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Research Article

Transdermal Permeation of *Kaempferia parviflora* Methoxyflavones from Isopropyl Myristate-Based Vehicles

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Abstract *Kaempferia parviflora* (*K. parviflora*) rhizomes have long been used in traditional folk medicines and as general health-promoting agents. Several biological activities of *K. parviflora*, especially its anti-inflammatory effect, are due to its major constituents, methoxyflavones. However, the oral bioavailability of these methoxyflavones has been shown to be low. The aim of this study was to investigate the permeation behaviors of *K. parviflora* methoxyflavones from isopropyl myristate (IPM)-based vehicles. We studied the effects of ethanol and propylene glycol (PG) as the hydrophilic, solvent-type vehicles as well as fatty acids as the permeation enhancers. A permeation experiment was performed *in vitro*, using side-by-side diffusion cells through the full thickness of pig ear skin. The solubility and permeation of methoxyflavones were able to be modified by choice and ratio of vehicles. The ethanol/IPM vehicle was shown to be more effective in enhancing the solubility and permeation of methoxyflavones when compared to the PG/IPM vehicle. Regarding an optimal balance between solubility or affinity to vehicle and skin to vehicle partition coefficient, the ethanol/IPM vehicle in the ratio of 1:9 maximized the flux. Among the investigated fatty acids, oleic acid showed the greatest enhancing effect on the permeation of methoxyflavones, indicating that saturated fatty acids are less effective than unsaturated fatty acids. Long chain fatty acids increased diffusion coefficient parameter and shortened the lag time. The number of carbon atoms and double bonds of fatty acids did not show direct relation to the profile of permeation of methoxyflavones.

KEY WORDS: fatty acid; isopropyl myristate; *K. parviflora*; methoxyflavones; permeation; vehicle.

INTRODUCTION

Kaempferia parviflora (*K. parviflora*) rhizomes are widely employed in folk medicine. Their major pharmacologically active constituents are methoxyflavones. At least 11 non-polar methoxyflavones in *K. parviflora* ethanolic extracts have been identified, of which the three major compounds are 3,5,7,3′,4′-pentamethoxyflavone (PMF), 5,7-dimethoxyflavone (DMF), and 5,7,4′-trimethoxyflavone (TMF) (1). Many potential therapeutic functions of methoxyflavones have been reported in the literature, including antiplasmodial and antifungal effects (2), anticholinesterase (3), anti-inflammatory (4–6), aphrodisiac (7), vasodilatory and antioxidant effects (8), antidepressant (9), and anti-obesity effects (10).

Ethanolic extracts of *K. parviflora* and methoxyflavones were reported for their anti-inflammatory effects against LPS-induced NO and PGE_2 release in RAW264.7 cells (4). Saewung et al. (2009) (5) had also demonstrated that an ethanolic extract of *K. parviflora* markedly inhibited PGE_2 release and suppressed mRNA expression of iNOS in dose-dependent manners, whereas COX-2 mRNA expression was partly affected. Furthermore, ten methoxyflavones were isolated from *K. parviflora extract* and PMF and 5,7,3'4'-tetramethoxyflavone showed inhibitory xanthine oxidase activity (6). With its strong anti-inflammatory effect, several *K. parviflora* products are available in the market including pills, capsules, tea, and wine. However, methoxyflavones are mostly lipophilic and show low aqueous solubility. Our pharmacokinetic study using male Wistar rats revealed the low oral bioavailability (about 1–4%) of methoxyflavones from *K. parviflora* ethanolic extract (250 mg/kg) (11). A novel delivery method, therefore, is desirable in order to increase the effectiveness of methoxyflavones from *K. parviflora*.

Due to their suitable physicochemical properties (12), including small molecular size (less than 300 Da), melting point (about 150–200°C), and log *K* (about 2–3), methoxyflavones (Fig. 1) are good candidates for transdermal delivery. Transdermal delivery of methoxyflavones offers many advantages, such as, sustaining drug level in blood circulation and reducing frequency of

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ABBREVIATIONS: C_0, Concentration of donor solution; D, The diffusion coefficient; DMF, 5,7-Dimethoxyflavone; *h*, The thickness of skin; HPLC, High performance liquid chromatography; *K*, The partition coefficient; *K. parviflora*, *Kaempferia parviflora*; IPM, Isopropyl myristate; *P*, The permeability coefficient; PBS, Phosphate-buffered saline; PEG 400, Polyethylene glycol 400; PG, Propylene glycol; PMF, 3,5,7,3′,4′-Pentamethoxyflavone; TMF, 5,7,4′-Trimethoxyflavone; T_{lag}, The lag time.

administration is to improve the patient compliance. This route may be good for the management of chronic inflammation. However, the barriers from the outermost layer of the skin could limit the effectiveness of transdermal transport. There are several simple and widely used techniques to overcome the barrier properties of the stratum corneum. One such technique is the combination of delivery vehicles and the use of permeation enhancers.

Various vehicles, lipophilic (such as isopropyl myristate (IPM), ethyl oleate) and hydrophilic (such as ethanol, propylene glycol (PG)), have been widely utilized for transdermal delivery. These vehicles enhance permeation by increasing both the thermodynamic activity of permeants and the skin to vehicle partition coefficient, as well as by changing the barrier properties of the skin. These vehicles were used alone or in combination with others. It has been reported that a combination of lipophilic and hydrophilic vehicles offers a synergistic permeation enhancement of lipophilic drugs through the skin, resulting in decreased skin irritation (13). Among available lipophilic vehicles, IPM is one of the most commonly used in topical and transdermal products. Its ability to enhance drug permeation has been extensively reported (14). A mechanistic study suggested that IPM act as a permeation enhancer resulted from incorporation into the stratum corneum lipids matrix, and perturbation of the multilamellar lipid arrangement (14).

