incorporated into nanocream. For stability testing, the particle size was increased when kept in heating-cooling condition. However, they were less than 300 nm. Their size distributions were broader and the zeta potential was less negative when compared with the initial stage. Their viscosity was increased but they also showed pseudoplastic flow with thixotropy property. At conditions of 4 and 27 °C, their particle size were also in range of nanometer (about 200 nm), however, their size distribution were broad. The zeta potential was changed but they were also near negative of 30 mV. Their viscosity tended to increase while their rheological behavior was not changed. At 45 °C, the results demonstrated that the particle size were larger with the range of 200-300 nm. The size distribution was increased while the zeta potential was decreased. Their viscosity was increased whereas their rheological behavior was not changed. In this case, it could be the effect of surfactant film rigidity by temperature [68]. All above results could be concluded that both 0.25 and 0.50% w/w TDE loaded SLN nanocream were rather stable when stored at conditions of 4 and 27 °C.

The study of TDE quantitative analysis indicated that the degradation of the TDE in TDE intact was increased, especially when kept under high temperature

condition. When compared with TDE loaded SLN nanocrems, the results indicated that the TDE in TDE loaded SLN nanocrems were degraded less than an intact. Moreover, it was found that the degradation of TDE in internal phase (TDE loaded into SLN) than that of TDE in external phase (free TDE). These obtained results could be the effect of solid lipid nanoparticles. Since, they helped to protect the TDE degradation from environment conditions such as light or heat. Thus, the TDE which were loaded with SLN was more stable than TDE in the free extract. For entrapment efficiency study, it was found that the % EE was decreased at both in heating-cooling and stability conditions. The reasons could be the effect of non entrapped TDE in external phase which were disintegrated by light and high temperature [43]. By the way, the TDE which loaded in SLN exhibited slow releasing out. From this view, it could be concluded that the TDE was unstable and simply disintegrated by heat. Therefore, the solid lipid nanoparticles could help to reduce degradation and enhance stability of the extract [15, 43].

Regarding to *in vitr*o release study, TDE solution showed slow releasing profile that it could be from its low solubility in ethanolic aqueous solution. The release rate of TDE was depended on the concentration of TDE in the nanocream. However, the profile of release was not different when the TDE concentration increased. It was found that the TDE loaded SLN in the nanocream was gradually released and maintained constant after 6 h. This result predicted that the SLN could help the TDE to be more stable and has long action from slow releasing in physical condition.

The irritation study indicated that the developed TDE loaded SLN nanocream had no irritation on the skin of tested animals.