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INTRODUCTION

Angelica sinensis (Umbelliferae) has been widely used in Thai traditional medicine for treatment of many illnesses (**1**). A major component in the dried roots of *A. sinensis* is ferulic acid (**2**) which possessed several activities such as increasing coronary blood flow, inhibiting vascular smooth muscle cell proliferation, and antioxidant (**3-7**). The extract and essential oil of clove (*Syzygium aromaticum*) are widely used because of their analgesic and antiseptic properties. Eugenol is the most important component of clove, showing several biological properties (**8-10**). So far clove and eugenol have been reported of immunomodulatory/anti-inflammatory effect on cytokine production (interleukin IL-1β, IL-6 and IL-10) *in vitro* (**11**). We are interested in combining the good antioxidant from *A. sinensis* and anti-inflammatory activity from clove oil in the form of microemulsion preparation. This clear transparency liquid will be used as topical anti-inflammation preparation.

MATERIALS AND METHODS

Material and reagents. The dried root of *A. sinensis* was purchased from traditional drug stores in Bangkok, Thailand, in May 2012. Ferulic acid was obtained from Aldrich (St. Louis, USA). Clove oil, peppermint oil, sage oil, neroli oil Tween 20 were obtained from Namsiang co., Ltd, Bangkok. All organic solvents were obtained from Merck (Germany). Spotting device - Linomat 5 automatic sample spotter (CAMAG, Muttenz, Switzerland). Syringe - 100 L (Hamilton, Bonaduz, Switzerland). TLC chamber -Glass twin-trough chamber (20 x 10 cm.)(CAMAG, Switzerland).Densitometer - TLC scanner 3 with winCATS software (CAMAG, Switzerland).TLC plates - 20.0 x 10.0 cm, 0.2 mm layer thickness precoated with silica gel 60 F_{254} , cat. No. 1.05554.0001(Merck, KGaA, Damstadt, Germany).

Extraction and analysis. Dried powder of root of *A. sinensis* (500 g) was weighed and transferred to a 2500 mL erlenmeyer flask. Ethanol (1000 mL) was added and the flask was placed in the carbinet for 7 days. The ethanol extract was collected and another portion of ethanol (1000 mL) was added to the marc and kept in the same manner. The extraction process was carried on until exhaust, monitored by TLC. The ethanol extracts were combined, filtered, and concentrated using a rotary evaporator.

Formulation of microemulsion using ternary phase diagram. In order to determine the proportions of a system consisting of oil (clove oil, peppermint oil, sage oil, and neroli oil), surfactant (Tween20/Cremophore RH40), and water, the ternary phase diagrams whose apexes respectively represent the pure components were constructed. Mixture of oil and surfactant at certain ratio were prepared in test tubes. Water then added drop wise to the given composition in ternary phase diagram. Vigorous stirring followed all the aqueous phase addition on vortex mixer. The indication of microemulsion formation was using visual observation for transparency, flow ability and physical stable state.

Prearation of *Angelica sinensis* root extract-loaded microemulsion. Microemulsion (oil : tween 20 : water , 1:4:5) of *Angelica sinensis* extract was prepared by adding clove oil (2 g) to *A. sinensis* extract (1 g) in the test tube and then added Tween 20 (8 g). The mixture was mixed by vortex mixer for a few minutes and the water phase (10 g) was added and mixed by vortex mixer to give a clear solution.

Particle size and zeta potential measurement. Particle size and zeta potential of developed microemulsion were analyzed by zetasizer model S4700 (Malvern, UK) at 25 °C. Samples were diluted with water and the sample was placed in the cuvette. A plot of particle size of microemulsion and zeta potential were performed and recorded.

***Ex vivo* drug diffusion study.** *In vitro* drug permeation study of selected formulation was performed using Franz diffusion cell through pig dorsal skin. The PBS (pH 7.0) containing tween 80 (20%) was used at the receptor medium in the diffusion cell. Skin was sandwiched between the receptor compartment and donor compartment so that the dermal portion was continuously bathed with the receptor fluid maintained at 37 ± 1 °C by circulating water bath and dorsal skin side exposed to ambient temperature. The receptor fluid was stirred continuously using a magnetic stirrer. Samples (5 mL) were withdrawn at different time intervals, replaced the same volume of fresh solution, and amount of drug was extracted with dichloromethane (5 mL × 2). The organic layers were combined and evaporated to dryness using rotary evaporator. The residue was dissolved with 1.0 mL of methanol and determined by TLC-densitometric method which was detected at 254 nm for ferulic acid. A plot showing release pattern of the microemulsion through pig dorsal skin was plotted.

RESULTS

The isotropic area in ternary phase diagrams of peppermint oil, sage oil, neroli oil, and clove oil with Tween 20 and water at 25 °C were 16, 18.3, 23.2, and 12.1 %, respectively. When used Cremophore RH40 as surfactant, the isotropic area were 8.1, 9.9, 7.2, and 17.4%, respectively. The three compositions which were clove oil/ Cremophore RH40/ water (1 : 5 : 4, 1 : 4 : 5, 2 : 6 : 2) and clove oil/ Tween 20/ water (1 : 5 : 4, 1 : 4 : 5, 2 : 6 : 2) were selected to form the 1% *A. sinensis* root extract microemulsions. After that they were subjected to measure the size and zeta potential by zetasizer model S4700. The results were showed in table 1.

