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# A comparison of skin delivery of ferulic acid and its derivatives: Evaluation of their efficacy and safety

Li-Wen Zhang[a], Saleh A. Al-Suwayeh[b], Pei-Wen Hsieh[c], Jia-You Fang[a,b,*]

[a] Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan
[b] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[c] Medicinal Chemistry Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan

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ABSTRACT

Ferulic acid (FA) can be used as an antioxidant to prevent damage from ultraviolet (UV) radiation and skin carcinogenesis. To this end, the feasibility of the skin absorption of FA and its derivatives was evaluated in the present study. The percutaneous absorption of five compounds into/across porcine skin was measured and compared using Franz diffusion cells. The skin delivery from pH 6 and 9.9 buffers was the highest for ferulic acid ethyl ether (FAEE), followed by coniferyl aldehyde (CD), coniferyl alcohol (CA), FA, and 3-hydroxy-4-methoxycinnamic acid (HMA). The skin deposition and flux of FAEE with a pH 6 buffer were 136 nmol/g and 26 nmol/cm$^2$/h, respectively. No significant difference in permeation profiles was observed between the two pH buffers. According to permeation via the skin with different treatments (delipidization, ethanol, and oleic acid treatments), it was determined that the lipid bilayers in the stratum corneum (SC) comprised the predominant barrier for FA permeation. On the other hand, FAEE could easily partition into and penetrate across the skin through intercellular pathways. Nude mouse was used as an in vivo animal model to examine the amount of permeants remaining in the skin. The in vivo skin deposition was generally correlated with the in vitro results. The in vivo skin deposition of FAEE (145 nmol/g) was comparable to that of CD (150 nmol/g). The safety study which examined transepidermal water loss (TEWL), erythema, and the skin pH value demonstrated that the topical application of FA and related compounds for up to 24 h did not cause skin irritation. It can be concluded that topical delivery may serve as an efficient and safe route for FA and its derivatives against photodamage.

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## 1. Introduction

Ultraviolet (UV) irradiation is a potent generator of oxidative stress in the skin. Exposure of skin to UV increases cellular levels of reactive oxygen species (ROS), which damage lipids, proteins, and nucleic acids in both epidermal and dermal cells and contributes to the sunburn reaction as well as photocarcinogenesis and photoaging (Pinnell, 2003). The use of sunscreens as the only measure to prevent the incidence of UV-mediated oxidative damage has proven to be insufficient. In this regard, prevention of UV damage using natural antioxidants is suggested as a promising approach and has generated enormous research efforts in recent times (Gu et al., 2005). Among many available antioxidants, ferulic acid (FA) has aroused great interest because of its strong antioxidant activity (Ou and Kwok, 2004). FA, 4-hydroxy-3-methoxycinnamic acid (Fig. 1), is an antioxidant naturally present in plant cell walls, especially in the bran of grasses such as wheat, rice, and oats (Alias et al., 2009). It has a phenolic nucleus and a long side chain so it readily forms a resonance stabilized phenoxy radical with high antioxidant and anti-inflammatory activities (Picone et al., 2009). FA was proven to afford significant protection to the skin against UV-induced erythema (Saija et al., 2000; Oresajo et al., 2008). FA has also been proposed for treating several age-related diseases such as neurodegenerative disorders, cardiovascular diseases, diabetes, and cancers (Barone et al., 2009).

Oral administration with several antioxidants was reported to provide protection to the skin and other organs (Di Giacomo et al., 2009). Most antioxidant protection depends on dietary intake and subsequent delivery to the skin. Because antioxidants are destroyed or altered by oxidation during neutralization, protection is often limited by the concentration of antioxidants remaining in the skin (Murray et al., 2008). FA undergoes a marked first-pass effect which limits its oral bioavailability. Only a low percentage of unmodified FA (9–20%) was found in the plasma (Bourne and Rice-Evans, 1998; Zhao et al., 2004). Delivery of antioxidants via the skin is an attractive alternative to oral dosing for both topical

* Corresponding author at: Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, 259 Wen-Hwa 1st Road, Kweishan, Taoyuan 333, Taiwan. Tel.: +886 3 2118800x5521; fax: +886 3 2118236.
*E-mail address:* fajy@mail.cgu.edu.tw (J.-Y. Fang).

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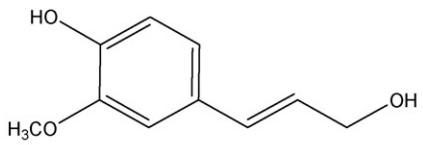
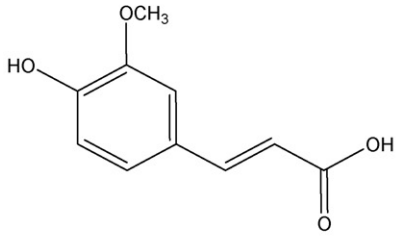
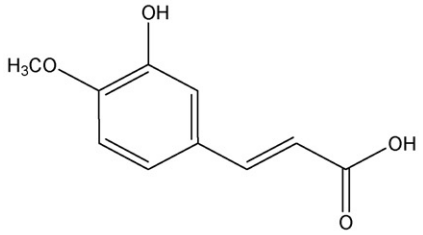


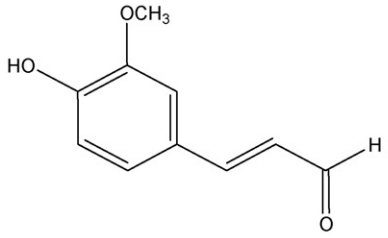

Coniferyl alcohol (CA)



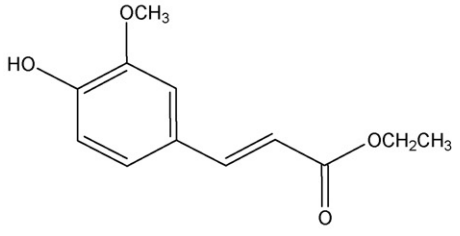

Ferulic acid (FA)




3-Hydroxy-4-methoxycinnamic acid (HMA)




Coniferyl aldehyde (CD)




Ferulic acid ethyl ether (FAEE)

**Fig. 1.** Chemical structures of ferulic acid and its derivatives.

and transdermal applications. Although FA was demonstrated to have significant activity on UV-irradiated skin, there is no information on the absorption and permeation parameters of FA in the skin.