Fatty acids are one of the most widely used permeation enhancers due to the fact that fatty acids appear as endogenous components of human skin, and thus their ability to enhance transdermal permeation of both lipophilic and hydrophilic substances. The enhancing effect of fatty acids depends on their structure and the number of carbon atoms. In general, fatty acids with carbon numbers of 10–18 have been used (15). However, the choice of a suitable fatty acid relies on the physicochemical properties of both the permeant and the vehicle used.

To our knowledge, there is currently no published information supporting the possibility of developing a transdermal application of *K. parviflora* ethanolic extracts, nor is there information regarding the permeation performance of methoxyflavones. Hence, the objective of this study was to investigate the permeation behavior of *K. parviflora* methoxyflavones using IPM-based vehicles. Furthermore, the effect of ethanol and PG on solubility and the permeation-enhancing effects of various fatty acids were examined.

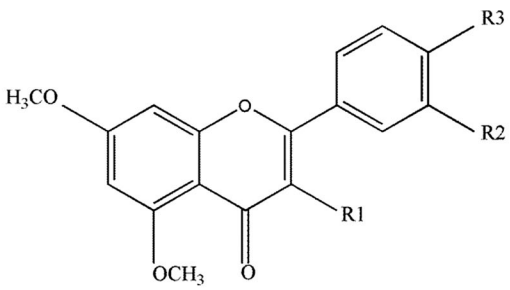
MATERIALS AND METHODS

Materials

Fresh *K. parviflora* rhizomes were obtained from Loei province (Thailand). Isopropyl myristate (IPM) was obtained from S. Tong Chemical Co. Ltd (Bangkok, Thailand). Propylene glycol (PG), capric acid, lauric acid, myristic acid, palmitic acid, linoleic acid, oleic acid, and polyethylene glycol 400 (PEG 400) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stearic acid was obtained from Labo chemie (Mumbai, India). Ethanol and methanol were purchased from Prolabo (Fontenay-sous-Bois, France) and Fisher® Scientific (Loughborough, England), respectively. All chemicals were analytical grade or better and were used as received.

Extraction of *K. parviflora* Ethanolic Extract

The *K. parviflora* ethanolic extract was prepared following our previously described procedure (11). In brief, the *K. parviflora* rhizomes were sliced, then dried at 45°C in a hot air oven. The dried powder was continuously extracted by maceration with 95% ethanol. After filtration, the residue was re-macerated. The combined filtrates were rotary evaporated and lyophilized to obtain a solid extract with 5.13% yield. Dry *K. parviflora* ethanolic extracts was kept at −20°C for further study. The amount of *K. parviflora* ethanolic extract applied in each experiment was standardized by the HPLC method described in "Quantitative Determination of Methoxyflavones in *K. parviflora* Ethanolic Extract".



Methoxyflavones	R1	R2	R3	MW	Calculated log *K*[a]	Melting point (°C)
3, 5, 7, 3′, 4′-Pentamethoxyflavone (PMF)	OCH_3	OCH_3	OCH_3	372	2.37	151.2
5,7-Dimethoxyflavone (DMF)	H	H	H	282	3.27	201.7
5, 7, 4′-Trimethoxyflavone (TMF)	OCH_3	H	H	312	3.53	156.9

[a] Log *K* was calculated using Advanced Chemistry Development (ACD/Labs) software.

Fig. 1. Structures and physicochemical properties of methoxyflavones (PMF, DMF, TMF): (modified from Sutthanut et al., 2009)

Quantitative Determination of Methoxyflavones in *K. parviflora* Ethanolic Extract

The HPLC assay to analyze the methoxyflavones (PMF, DMF and TMF) in the *K. parviflora* ethanolic extract was validated in term of sensitivity, linearity of the calibration curve, precision, and accuracy. The quantity of methoxyflavones in the *K. parviflora* extract was determined using a reverse phase HPLC system (Agilent® 1200 series, Germany) and an Agilent hypersil ODS column (C18, 5 μm, 4.6×250 mm). The analytical system included a quaternary pump with a VWD detector at a wavelength of 254 nm. The column temperature was set at 55°C. The mobile phases consisted of a mixture of water and methanol (35:65 to 70:30), at flow rate of 0.8–1.0 ml/min. The injection volume was 20 μl. The standards of PMF, DMF, and TMF were obtained from the *K. parviflora* extract using column chromatography as previously described (1).

Determination of the Solubility of Methoxyflavones

The solubilities of methoxyflavones in vehicles including IPM, ethanol/IPM, and PG/IPM systems, with or without fatty acids, were determined by dissolving an excess amount of *K. parviflora* ethanolic extract in known volumes of the vehicles. The mixtures were then shaken in a digital shaking water bath (Julabo SW-22, Seelbach, Germany) at 32±1°C and 100 rpm for at least 2 days until the concentration equilibrium of each major compound was achieved. After filtration through a nylon syringe filter (0.45 μm, 13 mm, Millipore filter, Millipore, Bedford, MA), the filtrate was analyzed for the methoxyflavones (PMF, DMF, and TMF) by HPLC as described in "Quantitative Determination of Methoxyflavones in *K. parviflora* Ethanolic Extract".

