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PREPARATION AND CHARACTERIZATIONS OF MICROEMULSION OF *ZINGIBER CASSUMUNAR* RHIZOMES EXTRACT

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INTRODUCTION

Zingiber cassumunar Roxb. (Zingiberaceae), a traditional herb commonly known in Thailand as Plai, has been widely used for asthma, muscle and joint pain (**1**). This plant was reported to have anti-inflammatory, antioxidant, insecticidal and uterine relaxant activities (**2-4**). Volatile oil from the rhizomes of *Z. cassumunar* has been formulated as cream and massage oil for relieving muscle pain. The major active compounds in this plant are (*E*)-1-(3,4-dimethoxyphenyl)but-3-en-1-ol (compound D), (*E*)-1-(3,4-dimethoxyphenyl)butadiene (DMPBD), cis-3-(2′,4′,5′-trimethoxyphenyl)-4-[(E)-2′′,4′′,5′′-trimethoxystyryl]-cyclohex-1-ene and curcumin (**5, 6**). All of these compounds have anti-inflammatory activity and we are interested in developing the *Z. cassumunar* rhizomes extract into microemulsions preparation. This preparation consists of an aqueous phase, a surfactant and co-surfactant component, which are thermodynamically stable and have been shown to have high solubilization capacity and to facilitate the skin permeation of both lipophilic and hydrophilic drug (**7, 8**). In this study, we first examined the ternary phase diagram of peppermint oil and surfactant in order to find the best composition in forming microemulsions. We furthermore examined the diffusion transport of the microemulsions using Franz diffusion cell method.

MATERIALS AND METHODS

Material and reagents. The rhizomes of *Z. cassumunar* was purchased from traditional drug stores in Bangkok, Thailand, in May 2011 and identified by Dr.Prasan Tangyuenyongwatana. Compound D was obtained from chemical synthesis in our laboratory (**9**). Methanol and Dichloromethane (AR grade) were obtained from JT Baker (USA). Spotting device - Linomat 5 automatic sample spotter (CAMAG, Muttenz, Switzerland). Syringe - 100 µL (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 rhizomes of *Z. cassumunar* (500 g) was accurately weighed and transferred to a 2500 mL erlenmeyer flask. Ethanol (1000 mL) was added and the flask was placed in the dark 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.

Ternary phase diagram at constant temperature. The phase behavior of a system consisting of peppermint oil, surfactant (Tween20), and water may be described on a ternary phase diagram whose apexes respectively represent the pure components. Mixture of peppermint 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, flowability and physical stable state.

Prearation of *Zigiber cassumunar* rhizomes extract. Microemulsion (oil : tween 20 : water , 2 : 5 : 3) of *Zigiber cassumunar* extract was prepared by adding peppermint oil (4 g) to *Z. cassumunar* extract (1 g) in the test tube and then added Tween 20 (10 g). The mixture was mixed by vortex mixer for a few minutes and the water phase (6 g) was added and mixed by vortex mixer to give a clear solution.

Characterization of developed formulations. 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 mucosa 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 compound D. A plot showing release pattern of the microemulsion through pig dorsal skin was plotted.

RESULTS

The ternary phase diagram of peppermint oil/ Tween 20/ water at 25 °C is depicted in figure 1. The isotropic and low viscosity compositions presented in the phase diagram as a dark spot. The remainder below these spots represents the turbid region, represented as multiphase conventional emulsion based on visual identification.

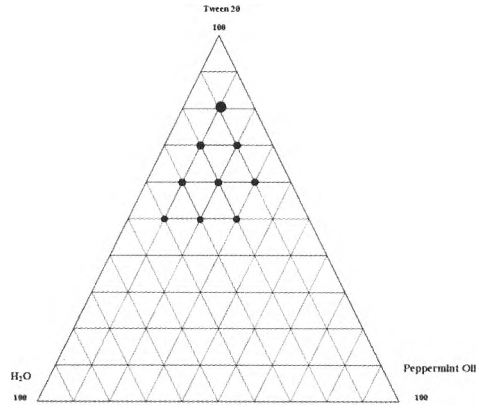

Figure 1. The ternary phase diagram of peppermint oil/ Tween 20/ water at 25 °C

The three compositions which were peppermint oil/ Tween 20/ water (1 : 5 : 4, 2 : 5 : 3, 3 : 5 : 2) represented the low amount of surfactant in the microemulsion system. These compositions were selected to form the 1% *Z. cassumunar* 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 (peppermint oil/ Tween 20/ water)	Size (nm.)	Zeta potential (mV)
1 : 5 : 4	199 ± 8.9	-9.8 ± 0.9
2 : 5 : 3	40 ± 0.8	-5.6 ± 0.4
3 : 5 : 2	130 ± 0.9	-4.9 ± 0.8

For the *In vitro* drug permeation study of selected formulation was performed using Franz diffusion cell through pig dorsal skin. The microemulsion of peppermint oil/ Tween 20/ water (2 : 5 : 3) was used in the experiment and the flux (*J*) was 104.6 ± 8.5 g/min.cm^2 which calculated from the slop of linear relationship in the graph (Figure 2.). The permeability was also calculated to obtain 1.34 ± 0.11 × 10^{-3} cm/min.