In an attempt to augment the protective strategy of FA, the aim of this work was to establish basic profiles of FA permeation into and across the skin. A group of biosynthetically related FA derivatives, including coniferyl alcohol (CA), 3-hydroxy-4-methoxycinnamic acid (HMA), coniferyl aldehyde (CD), and ferulic acid ethyl ether (FAEE) (Fig. 1), was chosen to examine whether different characteristic groups at the end of the propenoic side chain would affect the permeation performance. The present study utilized Franz cells to explore the in vitro skin permeation of FA and its derivatives. The drug deposition retained within the skin reservoir was determined by in vitro and in vivo methods. Possible pathways of the compounds via the skin were elucidated using skin treated by various strategies as permeation barriers. Possible irritation of the compounds was also studied by in vivo bioengineering methods such as transepidermal water loss (TEWL), erythema ($a^*$), and skin surface pH.

## 2. Materials and methods

### 2.1. Materials

CA, FA, HMA, CD, FAEE, and oleic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents were analytical grade and were used as received.

### 2.2. High-performance liquid chromatography (HPLC) analytical method

The HPLC system for FA and its derivatives included an L-2130 pump, an L-2200 sample processor, and an L-2400 UV–visible detector all from Hitachi (Tokyo, Japan). A 25-cm-long, 4-mm inner diameter stainless steel RP-18 column (Merck, Darmstadt, Germany) was used as the stationary phase. The mobile phase was a mixture of methanol and pH 2.1 water adjusted by phosphoric acid (45:55) at a flow rate of 1 ml/min. The UV–visible detector was set at 325 nm. The log $K'$ value (capacity factor) of the compounds was determined isocratically using HPLC. The retention time of each compound was measured, and the $K'$ value was calculated from the following equation:

$$\log\ K' = \log\left[\frac{t_r - t_0}{t_0}\right];$$

where $t_r$ is the retention time of each compound, and $t_0$ is the retention time of the non-retained solvent peak (water).

### 2.3. Animals

Specific pathogen-free (SPF) pigs (1 week old) were supplied by the Animal Technology Institute Taiwan (Miaoli, Taiwan). Female nude mice (8 weeks old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The animal experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University. Animals were housed and handled according to institutional guidelines.

### 2.4. Preparation of skin membranes

In the in vitro experiment, the animals were first sacrificed. Full-thickness skin was excised from the dorsal region of the animals. The subcutaneous fat, tissue, blood vessel, and epidermal hairs were carefully removed. To obtain delipidized skin, the stratum corneum (SC) side was pretreated with chloroform-methanol (2:1) for 2 h. Five percent oleic acid in a 25% ethanol/water vehicle was used to pretreat the skin mounted on a Franz cell for 2 h before the in vitro skin permeation experiment.

### 2.5. In vitro skin permeation

Porcine or nude mouse skin was mounted on the receptor compartment of a Franz cell with the SC side facing upwards into the donor compartment and dermis facing the receptor compartment. The latter compartment was filled with 5.5 ml of pH 7.4 citrate-phosphate buffer containing 30% (v/v) ethanol, and maintained at 37 °C under constant stirring. The donor compartment

was filled with 0.5 ml of the vehicle containing FA or its derivatives at a concentration of 2.6 μM and occluded by parafilm. The available diffusion area between the compartments was 0.785 $cm^2$. At appropriate intervals, 300-μl aliquots of the receptor medium were withdrawn and immediately replaced with an equal volume of fresh medium.

For quantification of the compounds in the skin at the end of the in vitro experiment (48 h), the skin was removed from the cell, and the skin surface was cleaned with a cotton wool swab immersed in water and methanol three times each. The skin was then weighed, cut with scissors, positioned in a glass homogenizer containing 1 ml of methanol, and homogenized for 10 min at 300 rpm. The resulting solution was centrifuged for 10 min at 10,000 rpm and then filtered through a polyvinylidene difluoride membrane with a pore size of 0.45 μm. All samples were analyzed by HPLC.

### 2.6. In vivo skin permeation

Nude mouse was used as the animal model in the in vivo experiment. All animals were starved overnight prior to the experiment. A glass cylinder with an available area of 0.785 $cm^2$ was placed on the dorsal skin with glue (Instant Super Glue®, Kokuyo, Japan). An aliquot of 0.2 ml of pH 6 buffer with FA or the derivatives (2.6 μM) was added to the cylinder. The application time was 8 h. The application region of the skin was excised at the end of the experiment. The procedures for washing and extraction of the compound from the skin were the same as for the in vitro experiment.