In Vitro Permeation of Methoxyflavones

In vitro permeation experiments were conducted using porcine ear skin and saturated solutions of *K. parviflora* ethanolic extract in IPM vehicles. IPM vehicles were as follows: IPM alone; ethanol/IPM prepared at volume ratios of 1:9, 2:8, and 3:7, and PG/IPM prepared at volume ratios of 1:9 and 2:8. In addition, the permeation enhancing capacity of fatty acids based on the vehicle effect was also studied. The experiments used five saturated fatty acids: capric acid, lauric acid, myristic acid, palmitic acid, and stearic acid; and two unsaturated fatty acids: oleic acid and linoleic acid.

Preparation of Saturated K. parviflora Extract Solution

The binary vehicles were prepared by thoroughly mixing the determined volume of IPM with that of ethanol or PG. In order to prepare the vehicles containing fatty acid, each fatty acid was weighted and dissolved in the vehicle to form a 5% *w/v* solution. Saturated solutions of *K. parviflora* extract were prepared by shaking an excess amount of *K. parviflora* ethanolic extract in the designated vehicles at 32°C using a digital shaking water bath until equilibrium was achieved.

Preparation of Pig Ear Skins

Porcine ears were obtained from a local slaughter house. Based on the guidelines of Khon Kaen University Animal Care committee, epidermal skin was prepared by the heat-separation technique. After cleaning the ears with tap water, the whole ear was immersed in water at 60°C for 45 s. The intact epidermis was subsequently teased off from dermis using forceps and a scalpel, washed with water, and then wrapped in aluminum foil. The epidermis was stored at −20°C until use, which occurred within 1 week of separation.

Procedure for In Vitro Permeation Study

The permeation experiment used side-by-side diffusion cells (Crown glass company, Inc, NJ) with an effective diffusion area of 0.64 cm^2 and a 3-ml cell volume maintained at 32±0.5°C. The frozen porcine ear epidermis was thawed and immersed in phosphate-buffered saline (PBS), pH 7.4, for 1 h. The thickness of the hydrated skin was determined by using a vernier caliper (Mitutoyo, Japan) before being securely mounted between the donor and receptor compartments. The receptor compartment was filled with pre-warmed (32±0.5°C) fresh medium. A saturated solution containing excess solid ethanolic extract of *K. parviflora* was placed in the donor compartment. PBS (pH 7.4) containing 40% PEG400 was used as a receiver medium. At predetermined times, a 1.0-ml sample was taken from the receptor compartment and an equal volume of receiver medium was immediately added. The concentration of methoxyflavones in the samples was determined using HPLC as described above (Quantitative Determination of Methoxyflavones in *K. parviflora* Ethanolic Extract).

Data Analysis

The resulting data were used to plot the cumulative amount of methoxyflavones permeating per unit area *versus* time. The slope of the linear portion of the plot was calculated as the flux (J) (μg/cm^2 h) from Fick's law of diffusion as described by Watkinson (2002) (16). The lag time (T_{lag}) was determined by extrapolating the linear portion of the cumulative amount of methoxyflavones permeated *versuss* time. The permeability coefficient (P), the partition coefficient of solute between skin and vehicle (K), and the diffusion coefficient (D) were calculated by using Eqs. (1), (2), and (3), respectively.

$$P = J/C_0 \tag{1}$$

$$K = \mathrm{Ph}/D \tag{2}$$

$$D = h^2/6T_{lag} \tag{3}$$

Where C_0 and h represent the constant concentration of donor solution and the thickness of skin, respectively.

Statistical Analysis

All data were expressed as mean±standard error (S.E.). Statistical analysis of the experimental data was carried out using SPSS (version 19). The significance of differences among groups was analyzed by using one-way ANOVA. The criterion for statistical significance was at $p<0.05$.

RESULTS AND DISCUSSION

Validation of HPLC

The HPLC assay was demonstrated to be of good linearity, precision, and high accuracy for the three target compounds PMF, DMF, and TMF (Table I). The linear regression equations of the three methoxyflavones showed good linear relationships with R^2 values greater than 0.9990 (linearity range 10–200 μg/ml). Intra-day and inter-day precision for PMF, DMF, and TMF at concentrations of 25–100 μg/ml ranged from 0.25 to 4.49%RSD and from 1.84 to 3.78%RSD, respectively, indicated that the proposed method is high precision. The accuracy of the method was determined for three concentrations (20, 50, and 100 μg/ml). The high percentage of recovery of PMF, DMF, and TMF ranging from 96.26–100.77 indicated that the proposed method is highly accurate. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.05–1.20 μg/ml and 0.15–2.00 μg/ml, respectively, which indicated the good sensitivity of the method. Figure 2 shows the HPLC chromatograms of PMF, DMF, and TMF with retention times of 8.2, 11.3, 22.3 min, respectively. Based on the HPLC analysis, it was determined that the crude *K. parviflora* ethanolic extract contained several methoxyflavones, of which PMF, DMF, and TMF were predominant with concentrations of 11.3, 12.7, and 13.2%, respectively. Therefore, PMF, DMF, and TMF were used as the chemical markers in this study.

