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Development of *Kaempferia parviflora* extract-loaded microemulsions for skin permeation enhancement

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Objectives: The aim of this study was to develop the novel microemulsion (ME) systems for transdermal delivery of *Kaempferia parviflora* (KP) extract. The effect of ME components on the intrinsic properties and *in vitro* skin permeation of KP extract-loaded ME was evaluated.
Methods: The solubility of KP in various oils (Oleic acid,caprylic/capric triglyceride, isopropyl myristate and isopropyl palmitate), surfactants (Tween 20, polyoxyethylene castor 35 oil and polyoxyethylene castor 40 oil) and co-surfactants (Propylene glycol(PG), ethoxydiglycol and butylene glycol) was studied in order to screen the suitable compositions of ME. The ME formulations composed of oil, surfactant, co-surfactant and water were selected by ME area of pseudo-ternary phase diagram. PG and ethoxydiglycol were found to be better than another investigated co-surfactant systems to construct the largest ME area of pseudo-ternary phase diagram. ME consisted of the mixture of surfactant/co-surfactant at the ratio of 1:2 was selected for incorporating KP extract. The effect of ME components on the mean droplet size, pH, conductivity and *in vitro* skin permeation of KP extract-loaded ME was determined.
Results: The results showed that the mixture of oil, water and the surfactant system played an important role on characteristics and *in vitro* skin permeation of KP extract-loaded ME. The mean droplet size of KP extract-loaded microemulsions was in the range of 20-30 nm. The pH value and conductivity of microemulsions after loading KP extract were 5.19-5.25 and 76.0-83.0 µS/cm, respectively. These results indicated that KP extract-loaded microemulsion were o/w microemulsions. The skin permeation flux of the appropriate KP extract-loaded ME formulations (oleic acid/tween 20/PG/water and oleic acid/tween 20/ethoxydiglycol/water) were significantly higher than the KP extract in water (control).
Conclusion: The results can be concluded that KP extract-loaded microemulsions were o/w microemulsions with nano-size range and enhanced skin permeation. Our finding ME system had a potential to be used for transdermal delivery of KP extract.

Introduction

Kaempferia parviflora Wall. Ex Baker (KP), a plant of Zingiberaceae family, is grown in the northern and northeastern region of Thailand. It is commonly referred as “Krachaidum” or “Black Ginger”. This plant is a perennial herb with dark purple to black rhizomes and these colors lead to its name. Its rhizome has been used as a folk medicine for the treatment of a wide variety of illnesses. There are many therapeutic functions of KP that have been reported in the antimicrobial, aphrodisiac effect, anti-gastric ulcer, antidepressant, anticholinesterase activity, anti-obesity effects, vasodilator and antioxidant effects.[1,2] In addition, KP extract demonstrated the efficacy of anti-inflammatory though the inhibition of nitric oxide and prostaglandin E_2 release in RAW 264.7 macrophage cells and decreased carrageenan-induced rat paw edema.[3,4] Therefore, KP extract was interested choice of herbal medicine product for anti-inflammatory effect.
KP is discovered chemical constituents at least 11 methoxyflavones analyzed by using gas chromatography.[5] The main methoxyflavone compounds of KP extract contain 3,5,7,3’,4’-pentamethoxyflavone (PMF), 5,7- dimethoxyflavone (DMF), and 5,7,4’-trimethoxyflavone (TMF) that are used as markers of KP extract. Nevertheless, these compounds characterize low water solubility, high lipophilicity and low bioavailability (about 1-4%).[7] Therefore, transdermal delivery systems are good choice to increase the effectiveness of KP extract. Moreover, the physicochemical properties of methoxyflavones are suitable for developing transdermal delivery in order to improve its therapeutic effects.[6,8]