### 2.7. In vivo skin tolerance test

A 0.6-ml aliquot of pH 6 buffer with FA or the derivatives was uniformly spread over a sheet of non-woven polyethylene cloth (1.5 cm × 1.5 cm), which was then applied to the back area of a nude mouse. The polyethylene cloth was fixed with Tegaderm® adhesive dressing (3 M, USA) and Fixomull® stretch adhesive tape (Beiersdorf AG, Germany). After 24 h, the cloth was removed, and the treated skin area was swabbed clean with a cotton wool swab. After withdrawal of the vehicle for 30 min, transepidermal water loss (TEWL), colorimetric parameters, and the pH of the applied skin were measured. TEWL was recorded using a Tewameter® (TM300, Courage & Khazaka, Köln, Germany). Measurements taken at a stable level were performed 30 s after application of the TEWL probe to the skin. TEWL was automatically calculated and expressed in $g/m^2/h$. A spectrocolorimeter (CD100, Yokogawa Electrical, Tokyo, Japan) was used to measure the skin erythema ($a^*$). The instrument records color reflectance three-dimensionally ($L^*$, $a^*$, $b^*$) as recommended by the CIE (Commission Internationale de l'Eclairage). When recording the color values, the measuring head was held perpendicular to the dorsal skin of the mouse, and the aperture was fitted with an applicator, to avoid compression of the subcutaneous capillaries. The reading was obtained within a few seconds on the display. The skin surface pH was determined by a Skin-pH-Meter® PH 905 (Courage & Khazaka, Germany). An adjacent untreated site was used as a baseline standard for each determination. The temperature and relative humidity in the laboratory were kept at 26 °C and 55%, respectively. The sample number for each experiment was five ($n = 5$).

### 2.8. Statistical analysis

A statistical analysis of differences between different treatments was performed using unpaired Student's $t$-test. A 0.05 level of probability was taken as the level of significance. An analysis of variance (ANOVA) test was also used if necessary.

**Table 1**
Physicochemical properties of ferulic acid and its derivatives.

| Compound | Formula | Molecular weight (Da) | log $K'$[a] | log $P$[b] | p$K_a$[b] |
|---|---|---|---|---|---|
| CA[c] | $C_{10}H_{12}O_3$ | 180.20 | 0.29 | 1.43 | 9.88 |
| FA[d] | $C_{10}H_{10}O_4$ | 194.18 | 0.47 | 1.67 | 4.58, 9.88 |
| HMA[e] | $C_{10}H_{10}O_4$ | 194.18 | 0.53 | 1.67 | 4.53, 9.00 |
| CD[f] | $C_{10}H_{10}O_3$ | 178.19 | 0.55 | 1.69 | 9.88 |
| FAEE[g] | $C_{12}H_{14}O_4$ | 222.24 | 1.45 | 2.24 | 9.88 |

[a] log $K'$, logarithm of $t_r - t_0/t_0$, $t_r$ is the retention time of product peak, $t_0$ is the retention time of solvent peak.
[b] log $P$ and p$K_a$, calculated from a molecular modeling software (Discovery Studio® version 2.0, Accelrys Inc., San Diego, USA).
[c] CA, coniferyl alcohol.
[d] FA, ferulic acid.
[e] HMA, 3-hydroxy-4-methoxycinnamic acid.
[f] CD, coniferyl aldehyde.
[g] FAEE, ferulic acid ethyl ether.

## 3. Results

### 3.1. Physicochemical characteristics of FA and its derivatives

The molecular weight (MW), lipophilicity (usually presented as the partition coefficient, log $P$), and ionization of compounds are important factors governing the percutaneous absorption of permeants. Some physicochemical properties of FA and its derivatives are summarized in Table 1. The lipophilicity ranking was evaluated by measuring the capacity factor (log $K'$), which indicates the relative retention of a compound in the HPLC system. According to those values, the relative order of decreasing polarity was CA < FA < HMA < CD < FAEE. The log $P$ values measured by molecular modeling (Discovery Studio® vers. 2.0, Accelys, San Diego, CA, USA) showed the same trend as the capacity factor. The FA derivatives are ionic compounds. FA and its isomer, HMA, exhibited two p$K_a$ values of ~4.5 and ~9.0. CA, while CD, and FAEE showed the same p$K_a$ of 9.88.

### 3.2. In vitro skin permeation via intact porcine skin

According to the p$K_a$ values, weakly acidic compounds were mainly in the non-ionized and ionized form at pH 6 and 9.9, respectively. We chose an aqueous buffer with both pH values as a safe vehicle for the topical delivery of FA and its derivatives. The solubility of FA in the aqueous vehicles was up to 3 mg/ml, which can be associated with a risk of local toxicity. The finite dose technique was applied in this study, although the maximal thermodynamic activity could not be obtained. All donor samples were prepared by dispersing 2.6 μM compounds in buffer. For topical formulations, the compound skin content was considered an important parameter. At the end of the in vitro experiment, the permeant was removed from the skin surface, and the amount of permeant absorbed in porcine skin (nmol/g) was determined as summarized in Table 2. Although there were similarities in the structures of these compounds, the skin deposition of these compounds showed discrepancies. It was observed that 136 nmol/g of FAEE from pH 6 buffer remained in the skin after 48 h, which was the greatest skin deposition ($p < 0.05$) among the compounds tested. The skin deposition decreased in the order of FAEE ≫ CD ≥ CA > FA = HMA. As clearly evidenced by permeation profiles, the findings demonstrate that, at both pH values, FA and related compounds are able to permeate via the skin. The pH factor had no marked influence on permeant skin deposition. There was no significant difference ($p > 0.05$) in skin deposition between pH 6 and 9.9 buffers for FA, CA, and FAEE.