Effect of Ethanol and PG in the IPM-Based Vehicle

The solubility of methoxyflavones in IPM alone was 15.6 ±0.4 mg/ml. Addition of ethanol to IPM significantly increased the solubility of methoxyflavones ($p<0.05$) at all ratios, and the solubility increased in proportion to the increase in the volume fraction of ethanol (Table II). The solubilities of methoxyflavones in vehicles containing 1:9, 2:8, and 3:7 ethanol/IPM volume ratios increased, as compared to the IPM alone by 6.0, 11.7, and 13.1 times, respectively. Adding PG to IPM also influenced the solubility of methoxyflavones. However, only the 1:9 PG/IPM vehicle yielded significantly higher methoxyflavones solubility (28.5±1.3 mg/ml) as compared to that of IPM (control). It should be noted that the solubility of methoxyflavones tended to decrease as the volume fraction of PG increased. Using the same ratio of hydrophilic solvents (ethanol or PG) in the IPM-based vehicle, methoxyflavones preferred to dissolve in the ethanol/IPM vehicle rather than the PG/IPM vehicle. The solubility of methoxyflavones in 1:9 ethanol/IPM and 1:9 PG/IPM was 94.2±1.4 and 28.5±1.3 mg/ml, respectively. Ethanol was therefore preferable to PG as a hydrophilic solvent for increasing the solubility of methoxyflavones.

The solubility of methoxyflavones in aqueous medium was also examined. It was not surprising that methoxyflavones were only very slightly soluble in 0.2 M PBS (pH 7.4) (0.47± 0.02 mg/ml), approximately 33 times lower than solubility in IPM. This can be explained simply by the solubility rule of thumb: "like dissolves like". IPM is a lipophilic solvent with a solubility parameter (δ) of 8.5 $(cal/cm^3)^{1/2}$, whereas water possesses a solubility parameter of 23.5 $(cal/cm^3)^{1/2}$. Ethanol and PG are commonly used hydrophilic solvents with modest polarity, and according to the solubility parameters, ethanol (12.9 $(cal/cm^3)^{1/2}$) is a less polar solvent than PG (14.8 $(cal/cm^3)^{1/2}$) (17). The binary combination of ethanol and IPM dissolved *K. parviflora* methoxyflavones more efficiently than PG/IPM. This result is in agreement with a previous study which compared the effects of similar ratios of ethanol/IPM and PG/IPM on the solubility of the antihypertensive lipophilic drug indapamide (log $K\approx2.52$) (18).

The permeation of methoxyflavones through porcine ear skin was performed by using side-by-side diffusion cells. Because porcine ear skin has histological characteristics and permeability properties comparable to human skin (19), it is widely used in permeation studies. Saturated solutions with excess solid extract of *K. parviflora* were used in order to equalize and maintain the maximum thermodynamic activity of the permeants in each donor phase throughout the permeation experiments. PBS (pH 7.4) containing 40% PEG 400 was used as a receptor medium in order to maintain the sink conditions. Addition of 40% PEG 400 into PBS resulted in an

Table I. Validation of HPLC

Methoxyflavones	Linearity (R^2)	LOD (μg/ml)	LOQ (μg/ml)	Accuracy: Concentration (μg/ml) 25	50	100	Precision: Intra-day concentration (μg/ml) 25	50	100	Inter-day concentration (μg/ml) 25	50	100
PMF	0.9990	0.1	0.2	97.2±0.0	99.9±0.1	99.6±0.1	23.5±0.0	49.8±0.1	99.6±0.1	23.5±0.4	49.2±0.7	99.2±1.4
DMF	0.9997	0.2	0.5	96.3±0.3	99.5±0.0	97.7±0.1	23.8±0.3	49.4±0.2	97.7±0.1	23.5±0.3	48.6±0.4	99.0±1.0
TMF	0.9986	1.2	2.0	100.6±0.2	100.8±0.6	99.9±0.5	24.7±0.5	51.1±0.6	98.5±1.0	25.1±0.3	50.6±0.8	104.3±1.1

Values are expressed as mean±S.E., $n=5$
LOD limit of detection, *LOQ* limit of quantitation