Table 1. Size and zeta potential of selected microemulsions

Composition (clove oil/ Tween 20 / water)	Size (nm.)	Zeta potential (mV)
1 : 5 : 4	13 ± 0.0	-6.6 ± 1.6
1 : 4 : 5	22 ± 1.3	-5.3 ± 1.1
2 : 6 : 2	17 ± 0.2	-2.1 ± 0.5
Composition (clove oil/ Cremophore RH40 / water)	Size (nm.)	Zeta potential (mV)
1 : 5 : 4	18 ± 0.0	-7.6 ± 1.2
1 : 4 : 5	21 ± 0.0	-4.6 ± 0.4
2 : 6 : 2	22 ± 1.3	-4.7 ± 0.5

For the *In vitro* drug permeation study of selected formulation was performed using Franz diffusion cell through pig dorsal skin. The microemulsion of clove oil/ Tween 20/ water (1 : 4 : 5) was used in the experiment and the flux (*J*) was 852.9 ± 14.6 g/min.cm^2 which calculated from the slop of linear relationship in the graph (Figure 1.). The permeability value was also calculated to obtain 3.83 ± 0.07 × 10^{-3} cm/min.

DISCUSSION

The microemulsion area of each oil ternary phase diagram suggested that ability of a surfactant to initiate microemulsion also depended on compositions consisting in the mixture. This is due to changes in microemulsion composition change the microenvironment of surfactant leading to modification of the apparent critical packing parameter of the surfactant (**12**). However, in this study, the microemulsion systems could be obtained by using Tween 20 or Cremophore RH40 at high concentration (≥ 40%). This might be that lipophilic chains of these surfactants were quite long and contained saturated bonds leading to low fluidity lipophilic chains. Therefore, they could not initiate microemulsion at low concentration (**13**).

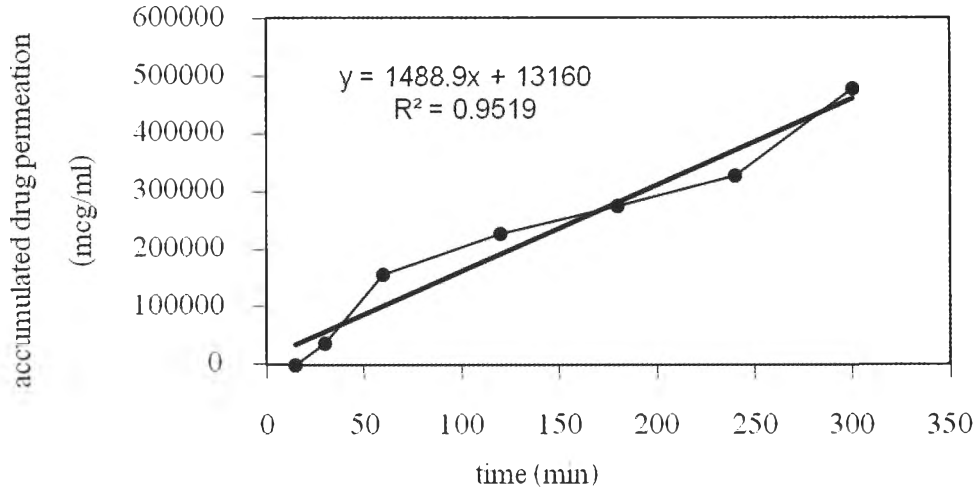

Figure 1. Accumulated drug permeation versus time of the microemulsion

The droplet size and zeta potential of representative formulations presented in Table 1 showed their droplet size were in submicron range with negative zeta potential. This indicated the ability of these microemulsions could facilitate skin penetration of ferulic acid properly. Because of high concentration of surfactant consisting in microemulsion, it could alter the structure of stratum corneum causing poor barrier property of stratum corneum and effective permeation of drug through the skin (**14**). Therefore, the submicron droplets of these microemulsions could penetrate through the skin easily without an effective barrier. These conclusions were confirmed by the results of permeation study through pig skin. The microemulsion containing clove oil/Tween 20/water at the ratio of 1:4:5 could facilitate ferulic acid through the skin 852.9 ± 14.6 μg/min.cm^2 with velocity of 3.83 ± 0.07 × 10^{-3} cm/min.

CONCLUSION

The present study demonstrated that microemulsions of *A. sinensis* extract could be developed to be a topical anti-inflammatory preparation. According to the results of this study, clove oil had a good combination with the microemulsions system for both Cremophore RH40 and Tween 20. They all have droplet size and zeta potential in nanometer range with negative zeta potential. These indicated the possible of being good carriers facilitating skin penetration for ferulic acid. For the release and the diffusion of ferulic acid from microemulsion through a dorsal pig skin, we selected Tween 20 as a surfactant because it had lower viscosity and our study showed that this microemulsion can facilitate ferulic acid through the pig skin.

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