Fig. 2 shows the cumulative amounts of permeants which penetrated through porcine skin. The flux ($nmol/cm^2/h$) is equal to the slope of the linear penetration profile and was calculated from

**Table 2**
In vitro porcine skin permeation data of ferulic acid and its derivatives from aqueous solutions with various pH values.

| Compound | pH 6 | | | pH 9.9 | | |
|---|---|---|---|---|---|---|
| | Skin deposition (nmol/g) | Flux (nmol/cm$^2$/h) | $S$[a] | Skin deposition (nmol/g) | Flux (nmol/cm$^2$/h) | $S$[a] |
| CA[b] | 33.32 ± 7.11 | 6.24 ± 2.33 | 5.3 | 40.04 ± 9.83 | 10.62 ± 2.97 | 3.8 |
| FA[c] | 24.96 ± 6.27 | 1.16 ± 0.30 | 21.5 | 15.18 ± 0.66 | 0.48 ± 0.10 | 31.6 |
| HMA[d] | 23.80 ± 4.82 | 1.79 ± 0.42 | 13.3 | 12.74 ± 5.14 | 0.39 ± 0.04 | 32.6 |
| CD[e] | 39.64 ± 8.36 | 15.06 ± 2.13 | 2.6 | 51.39 ± 8.77 | 9.04 ± 1.60 | 5.7 |
| FAEE[f] | 135.95 ± 24.20 | 25.96 ± 0.64 | 5.2 | 209.66 ± 109.27 | 24.42 ± 1.86 | 10.5 |

Each value represents the mean ± S.D. ($n$ = 4).
[a] $S$, the dermal/transdermal selectivity index ($S$) was calculated as a ratio of the absolute compound amount in the skin and the flux value.
[b] CA, coniferyl alcohol.
[c] FA, ferulic acid.
[d] HMA, 3-hydroxy-4-methoxycinnamic acid.
[e] CD, coniferyl aldehyde.
[f] FAEE, ferulic acid ethyl ether.

the experimental curves shown in Fig. 2. This flux, i.e., the amount of compound traversing across a unit area of skin per unit time, in fact predicts the efficiency of transdermal delivery to the systemic circulation. As depicted in Table 2, a positive correlation was noted between the flux and deposition of these compounds (correlation coefficients $r$ = 0.91 for pH 6 and $r$ = 0.85 for pH 9.9). FAEE, the compound with the highest flux, showed 22- and 51-fold greater transdermal penetration levels compared to FA at pH 6 and 9.9, respectively. The $S$-value in Table 2 is the dermal/transdermal selectivity. A higher $S$-value implies higher permeant retention in the skin than penetration through it. A lower $S$ means that such compound favors transdermal transport. From this point of view, FA and its isomer, HMA, appeared to be applicable permeants for topical but not transdermal treatment.

According to a previous study (Nenadis et al., 2003), the relative order of the scavenging activity toward free radicals derived from a kinetic study was CA ≫ FA ∼ CD ∼ FAEE. The antiradical efficiencies (AEs) of these four compounds are listed in Table 3. Since HMA is an isomer of FA, the AE of HMA was set to 0.04 [(mol of antioxidant/mol of DPPH) × min]$^{-1}$, which is the same as the value of FA. The antioxidant indices for the dermal delivery of FA and its derivatives were determined by the results of skin deposition multiplied by the AE as shown in Table 3. CA exhibited the highest antioxidant index among all permeants, followed by FAEE. The antioxidant indices of FA, HMA, and CD were comparable to each other.

**Table 3**
The antioxidant index of ferulic acid and its derivatives by multiplying the antiradical efficiency (AE) and skin deposition.

| Compound | AE[a] | pH 6 | pH 9.9 |
|---|---|---|---|
| CA[b] | 0.86 | 28.7 | 34.4 |
| FA[c] | 0.04 | 1.0 | 0.6 |
| HMA[d] | 0.04 | 1.0 | 0.5 |
| CD[e] | 0.03 | 1.2 | 1.5 |
| FAEE[f] | 0.03 | 4.1 | 7.7 |

[a] AE, antiradical efficiency from the reference of Nenadis et al. (2003).
[b] CA, coniferyl alcohol.
[c] FA, ferulic acid.
[d] HMA, 3-hydroxy-4-methoxycinnamic acid.
[e] CD, coniferyl aldehyde.
[f] FAEE, ferulic acid ethyl ether.

### 3.3. *In vitro skin permeation via various skin types*

In order to elucidate the mechanisms involved in the skin permeation of FA derivatives, an in vitro permeation experiment was performed using skin membranes with various treatments. A pH 6 buffer was used as the vehicle. FA, CD, and FAEE were chosen as the representative permeants because of their poor, moderate, and high permeation rates, respectively. Table 4 summarizes the skin deposition via various skin membranes. The treatment ratio (TR) was obtained by the skin deposition of a compound in treated skin divided by the compound deposition in intact skin. The skin deposition of FA in delipidized skin showed a 3.1-fold ($p$ < 0.05) increase compared to that in intact porcine skin. As shown in Table 5, FA flux across delipidized skin was ∼14 times greater ($p$ < 0.05) than that across intact skin. To further explore the permeation mechanisms, 25% ethanol and 5% oleic acid in 25% ethanol were used to pretreat the skin. Ethanol at 25% was used as the pretreatment medium of oleic acid for solubility considerations. Pretreatment with 25% ethanol significantly reduced ($p$ < 0.05) FA deposition. Treatment with oleic acid increased FA deposition by a twofold greater factor