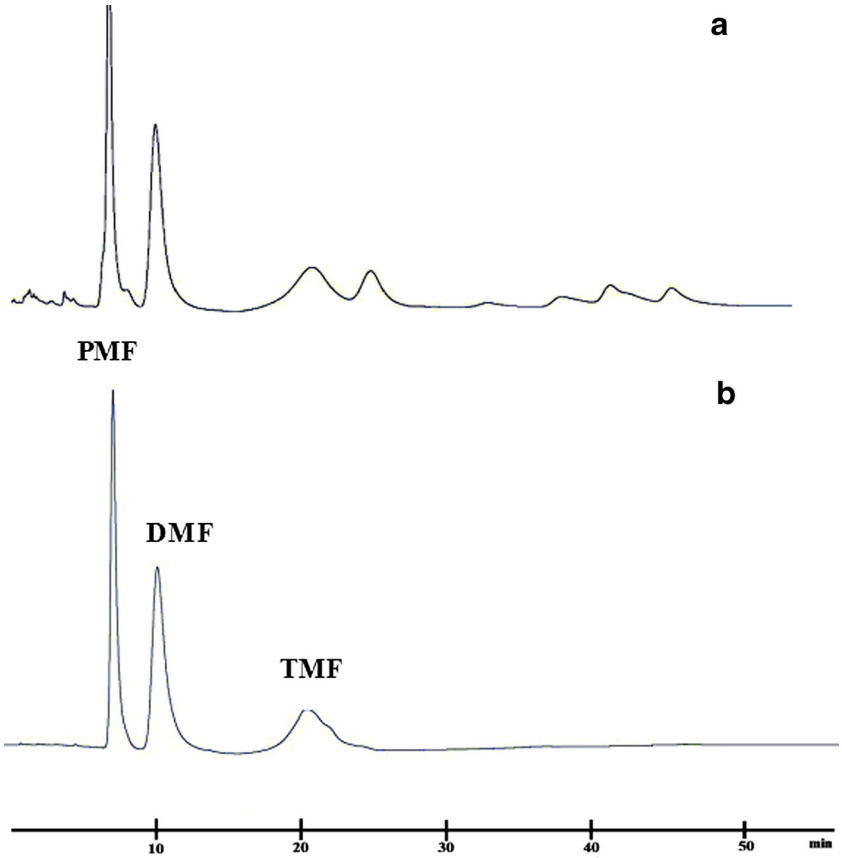


Fig. 2. HPLC chromatograms of *K. parviflora* ethanolic extract (**a**) and standard methoxyflavones (**b**). The retention times of PMF, DMF, and TMF were 8.2, 11.3 and 22.3 min, respectively

increase in solubility of the methoxyflavones to 40.6±0.7 mg/ml, which was approximately an 80-fold increase to that of PBS alone.

The permeation profiles of methoxyflavones in various ethanol/IPM and PG/IPM vehicles are shown in Fig. 3. The permeations of methoxyflavones from ethanol/IPM vehicles were higher than those obtained from IPM alone (Fig. 3a). Interestingly, when different ethanol/IPM ratios are compared, the methoxyflavones permeations decreased when the volume ratio of ethanol in the vehicles increased. The permeation profiles of methoxyflavones obtained from PG/IPM vehicles were only slightly higher than those from IPM alone (Fig. 3b). For all ethanol/IPM mixtures investigated, the steady state fluxes of methoxyflavones were significantly increased as compared with IPM ($p<0.05$). The flux of three methoxyflavones in the 1:9 ethanol/IPM vehicle increased from 62.7±5.2 μg/cm^2/h to 314.7±9.7 μg/cm^2/h, which was fivefold greater than IPM alone. The flux of each methoxyflavones increased from 30.6±1.9 μg/cm^2/h to 118.2 ±2.5 μg/cm^2/h for PMF, 17.3±2.2 μg/cm^2/h to 94.6±3.2 μg/cm^2/h for DMF, and 11.0±1.31 μg/cm^2/h to 77.6±3.9 μg/cm^2/h for TMF. When different ethanol/IPM ratios are

Table II. Solubility and permeation parameters of methoxyflavones from saturated solutions of *K. parviflora* ethanolic extract in various IPM-based vehicles through porcine ear skin

Vehicle	Vehicle *S*olubility (mg/ml)	Flux (μg/cm^2/h)	Permeability coefficient (cm/h)×10^{-3}	Lag time (*h*)	Diffusion coefficient (cm^2/h)×10^{-3}	Partition coefficient
IPM (control)	15.6±0.4	62.7±5.2	4.0±0.3	3.2±0.1	0.77±0.05	0.64±0.07
1:9 Ethanol/IPM	94.1±1.4*	314.7±9.7*	3.3±0.1	3.6±0.2	0.75±0.01	0.57±0.03
2:8 Ethanol/IPM	182.4±0.4*	264.2±19.4*	1.4±0.1*	3.3±0.0	0.77±0.04	0.23±0.02*
3:7 Ethanol/IPM	204.4±4.5*	198.9±15.0*	1.0±0.1*	2.6±0.4	0.86±0.22	0.14±0.03*
1:9 PG/IPM	28.5±1.3*	78.0±0.6	2.7±0.0*	4.0±0.1*	0.70±0.01	0.51±0.01
2:8 PG/IPM	24.6±2.4	81.6±0.3	3.3±0.0	4.2±0.2*	0.67±0.04	0.64±0.03

Values are expressed as mean±S.E., $n=3$
IPM isopropyl myristate, *PG* propylene glycol
*Significantly different from control at $p<0.05$