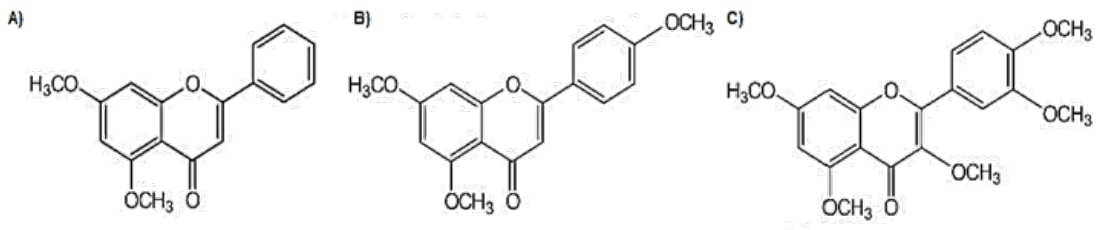

Figure 1. The main methoxyflavone compounds of KP extract A) DMF, B) TMF and C) PMF.

Microemulsions (ME) are transparent systems composed of two immiscible phases which was stabilized by the mixture of surfactant and co-surfactant. ME was widely used in transdermal drug delivery because ME offered several advantages including simplicity of preparation, high loading capacity for hydrophilic and lipophilic drugs, thermodynamically stable and high potential for skin permeation enhancement. Many researches were studies in the design of transdermal delivery system with improving drug permeation for low water solubility and high lipophilicity.[9] The aim of this study was to develop microemulsions for improving skin permeation of KP extract.

Materials & Methods

Chemicals: Acetonitrile (LabScan, Bangkok, Thailand), formic acid (Thermo Fisher Scientific, Leicester, UK), oleic acid (Aldrich/USA), isopropyl myristate (KLK Oleo/Malaysia), isopropyl palmitate (Nikkol/Singapore), caprylic/capric triglyceride (KLK Oleo/Malaysia), ethoxydiglycol (Transcutol CG; Getteffosse/France), butylene glycol (KH Neo Chem Co.,Ltd/Japan), propylene glycol (SKC Co.,Ltd/Korea), Tween 20 (Lonza/USA), polyoxyethylene castor 35 oil (Cremophor EL®; BASF/Germany) and polyoxyethylene castor 40 oil (Cremophor RH 40®; BASF/Germany) were used in this study.

Preparation of KP extract: The dried rhizomes of KP were purchased from Loei province, Thailand. The rhizomes were grounded and passed through a sieve until obtain powder. KP rhizome powder was extracted by percolation with ethanol. The solvent was removed under reduced pressure at 50 ºC until obtain the extract (5.5% w/w yield).

Solubility of KP extract: The solubility of KP in various oils, surfactants and co-surfactants was studied in order to screen the suitable compositions of microemulsions. Each experiment was performed in triplicate. An excess amount of KP extract was added to 5 mL of water, oils, surfactants or co-surfactants. The mixtures were shaken at 30±2°C for 48 h. Then the mixtures were centrifuged (9,000 rpm, 15 min) and supernatants were collected. The concentrations of PMF, TMF, and DMF in supernatants were quantified by using Ultra-Performance Liquid Chromatography (UPLC).

Construction of pseudo-ternary phase diagrams: Pseudo-ternary phase diagrams were constructed using the water titration method in order to optimize the concentrations of surfactants, co-surfactants and oils. The ratios of surfactant and co-surfactant (S_{mix}) used was 1:1, 1:2, 2:1 and 1:3. For each phase diagram at a specific ratio of S_{mix}, aliquots of each S_{mix} were mixed with oil at 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. Water was added drop by drop to the oil and S_{mix} mixture under magnetic stirring at ambient temperature. Microemulsion was taken as the end point of aqueous titration method. The concentrations of components were then calculated in order to plot the pseudo-ternary phase diagram. The ratio of oil, surfactant and co-surfactant which gave the greatest formation stability and broadest microemulsion regions was selected.

Preparation of microemulsions: Microemulsions were prepared by mixing surfactant, co-surfactant, oil and water by volume ratio using magnetic stirrer at ambient temperature. KP extract was accurately weighed (1 g) and adjusted to weight (10 g) with the microemulsions, followed by stirring with magnetic stirrer at ambient temperature.