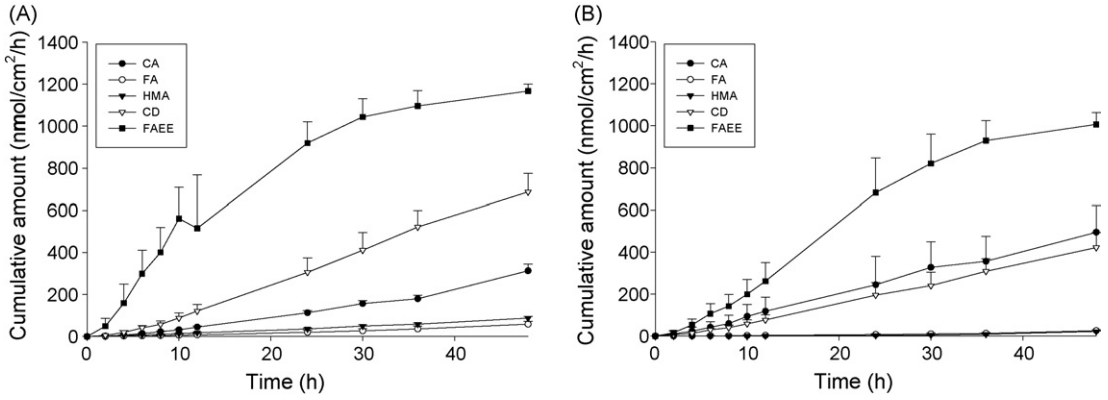



**Fig. 2.** In vitro cumulative amount versus time profiles of in vitro topical application of ferulic acid and its derivatives permeating across porcine skin from aqueous buffers of (A) pH 6 and (B) 9.9. All data are presented as the mean of four experiments ± SD.

**Table 4**
In vitro skin deposition of ferulic acid and its derivatives from pH 6 aqueous solution in various skin types.

| Compound | Skin type | Skin deposition (nmol/g) | Treatment ratio (TR)[a] |
|---|---|---|---|
| FA[b] | Intact skin | 24.96 ± 6.27 | – |
| | Delipid skin | 78.32 ± 20.18 | 3.1 |
| | 25% ethanol treatment | 14.72 ± 2.72 | 0.6 |
| | Oleic acid treatment[c] | 52.97 ± 12.91 | 2.1 |
| CD[d] | Intact skin | 39.64 ± 8.36 | – |
| | Delipid skin | 44.78 ± 3.74 | 1.1 |
| | 25% ethanol treatment | 25.27 ± 8.84 | 0.6 |
| | Oleic acid treatment[c] | 44.31 ± 17.70 | 1.0 |
| FAEE[e] | Intact skin | 135.95 ± 24.20 | – |
| | Delipid skin | 152.50 ± 39.84 | 1.1 |
| | 25% ethanol treatment | 154.13 ± 16.31 | 1.1 |
| | Oleic acid treatment[c] | 101.15 ± 24.04 | 0.7 |

Each value represents the mean ± S.D. ($n$ = 3–5).
[a] Treatment ratio (TR), skin deposition in treated skin/skin deposition in intact skin.
[b] FA, ferulic acid.
[c] Oleic acid at 5% (v/v) was dissolved in 25% ethanol in water.
[d] CD, coniferyl aldehyde.
[e] FAEE, ferulic acid ethyl ether.

compared to the untreated group. FA flux across the skin treated with ethanol and oleic acid was comparable ($p > 0.05$) to that across intact skin.

CD and FAEE revealed similar permeation behaviors after skin treatment. The delipidization process did not change the amount of CD or FAEE in the skin reservoir or receptor (Tables 4 and 5). The same result was observed in skin with 25% ethanol treatment. The TR was 0.6 for CD deposition in ethanol-treated skin, but the statistical analysis revealed no significant difference ($p > 0.05$) with intact skin. Pretreatment with oleic acid also did not change the permeation profiles of CD or FAEE ($p > 0.05$).

### 3.4. In vivo skin permeation

In vivo percutaneous absorption of FA and its derivatives in skin was evaluated using nude mouse because of its easy handling. In order to ascertain the similarity of permeation trends between nude mouse skin and porcine skin, an in vitro permeation study using nude mouse skin was performed with FA, CD, and FAEE.

**Table 5**
In vitro flux of ferulic acid and its derivatives from pH 6 aqueous solution across various skin types.

| Compound | Skin type | Flux (nmol/cm$^2$/h) | Treatment ratio (TR)[a] |
|---|---|---|---|
| FA[b] | Intact skin | 1.16 ± 0.30 | – |
| | Delipid skin | 16.02 ± 4.75 | 13.8 |
| | 25% ethanol treatment | 1.01 ± 0.20 | 0.9 |
| | Oleic acid treatment[c] | 0.94 ± 0.38 | 0.8 |
| CD[d] | Intact skin | 15.06 ± 2.13 | – |
| | Delipid skin | 12.03 ± 2.76 | 0.8 |
| | 25% ethanol treatment | 1.36 ± 0.63 | 1.1 |
| | Oleic acid treatment[c] | 17.96 ± 3.31 | 1.2 |
| FAEE[e] | Intact skin | 25.96 ± 0.64 | – |
| | Delipid skin | 25.08 ± 3.85 | 1.0 |
| | 25% ethanol treatment | 22.07 ± 1.20 | 0.9 |
| | Oleic acid treatment[d] | 24.50 ± 1.15 | 0.9 |

Each value represents the mean ± S.D. ($n$ = 3–5).
[a] Treatment ratio (TR), Skin deposition in treated skin/skin deposition in intact skin.
[b] FA, ferulic acid.
[c] Oleic acid at 5% (v/v) was dissolved in 25% ethanol in water.
[d] CD, coniferyl aldehyde.
[e] FAEE, ferulic acid ethyl ether.

**Table 6**
In vitro nude mouse skin permeation data of ferulic acid and its derivatives from pH 6 aqueous solution.

| | Skin deposition (nmol/g) | Flux (nmol/cm$^2$/h) |
|---|---|---|
| FA[a] | 137.77 ± 17.10 | 11.87 ± 4.91 |
| CD[b] | 207.48 ± 176.18 | 20.57 ± 3.07 |
| FAEE[c] | 717.01 ± 46.09 | 20.45 ± 3.02 |

Each value represents the mean ± S.D. ($n$ = 3∼5).
[a] FA, ferulic acid.
[b] CD, coniferyl aldehyde.
[c] FAEE, ferulic acid ethyl ether.