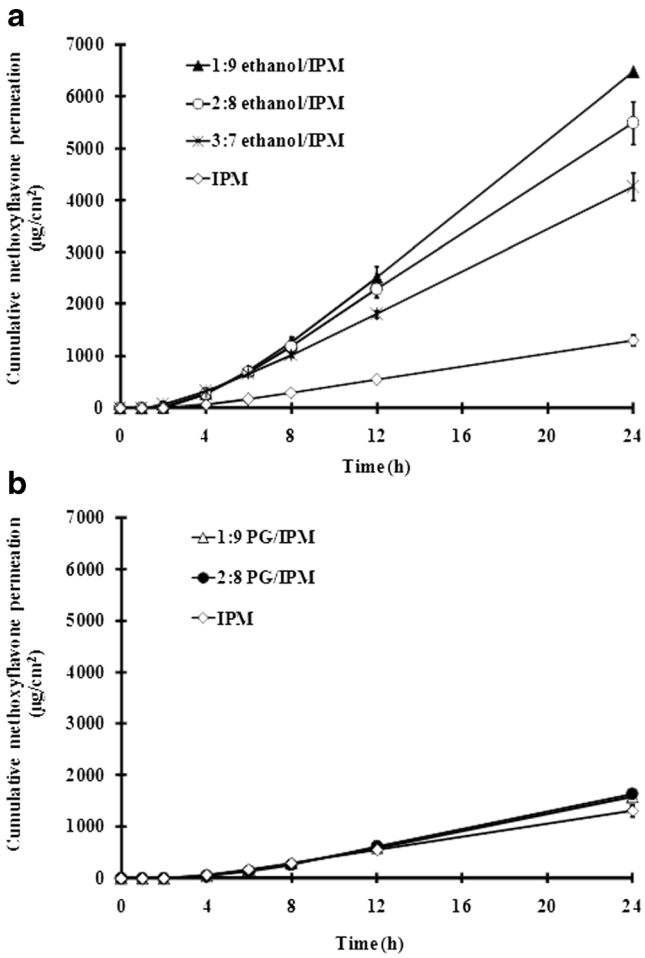


Fig. 3. Permeation profiles of methoxyflavones through porcine ear skin from ethanol/IPM (**a**) and PG/IPM (**b**) vehicles (each point represents the mean±S.E., n=3)

compared, the fluxes and the corresponding permeability coefficients were in the rank order of 1:9>2:8>3:7 ethanol/IPM vehicles. Interestingly, the permeability coefficients of methoxyflavones from all ethanol/IPM vehicles were lower than that from IPM alone and decreased with the volume ratio of ethanol. This can be attributed to the lower partition coefficient effect of these vehicles. The partition coefficient of vehicle to skin depends upon the solubility of the substance in its vehicle, which in turn affects the permeation rate of the substance. Adding ethanol to IPM increased the methoxyflavones solubility which consequently decreased the partition coefficient of methoxyflavones between skin and vehicle. The significant increases in the flux values of ethanol/IPM vehicles can therefore be attributed primarily to the enhanced solubility of methoxyflavones in ethanol/IPM vehicles, which provided an increase in the concentration gradient across the skin.

There were no significant differences in the lag times between ethanol/IPM vehicles and IPM. The diffusion coefficients of methoxyflavones from ethanol/IPM vehicles (1:9 to 3:7) were therefore assumed to be comparable to those from IPM as lag time is a permeation parameter that depends mainly on the diffusion coefficient of the permeant through the skin.

IPM, the most common fatty acid ester used in topical and transdermal formulations, was previously assumed to be able to perturb the structure of lipids, resulting in a lower permeation resistance of the stratum corneum (14). Ethanol, a solvent-type enhancer, was reported to function as a permeation enhancer by altering the barrier structure of the stratum corneum through lipid extraction and lipid fluidization mechanisms (20). The application of an ethanol and IPM vehicle to enhance the permeation capacity of drugs was previously reported. The fluxes across porcine ear skin of the hydrophilic zidovudine from ethanol/IPM vehicles (20, 30, and 40% ethanol in IPM) were increased with the volume fraction of ethanol (13). El Maghraby et al. (2009) (21) had reported that the transdermal delivery of tadalafil, a lipophilic compound, was higher in ethanol/IPM vehicles than in ethanol or IPM, with the fluxes increased with increasing ethanol concentration in the mixtures (2:1>1:1>1:2 ethanol/IPM vehicles). Similar enhancement of IPM-based vehicles with short-chain alkanols in transdermal delivery has been demonstrated. The synergistic mechanisms as permeation enhancers of binary vehicles are related to the increase in the solubility in the vehicles and the skin-to-vehicle partition coefficient, as well as the modulation of the skin barrier property (13).

Although an increase in the volume ratio of ethanol in the IPM-based vehicles can increase the degree of permeation for some substances (21), our results did not show an increase for methoxyflavones. Table II showed that a higher ethanol volume ratio resulted in a decreased skin-to-vehicle partition coefficient for methoxyflavones when compared with the IPM-only control vehicle. For example, skin-to-vehicle partition coefficient of methoxyflavones in 3:7 ethanol/IPM and 1:9 ethanol /IPM was 0.14±0.03 and 0.57±0.03, respectively. The apparent solubility of methoxyflavones in the skin could be estimated from their correlating solubility in the vehicles. The correlations between vehicle solubility, apparent skin-to-vehicle partition coefficient and apparent solubility of methoxyflavones in the skin are shown in Fig. 4. It was clearly demonstrated that addition of ethanol into the IPM-based vehicles can enhance the methoxyflavones solubility and decrease the partition coefficient, which consequently affects the apparent solubility of methoxyflavones in the skin. Previously, an inverse relationship between the solubility and the epidermal partition coefficient has been reported (21). The epidermal partition coefficients were inversely proportional to the ratio of ethanol to IPM and the solubility of paclitaxel in ethanol/IPM vehicles (22). Transdermal delivery is a complex phenomenon influenced by the permeation-enhancing potency of the vehicle, the release from the vehicle, and the partitioning into the skin. Therefore, the lower methoxyflavones permeation flux in the higher ratio ethanol/IPM vehicles could be explained by the higher affinity of methoxyflavones to higher ratio ethanol/IPM vehicles, causing the lowered tendency of methoxyflavones to migrate and partition into the skin.