Evaluation of microemulsions: The blank microemulsions and 10% KP extract-loaded microemulsions were determined their droplet size in triplicate at 25°C by using the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The pH was determined at 25°C using pH meter (Mettler Toledo, Sevencompact S220, USA). The electrical conductivity of the microemulsions was determined using conductivity meter (EC Testr 11+, USA) at 25°C. The measurements were performed in triplicate.

In vitro Skin permeation studies: The permeability of methoxyflavones from KP extract-loaded microemulsions and KP extract in water (KP-H_2O) were determined by using Franz diffusion cells with a penetration area of 2.31 cm^2. The model skin was porcine abdominal skin. The skins were washed with the pre-warmed phosphate buffer saline pH 7.4 (PBS pH 7.4) and mounted in the diffusion chamber of the cell, with the stratum corneum facing the microemulsion formulations. Diffusion cells were connected with a circulating water bath to maintain the temperature at 32°C. Each microemulsion formulation (1 g) was applied into the donor compartment. The receptor compartment was filled with 6 mL of PBS (pH 7.4) and stirred with a magnetic bar at a rate of 500 rpm. At the predetermined times of 15, 30 min, 1, 2, 4, 8 and 24 h, 1.0 mL of receiver medium was withdrawn, and KP content was determined by UPLC. The same volume of PBS was added into the receiver compartment to maintain a constant volume. Each sample was analyzed in triplicate.

UPLC analysis: UPLC analysis was carried out using ACQUITY UPLC BEH C18 reversed phase column (100 x 2.1 mm, 1.7 µm). The isocratic mobile phase consisted of 0.5% formic acid in water and acetonitrile. Flow rate and injection volume were 0.5 ml/min and 10 µl. Detection was performed by measuring absorbance at 335 nm using an UV/Vis detector.

Statistical analysis: Results were expressed as the means ± SD. Statistical data were analysed by independent sample t-test with $P<0.05$ as minimal level of significance.

Results and Discussion

Solubility of KP extract: The solubility of methoxyflavones (DMF, TMF and PMF) at 30±2 °C in water, surfactants, co-surfactants and oils, is shown in Table 1. The solubility of total methoxyflavones in water was found to be extremely low (0.121±0.005 mg/mL). The solubility of total methoxyflavones in surfactants was in the order of Tween 20> polyoxyethylene castor 35 oil> polyoxyethylene castor 40 oil. The solubility total methoxyflavones in co-surfactants was in the order of ethoxydiglycol> propylene glycol> butylene glycol and in oils was in the order of oleic acid> caprylic/capric triglyceride> isopropyl myristate> Isopropyl palmitate. The solubility of methoxyflavones in oleic acid showed the highest value of 23.668±0.147 mg/ml.

Construction of pseudo-ternary phase diagrams: From the solubility result, oleic acid was chosen as the oil phase. Polyoxyethylene castor 35 oil and Tween 20 were selected as surfactant. Ethoxydiglycol and propylene glycol were selected as co-surfactant. In preliminary studies, a total of four phase diagrams prepared by two surfactants and two co-

surfactants at the 1:1 ratio were constructed (data not shown). After that, two compositions (Tween 20/propylene glycol and Tween 20/ethoxydiglycol) which formed a stable and broad microemulsion area were selected. Eight diagrams were constructed by varying the ratios of S_{mix} at 1:1, 1:2, 2:1 and 1:3 (data not shown). Finally, the ratio of S_{mix} of 1:2 was selected and two phase diagrams were constructed and showed in Figure2.

Table 1. The solubility of methoxyflavones in KP extract at 30±2°C in water, surfactants, co-surfactants and oils.