As shown in Table 6, similar trends of skin deposition and flux (FAEE > CD > FA) were detected between the skins of both animals. Uptake of the compounds by nude mouse skin was significantly higher ($p < 0.05$) compared to porcine skin. FA flux across nude mouse skin was also greater than that across porcine skin. However, the fluxes across nude mouse and porcine skin were comparable ($p > 0.05$) for CD and FAEE.

The in vivo skin uptake of the compounds from pH 6 buffer into nude mouse skin was studied. Levels of permeants in the skin reservoir were determined following a single application to the dorsal surface of nude mouse as shown in Fig. 3. Levels of skin deposition of FA, CD, and FAEE 8 h after in vivo topical application were 92, 150, and 145 nmol/g, respectively. The results clearly indicated that CD and FAEE exhibited better percutaneous absorption ($p < 0.05$) than FA. The difference between CD and FAEE was not significant according to a $t$-test.

### 3.5. In vivo skin tolerance test

Because of the importance of developing topical formulations with low toxicity, the possibility of skin irritation of the compounds was assessed based on in vivo bioengineering techniques. The Δ values (the value of the treated site minus the value of an adjacent untreated site) of TEWL, erythema ($a^*$), and pH were determined after 24-h topical application of FA, CD, and FAEE as shown in Fig. 4. No significant skin irritation was determined when the bar of the standard deviation (SD) passes across the zero line in Fig. 4. We did not see an irritant response with these compounds according to TEWL, suggesting tolerance of the skin to these topically applied vehicles. The results of $\Delta a^*$ showed that the three compounds produced negligible erythema. The same result was observed for the determination of the skin surface pH.

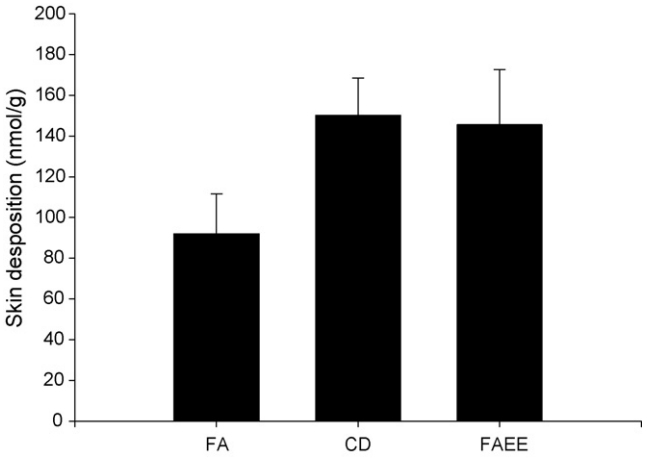



**Fig. 3.** In vivo skin deposition of ferulic acid, coniferyl aldehyde, and ferulic acid ethyl ether from a pH 6 aqueous solution. All data are presented as the mean of five experiments ± SD.

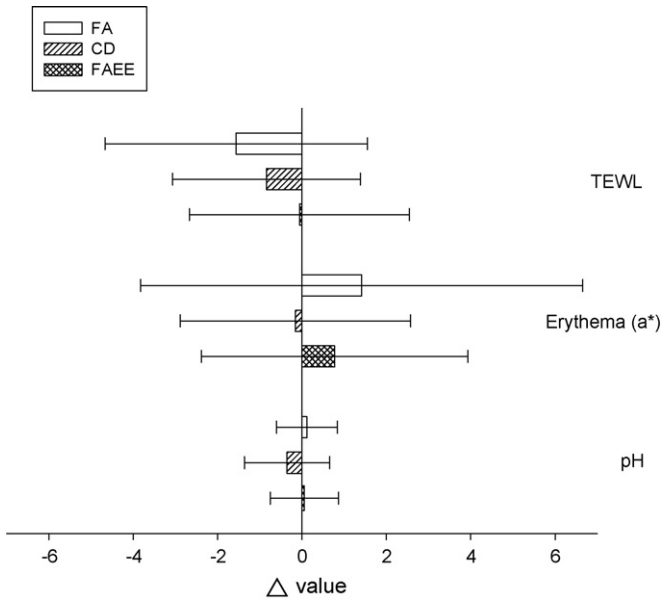



**Fig. 4.** In vivo skin irritation examination determined by transepidermal water loss (TEWL), erythema ($a^*$), and the pH value after a 24-h application of topically applied ferulic acid, coniferyl aldehyde, and ferulic acid ethyl ether from a pH 6 aqueous solution. The Δ value indicates the value of the treated site minus the value of an adjacent untreated site. All data are presented as the mean of five experiments ± SD.

## 4. Discussion

The skin is one of the main targets for ROS. ROS can induce damage, and protein and lipid modifications can occur. As a result of these modifications, the skin can develop a variety of diseases ranging from photosensitivity to cancer (Valko et al., 2006). FA and related compounds show photoprotective efficacy against skin carcinogenesis. However, there are many antioxidants that have been demonstrated to provide photoprotection for in vitro and cellular systems but fail in vivo mostly because they are unable to enter the skin (Tournas et al., 2006; Murray et al., 2008). Despite various reports linking many of the beneficial properties of FA to its use, no comprehensive study has been conducted investigating the skin absorption ability of related compounds. The objective of this work was to examine both the local skin concentration and transdermal flux of FA and its derivatives and the conditions that influence these processes. We found that the structures of these compounds largely affected their skin permeation. The results showed that FAEE exhibited the highest absorption among all compounds. On the other hand, CA revealed the greatest antioxidant activity by skin delivery. Negligible skin irritation was detected, indicating the feasibility of their dermal/transdermal use.