In the case of PG/IPM vehicles, no significant increase in the methoxyflavones flux was observed for both the 1:9 and 2:8 PG/IPM vehicles when compared with that of the IPM-only control vehicle. These results may be due to the low solubility enhancement power of PG/IPM vehicles. The lag

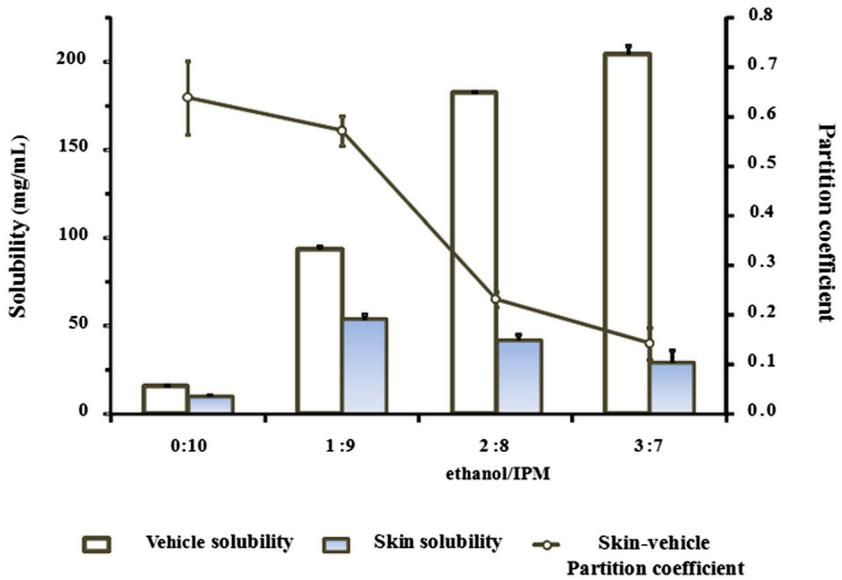


Fig. 4. Correlation between vehicle solubility, skin solubility, and skin-to-vehicle partition coefficient of methoxyflavones permeation ($n=3$)

times of methoxyflavones permeation for the 1:9 and 2:8 PG/IPM vehicles increased significantly to be 4.0±0.1 and 4.2±0.2 h, respectively, whereas the lag time for the IPM-only control vehicle was 3.2±0.1 h. The longer lag time has been attributed to the decrease in the diffusion coefficient. Nevertheless, in order for diffusion to take place, the permeant has to be released and partitioned into the skin. Therefore, lag time relies indirectly on the release process. Our finding demonstrated that the vehicle composition was an important factor to affect the skin permeation and lag time of methoxyflavones.

Effect of Fatty Acids in the Ethanol/IPM Vehicle

The solubilities of methoxyflavones in the 1:9 ethanol/IPM vehicle containing 5% of various fatty acids were shown in Table III. Among the fatty acids investigated, the solubility of methoxyflavones in the 1:9 ethanol/IPM vehicle was significantly affected only when adding stearic acid and linoleic acid, which increased to 121.4±1.4 and 135.3±6.6 mg/ml, respectively.

Addition of 5% fatty acid to the 1:9 ethanol/IPM vehicle had no effect on the pattern of methoxyflavones permeation through porcine ear skin (data not shown). The permeation flux of methoxyflavones was significantly enhanced only when oleic acid was used; however, this was only a 1.1-fold increase in the permeation enhancement as compared to that of the control. The addition of other fatty acids showed minor effect on solubility, and flux of methoxyflavones when compared to the control vehicle (1:9 ethanol/IPM vehicle). Since several mechanisms have been proposed for the permeation enhancement effect of fatty acids, including an increase in drug solubility, a reduction of the skin permeation resistance by interacting with stratum corneum components and disrupting their highly ordered domains, an increase the skin to vehicle partitioning, and increase the transport of solvents into/across the skin (23). This implies that fatty acids may affect the cutaneous permeation via the similar mechanisms as IPM and ethanol already did to the skin structure.

On the other hand, the lag time was influenced by all of the fatty acids investigated. The lag time was significantly

Table III. Effect of 5% fatty acids (*w/v*) on the solubility and permeation parameters of methoxyflavones from saturated solutions of *K. parviflora* ethanolic extract in 1:9 ethanol/IPM (control) vehicles through porcine ear skin

Vehicle	Vehicle solubility (mg/ml)	Flux ($\mu g/cm^2/h$)	Permeability coefficient $(cm/h)\times10^{-3}$	Lag time (h)	Diffusion coefficient $(cm^2/h)\times10^{-3}$	Partition coefficient
Control	94.1±1.4	314.7±9.7	3.3±0.1	3.6±0.2	0.75±0.01	0.57±0.03
Capric acid (C10)	86.0±2.8	293.5±8.2	3.4±0.1	2.8±0.3	0.83±0.06	0.49±0.03
Lauric acid (C12)	104.4±8.2	288.3±13.5	2.8±0.1	2.9±0.2	0.82±0.16	0.42±0.06
Myristic acid (C14)	83.2±4.4	282.7±15.5	3.4±0.2	2.7±0.1*	0.84±0.04	0.47±0.02
Palmitic acid (C16)	107.8±8.9	338.9±16.1	3.1±0.1	2.2±0.2*	0.91±0.03*	0.38±0.02*
Stearic acid (C18)	121.4±1.4*	328.6±23.8	2.7±0.2*	2.3±0.4*	0.91±0.03*	0.33±0.01*
Oleic acid (C18:1)	109.8±5.3	358.5±12.1*	3.3±0.1	2.7±0.2*	0.85±0.02*	0.45±0.03*
Linoleic acid (C18:2)	135.3±6.5*	338.9±25.6	2.5±0.2*	2.7±0.3*	0.84±0.09	0.35±0.03*