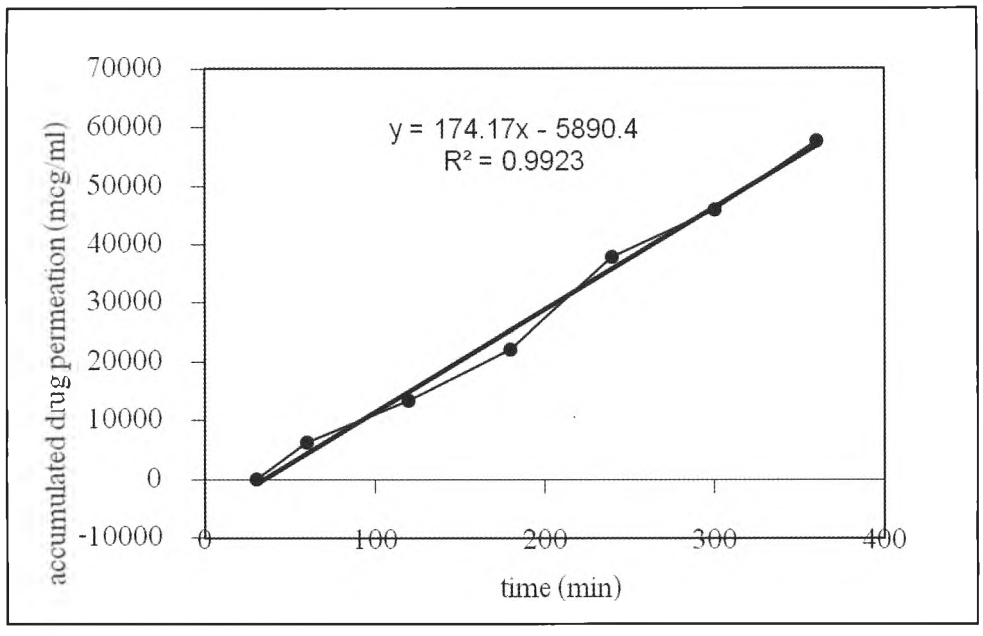

Figure 2. Accumulated amount of compound D permeation versus time

DISCUSSION

The ternary phase diagram of peppermint oil/Tween 20/water at 25 °C shown in Figure 1. suggested that microemulsion systems could be obtained by using Tween 20 at varied concentration (50- 80%). This might be that Tween 20 at effective concentrations could lower the surface tension at the interface between peppermint oil and water. Furthermore, due to the proper orientation of Tween 20 molecules at interface, Tween 20 molecules could form highly fluid interface film, penetrated and associated with oil molecules and initiate microemulsion formation (**10**). However, since high concentration of surfactant could lead to skin irritation and toxicity (**11**), the microemulsions consisting of low concentration of Tween 20 (50%) were selected as representative for the further study. The droplet size and zeta potential of representative formulations in Table 1 were in nanometer range with negative zeta potential. These indicated the potential of being good carriers facilitating skin penetration for compound D of these microemulsions. Because microemulsion contains high concentration of surfactant, it could destroy the structure of stratum corneum and increase the permeation of drug through the skin (**12**). Therefore, the submicron droplets of these microemulsions could penetrate through the skin without an effective barrier. This conclusions are reinforced by the results of permeation study showed that the microemulsion containing peppermint oil/Tween 20/water at the ratio of 2 : 5 : 3 could facilitate compound D through the skin 104.6 ± 8.5 μg/min.cm^2 with velocity of 1.34 ± 0.11 × 10^{-3} cm/min.

CONCLUSION

The results showed the possibility of using microemulsions as topical drug delivery vehicles of *Zigiber cassumunar* extract. According to the results of this study, the microemulsions 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 compound D. For the release and the diffusion of compound D from microemulsions through a dorsal pig skin, our study showed that microemulsion can facilitate compound D through the pig skin.

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