The assessment of percutaneous permeation of molecules is one of the main steps in the design and evaluation of dermal/transdermal delivery systems. It is important to emphasize that in vitro and animal models provide important tools for estimating the ranking of skin transport for a series of permeants (Godin and Touitou, 2007). Full-thickness neonate porcine skin, a good model for human skin in terms of hair density and distribution of blood vessels (Riviere and Papich, 2001; Donnelly et al., 2008), was initially used as the permeation barrier in this study. Certain levels of the five compounds examined could permeate into and across porcine skin. Skin absorption of a compound is determined by its physicochemical properties, in particular, MW and lipophilicity, which play major roles (Marti-Mestres et al., 2007). Finnin and Morgan (1999) indicated that molecules with a MW of <500 Da can penetrate across the skin because of their small molecular volumes. FA and its derivatives fit this criterion. Different species exhibit different degrees of ability to permeate the skin. Statistical analysis of the in vitro data suggested that the amount of permeants remaining in and passing through the skin tended to increase with increasing lipophilicity except for CA. The lipophilicity of the test compounds, frequently measured as $\log K'$ and $\log P$, is expected to be a strong predictor of skin absorption (Huang et al., 2008; Doan et al., 2010). The partitioning indicates the relative magnitude of the escape tendency of the permeant from the aqueous vehicle to the SC (Cal, 2006).

The lowest lipophilicity of CA may be due to the fact that a lesser amount of it permeated into/across the skin than the other compounds. The experimental results of CA permeation did not follow this expectation. CA and CD had similar skin deposition characteristics, but greatly differed with respect to lipophilicity. The structure of CA lacks the =O moiety in the alkyl chain, which is found in FA, HMA, CD, and FAEE. The branching in the alkyl chain hinders the permeant penetration through the lipid bilayers of the SC (Vaddi et al., 2005; Baert et al., 2007). CA molecules can easily pass through the SC, resulting in moderate skin permeation, although it exhibits low lipophilicity. Hence both the lipophilicity and molecular stereochemical complexity predominate the skin delivery of FA and its derivatives. Another possibility is the low MW of CA. One might expect higher permeation to be associated with lower-MW compounds. However, this was not a general rule for all FA derivatives since FAEE showed both the highest skin absorption and MW. FAEE, which is naturally occurring and a more-lipophilic form of FA, demonstrates strong photoprotective activity in the skin (Calabrese et al., 2008; Choquenet et al., 2008). It can be seen in the cumulative amount-time curves of FAEE that the initial penetration across the skin to the receptor was fast and then became slower with time. This may indicate that FAEE penetration achieved a maximum level because of its high lipophilicity and partitioning. FA and HMA are isomers of each other. The difference in the structures of these two compounds is simply the positions of the hydroxyl and methoxyl groups in the ring. This structural similarity contributed to the comparable skin permeation characteristics of both compounds.

Percutaneous absorption of a weakly acidic compound is always higher at lower pH values since the non-ionized form is beneficial to skin partitioning due to its lipophilicity (Saija et al., 2000; Hung et al., 2010). Vehicle pH values proved to have little influence on skin permeation profiles in the present study. Because the FA derivatives are mainly in ionized form at higher pH values, the present findings suggest that the corresponding anions are also skin permeable. Increased pH can ionize a greater part of the intercellular fatty acids, changing the phase behavior and packing of the barrier lipid mixture (Vávrová et al., 2008). The SC might be much more permeable to molecules in an alkaline vehicle.

Delivery of chemicals to the skin serves different functional purposes. The first purpose is to design formulations for dermal delivery. These preparations allow the permeant to locate in the skin reservoir without penetration into the systemic circulation. The second purpose is to design transdermal delivery systems to provide high and appropriately timed plasma concentrations of a compound without inducing local adverse reactions (Baert et al., 2007). In order to evaluate whether FA and related compounds are feasible for dermal or transdermal delivery, the $S$-value (skin deposition/flux) was measured (Vávrová et al., 2008; Frelichowska et al., 2009). A direct correlation between the skin deposition and flux was achieved for all permeants tested. The concentration gradient between the skin and receptor may have contributed to this result. FA and HMA showed higher $S$-values compared to the others, indicating the feasibility of dermal delivery for photoprotective treatment without penetration into the circulation. On the other hand, successful transdermal delivery can be attained by using CA, CD, and FAEE as the permeants. Thus the skin route can be an alternative administration to the oral route to improve the low

bioavailability of FA derivatives. Although the *S*-values of FA and HMA were greater than those of the other compounds, the low skin deposition of FA and HMA may be insufficient to induce antioxidant activity. Further strategies to enhance the topical delivery of FA and HMA are needed to promote the dermal use of these compounds.

Successful skin delivery is a prerequisite for successful prevention/therapy. The antioxidant index was calculated to observe the possible antioxidant activity of FA and its derivatives after skin delivery. Although the skin deposition of CA was relatively lower than that of FAEE, CA revealed a 29-fold greater potency of antioxidant activity (AE) than FAEE. Consequently CA achieved a 5–7-fold higher antioxidant index compared to FAEE. Although FAEE showed a comparable AE value to FA, HMA, and CD, the greater skin uptake indicated that FAEE is a superior candidate for preventing oxidative stress-mediated skin damage. The antioxidant index assay confirms the scant ability of FA and HMA to permeate the skin by passive diffusion.