Values are expressed as mean±S.E., $n=3$
IPM isopropyl myristate
*Significantly different from control at $p<0.05$

decreased to 2.2–2.7 h by addition of myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid. The decrease in the lag time clearly resulted from the increase in the diffusion coefficients. The calculated diffusion coefficients based on the permeation data revealed that the addition of fatty acids with 10–18 carbon atoms resulted in an increase in the diffusion coefficients compared to that of the control (1:9 ethanol/IPM vehicle). This effect was more pronounced with the longer fatty acid chain. The increase in diffusion coefficients caused by enhancers like fatty acids is attributed to the disruption of stratum corneum lipid organization, increasing the free volume available for drug diffusion (24). Our findings are in accordance with previous reports that fatty acids decrease the permeation lag time of drugs such as melatonin (15).

Addition of fatty acids also lowered the apparent partition coefficients, of which the longer alkyl chain fatty acids (palmitic acid, stearic acid, and linoleic acid) showed the greatest effect. This might be attributed to the higher affinity of methoxyflavones to vehicles containing fatty acids. The decrease in partition coefficients with addition of fatty acids (C8–C12, C18:1, C18:2) was previously observed with physostigmine permeation in a mineral oil base vehicle (25).

The enhancement capability and the ultimate permeation effects of fatty acids depends on the physicochemical properties of the permeants and the enhancers, as well as the vehicle used (26). It has been reported that the enhancing capacity of fatty acids is influenced by the alkyl chain length, bond saturation, and the branching of the fatty acids. Kim and coworkers (2008) (27) reported that among the saturated fatty acids with 12–20 carbon units, palmitic acid was the most effective for increasing the permeation rate of diclofenac. The permeation of propranolol was enhanced by fatty acids with a certain chain length (approximately 16 carbons) due to an optimal balance between partition coefficient or solubility parameter and affinity to skin (28). The permeation of lornoxicam through excised hairless mouse skin was reported to increase with the chain length of the fatty acids, in the rank order of linoleic acid > oleic acid > lauric acid > capric acid (29).

Moreover, the results show that only oleic acid significantly enhanced the flux of methoxyflavones which is consistent with previous studies. Chi et al. (1995) (30) reported an increase of 6.5-fold to 17.5-fold in the permeation rate of flurbiprofen through rat skin by unsaturated fatty acids, while no significant increase was observed with saturated fatty acids. Generally, saturated fatty acids are less effective than unsaturated derivatives when comparing the same chain length. Unsaturated fatty acids are likely to be more effective in enhancing percutaneous absorption of drugs. It has been suggested that the presence of double bonds causes the formation of kinks in the lipid structure. This causes a mobile structural defect representing free volumes in the hydrocarbon phase of the membrane, resulting in increased fluidity, permitting small molecules to enter the free volumes of the kinks and migrate across the membrane together with these kinks (31).

However, the permeation of methoxyflavones in the 1:9 ethanol/IPM vehicle when adding investigated fatty acids showed no distinct relationship between the number of carbon atoms (C10-C18) or the number of double bonds (0, 1, or 2) in the chain of fatty acids and its permeation-enhancing ability. There was no significant difference in the flux, the lag time or the diffusion coefficient between the investigated fatty acids. However, because of the significant increase of methoxyflavones solubility in stearic acid (C18) and linoleic acid (C18:2) containing vehicles, the partition coefficients and consequently the permeability coefficients were significantly altered as compared to those of capric acid and myristic acid.

The multicomponent vehicles (5% oleic acid in the 1:9 ethanol/IPM vehicle) provided a high flux of methoxyflavones and decreased lag time. The flux of total methoxyflavones was in a considerable amount, which was 358.5 ± 12.1 μg/cm^2/h (152.1 ± 11.3 μg/cm^2/h, 90.9 ± 7.7 μg/cm^2/h, and 81.1 ± 7.7 μg/cm^2/h for PMF, DMF, and TMF, respectively), suggesting the possibility to exhibit anti-inflammatory effect.

CONCLUSION

The present study is a first analysis of the transdermal permeation of methoxyflavones from *K. parviflora* ethanolic extract. We have demonstrated effective transdermal delivery of the *K. parviflora* ethanolic extract via the enhancement effect of multicomponent vehicles. The solubility and consequently the permeation of methoxyflavones can be effectively modulated by alternating delivery vehicles and changing their ratios. The binary vehicles containing ethanol and IPM were more appropriate for methoxyflavones, with the 1:9 ethanol/IPM vehicle having the maximum associated flux. Addition of 5% fatty acids into the 1:9 ethanol/IPM vehicle could further alter the permeation by modulating the diffusion coefficient and partition coefficient parameters. Among the investigated fatty acids, in terms of flux and lag time, oleic acid showed the greatest enhancing effect on the permeation of methoxyflavones. All of the obtained permeation data for methoxyflavones provided the permeation behavior of herbal flavones component, indicating the feasibility of a skin application delivery method of the plant extract. Transdermal delivery of methoxyflavones might be an alternative delivery to minimize the high oral dose of methoxyflavones. The advantages by using this route will help to improve the patient compliance; also, transdermal products should be further developed to provide an optimal formulation for delivering *K. parviflora* extract to systemic systems.

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Conflict of Interest All authors declare no conflict of interest with the content of this article.

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