Compounds	Solubility (mg/mL)			
	DMF	TMF	PMF	Total
Water	0.044±0.002	0.034±0.001	0.043±0.001	0.121±0.005
Surfactants				
Polyoxyethylene castor 35 oil	2.526±0.136	2.468±0.142	2.564±0.156	7.558±0.048
Polyoxyethylene castor 40 oil	2.194±0.116	2.122±0.149	2.187±0.149	6.504±0.040
Tween 20	4.335±0.295	4.179±0.292	4.052±0.286	12.566±0.141
Co-surfactants				
Propylene glycol	7.424±0.504	7.341±0.496	7.184±0.493	21.949±0.122
Butylene glycol	6.709±3.015	4.961±0.245	4.887±0.311	16.557±1.031
Ethoxydiglycol	7.586±0.123	7.535±0.127	7.374±0.118	22.496±0.111
Oils				
Isopropyl palmitate	0.788±0.262	0.487±0.165	0.456±0.194	1.731±0.183
Isopropyl myristate	1.102±0.013	0.364±0.005	0.478±0.009	1.943±0.398
Oleic acid	8.026±0.104	7.908±0.110	7.734±0.142	23.668±0.147
Caprylic/capric triglyceride	1.604±0.048	1.216±0.035	1.286±0.045	4.107±0.207

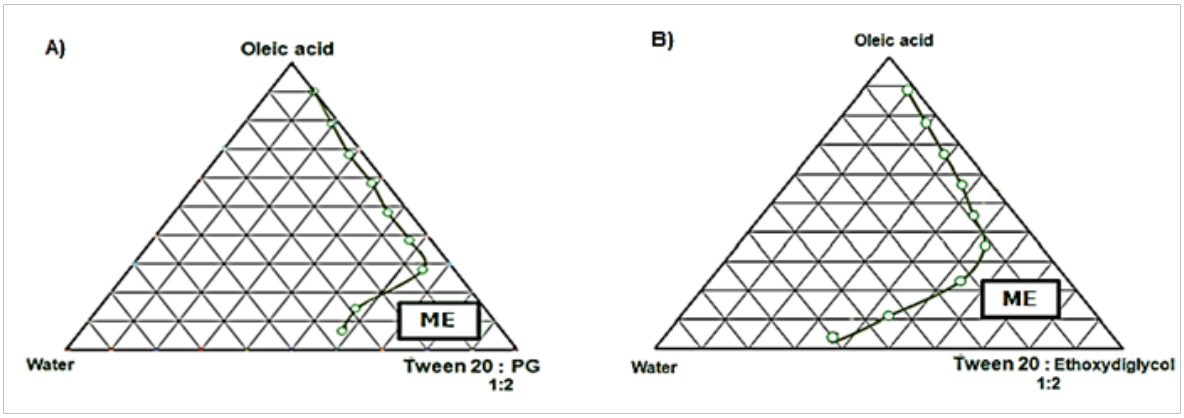

Figure 2. Pseudo-ternary phase diagrams of microemulsions composed of (A) oleic acid/tween 20/ propylene glycol/water (B) oleic acid/tween 20/ethoxydiglycol/water.

Preparation of KP extract-loaded microemulsions: It has been reported that 10% w/w KP extract showed an anti-inflammatory effect[1]. Therefore, 10% w/w KP extract was used in this study. Microemulsion composed of oleic acid as oil phase, tween 20 as surfactant, propylene glycol (ME1) or ethoxydiglycol (ME2) as co-surfactant and water as aqueous phase were formulated at the ratio of 1:4:8:7. After that, 10% w/w KP extract was added in the ME1 and ME2 and coded as KPME1 and KPME2, respectively.

Characterization of KP extract-loaded microemulsions: The mean droplet size of KPME1 and KPME2 was 28.69±0.27 and 29.29±0.23 nm, respectively. Whereas, the mean droplet size of ME1 and ME2 was14.83±0.24 and 16.19±0.26 nm, respectively, which were smaller than those of KPME1 and KPME2. The pH value of microemulsions with and without 10 % KP extract were 5.20-5.22 and 5.19-5.25, respectively. The conductivity of ME1, ME2, KPME1 and KPME2 was 37.6±0.9 and 54.3±2.1 µS/cm,77.1±0.8 and 82.5±1.3 µS/cm, respectively. The result indicated all microemulsions were o/w microemulsions.