The SC comprises the outermost layer of the skin, which constitutes the major barrier to percutaneous absorption. Skin entry is generally described as being intercellular or transcellular, but the latter is of little, if any, practical importance. The lipidation process can remove intercellular lipids from the SC. The SC is principally lipophilic in nature and far more resistant to polar than non-polar compounds. This speculation is consistent with the permeation profiles after the removal of lipids which largely increased FA, but not CD or FAEE, permeation. This indicates that the SC is a major contributor to the barrier function against skin absorption of FA. Pretreatment with 25% ethanol resulted in a retardation of the FA reservoir in the skin. Changes in the skin's structure induced by ethanol reduced the permeation of some drugs (Fang et al., 2003; Wang et al., 2007). Protein denaturalization in the SC may have been involved in this reduced permeation. This result indicates that the hydrophilic pathway through corneocytes can play a role in the skin delivery of FA via the SC. Oleic acid is used as a permeation enhancer for dermal/transdermal drug delivery. Oleic acid can act at the lipidic tail portion of intercellular lipid bilayers (Gwak and Chun, 2002; Jain and Panchagnula, 2003). According to our previous report (Huang et al., 2008), a 5% oleic acid in 25% ethanol could completely disrupt the lipophilic region of the intercellular lipids. Thus the pathways of FA permeation via skin can be elucidated by examining the permeability in the absence of lipophilic tail of bilayers. FA permeation showed the highest enhancement among the three permeants when the skin was treated with oleic acid. This suggests that the alkyl chain of the lipids in the SC is the main barrier blocking the transit of FA. It also confirms that the intracellular route may be essential for FA since oleic acid can disrupt corneocytes (Touitou et al., 2002).

The results for CD and FAEE suggest that the compound concentration in delipidized skin and receptor did not significantly differ compared to intact skin. Moreover, neither ethanol nor oleic acid could elaborate the activity of CD and FAEE permeation. This suggests that CD and FAEE are easily partitioned and penetrated into the SC with no need to increase the permeation by enhancers. The SC layer was not a rate-limiting process for these two compounds with higher lipophilicity than FA.

Due to its availability and easy handling, the skin of rodents is most commonly used for in vivo percutaneous absorption experiments. There are a number of hairless species (nude mice and hairless rats) in which the absence of a coat of hair mimics the human skin better than hairy skin (Godin and Touitou, 2007). A similar trend was seen between porcine skin and nude mouse skin in the present work. Although nude mouse skin is more permeable than porcine skin, it is still a good model for examining the ranking of skin transport of FA derivatives. The in vivo nude mouse skin absorption results were in good accordance with the results of in vitro skin deposition. The in vivo absorption levels of CD and FAEE were greater than that of FA, although there was no significant difference between the levels of CD and FAEE. FAEE did not show notable skin entry in an in vivo status. A possible mechanism is saturation of the skin reservoir; hence, further diffusion of FAEE into the skin may quickly pass through the skin into the systemic circulation or deeper tissues. The in vivo skin deposition was less than the in vitro profile, especially for FAEE. This was due to a significant diffusion and distribution of the permeants from the skin to the circulatory system or other tissues after in vivo topical administration, thus reducing the skin accumulation in an in vivo status (Hung et al., 2008).

Besides the efficiency of diffusion into the skin, the skin tolerance is another concern for topical delivery systems. Many cutaneous reactions to herbal preparations and natural products were reported (Stratton et al., 2000; Bedi and Shenefelt, 2002). By evaluating established endpoints of skin irritation, the present work demonstrated that the topical application of FA and its derivatives for up to 24 h did not cause an adverse skin response. TEWL is used to assess the degree of disruption of the SC. The $a^*$-coordinate of colorimetry (which indicates erythema) correlates well with inflammatory interactions of the skin, especially viable skin (Hung et al., 2008). FA compounds delivered by topical delivery may be safe for both the epidermal and dermal layers. Prolonged hydration can lead to skin degradation where such a catastrophic breakdown would be expected to occur (Heard et al., 2006). That was not the case in this study using the pH 6 buffer as the topical vehicle for 24-h treatment. A previous study (Ou and Kwok, 2004) indicated low toxicity of FA by oral administration. The same phenomenon was also observed in skin tissue. Yet it has to be kept in mind that the tolerance test used here was established to detect macroscopic changes induced by compounds not microscopic observations. Further study is needed to fully explore the skin tolerance of FA and related compounds.

## 5. Conclusions

As demonstrated in this study, compounds related to FA were readily absorbed by the skin in both in vitro and in vivo experiments. Our data suggest that the ester derivative of FA, FAEE, showed the highest transport via the skin. The results confirm that lipophilicity is one of the main parameters determining the permeation behaviors of these compounds. CA was an exception because of its low MW and the lack of a branched alkyl chain. According to the antioxidant index, CA and FAEE exhibited promising prevention or therapy by the skin delivery route. Although FA was the most-investigated compound for biological activities in previous manuscripts, its low permeation into/across the skin may be insufficient to trigger a pharmacological effect. FA and its derivatives retained within the skin after topical application can be an efficient therapy for the prevention of UV exposure and skin carcinogenesis. Moreover, delivery by the skin route can avoid degradation because of the low metabolism in the skin, resulting in the possible prolongation of the half-life and a sufficient concentration in the systemic circulation. Summarizing the results of the present work, it was concluded that FA and HMA may be suitable to develop dermal preparations for protection against the harmful effects of UV, although further enhancement of their absorption is necessary. The other compounds can be considered for a transdermal route to overcome the low bioavailability by the oral route. More in vivo and clinical information on the efficacy and safety of the percutaneous absorption of FA derivatives is required to assess future practicability.

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