***In vitro* skin permeation studies:** The skin permeation profiles of methoxyflavones, DMF, TMF and PMF through abdominal porcine skin are shown in Figure 3. The skin permeation fluxes of methoxyflavones from KPME1 and KPME2 were 0.41±0.07 and 0.37±0.11 µg/cm^2/h, respectively. Moreover, the skin permeation fluxes of the KP extract-loaded ME formulations were significantly higher than the KP extract in water (0.012 µg/cm^2/h). Various mechanisms for skin permeation enhancement of permeants by microemulsions have been proposed. Firstly, microemulsions act as drug reservoirs. Secondly, microemulsion droplets might breakdown on the surface of stratum corneum and then release their content into skin. Thirdly, skin permeation of loaded drug occurs directly from the microemulsion droplets to the stratum corneum. The last mechanism is caused by the nano-sized droplets dispersed in continuous phase which can move easily into the stratum corneum and carry the drug through the skin barrier.[10]

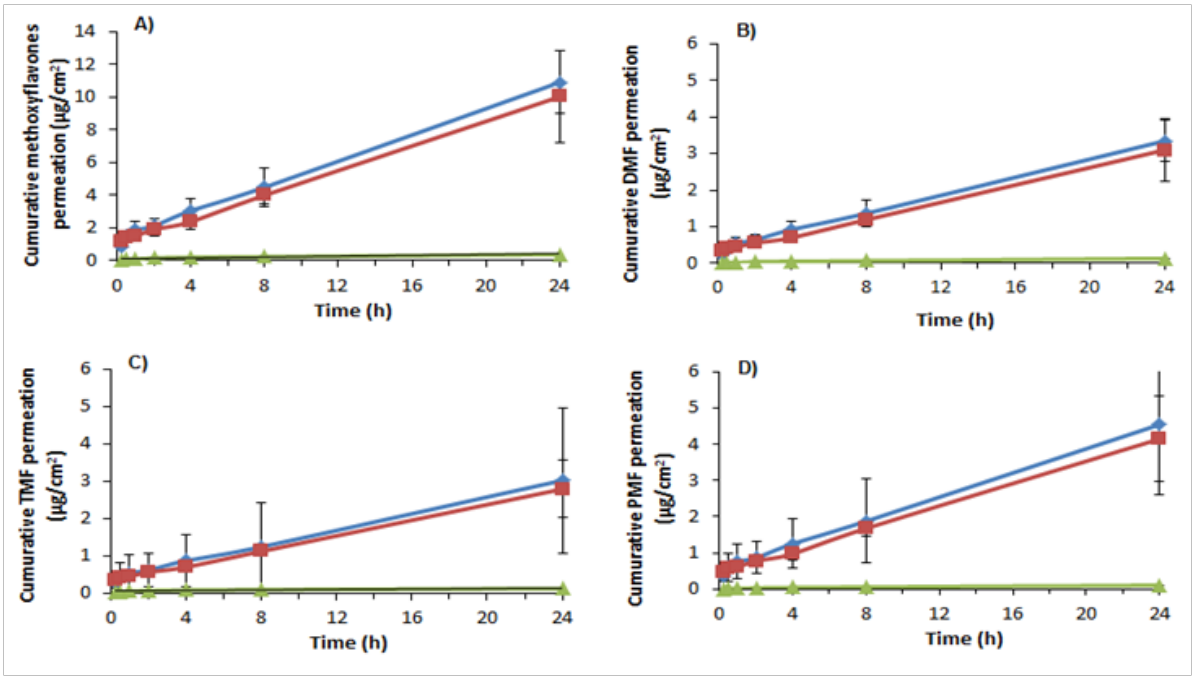

Figure 3. *In vitro* skin permeation profiles ofA) Methoxyflavones, B) DMF, C) TMF and D) PMF from KPME1 (◆), KPME2 (■) and KP extract in water (▲).

Conclusion

In this study, two KP extract-loaded microemulsions composed of 4.5% oil (oleic acid), 18% surfactant (Tween 20), 36% co-surfactant (Propylene glycol (KPME1) and Ethoxydiglycol (KPME2)), 31.5% water and 10% w/w KP extract were successfully prepared. Both KP extract-loaded microemulsions were o/w microemulsions with nano-size range and enhanced skin permeation. Our finding ME system had a potential to be used for transdermal delivery of KP.

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