Percutaneous penetration enhancement effect of essential oil of mint (Mentha haplocalyx Briq.) on Chinese herbal components with different lipophilicity

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Abstract  Objective: To investigate the percutaneous penetration effect of essential oil of mint from Mentha haplocalyx Briq. on the complex active components in Chinese herbal external preparations, and assess its toxicity on the skin cells.

Methods: The cytotoxicity of mint oil on HaCaT keratinocytes and CCC-ESF-1 fibroblasts was measured using an MTT assay. Five model drugs with a wide range of lipophilicity, namely osthole, tetramethylpyrazine, ferulic acid, puerarin, and geniposide, were tested using in vitro permeation studies to investigate the percutaneous penetration enhancement effect of mint oil. Secondary structure alterations of skin stratum corneum (SC) were measured using Fourier transform infrared spectroscopy (FTIR). Saturation solubilities and SC/vehicle partition coefficients of the five model drugs with and without mint oil were also determined to understand the potential mechanisms of the essential oil.

Results: Half maximal inhibitory concentration (IC50) values of mint oil were significantly higher in HaCaT and CCC-ESF-1 cell lines than values in the well-established and standard penetration enhancer Azone.

Conclusions: Mint oil at proper concentration could effectively facilitate percutaneous penetration of both lipophilic and hydrophilic drugs, and exhibit higher efficiency for moderate hydrophilic drugs. Mechanisms of penetration enhancement by mint oil could be explained with

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Introduction

Mint (Mentha haplocalyx Briq.) belongs to the labiatae family and is widely used as a traditional Chinese medicine (TCM) for its medicative properties and is also an important ingredient in health care products and foods. The Chinese classic Rhymed Discourse on Topical Remedies (Li Yue Pian Wen; 1870) mentions herbs that are rich in essential oil, such as mint, have unique properties that can facilitate absorption through the skin of active components in a topical herbal prescription. Thus, in addition to its therapeutics, the essential oil from mint is used extensively in traditional Chinese external preparations for its purported ability to promote absorption of other herbal ingredients.

Despite some studies have reported the activity of essential oil of mint as a transdermal penetration enhancer,\(^1\)\(^2\) a single Chinese herbal ingredient or pharmaceutical component is commonly used to evaluate its penetration enhancement activity, thus it is difficult to fully assess its enhancement properties in multi-ingredient external preparations. Due to the complexity of Chinese herbal formulas, there is currently no systematic information available about the skin permeation effect of mint oil on the complex active components in Chinese external preparations.

Although certain physicochemical parameters of drug molecules are associated with their skin permeability, including molecular size, lipophilicity, acidity or basicity, among others, the quantitative structure-activity relationship (Q SAR) has revealed that drug lipophilicity is the predominant factor which affects the skin permeability of drugs,\(^3\)\(^4\) and there is little correlation between the drug molecular size and penetration rate within a narrow range of molecular weight (200–500).\(^5\) Therefore, based on these studies, the complex active components in Chinese herbal external preparations can be roughly represented with herbal components with a wide range of different lipophilicities.

In this study, to investigate the penetration enhancement effect of mint oil on the complex active components in Chinese herbal external preparations, a series of TCM model drugs with a wide range of lipophilicity represented by n-octanol/water partition coefficient (logK\(_{\text{o/w}}\)) were selected, namely osthole (OT, logK\(_{\text{o/w}}\) = 3.85), tetramethylyprazine (TMP, logK\(_{\text{o/w}}\) = 2.34), ferulic acid (FA, logK\(_{\text{o/w}}\) = 1.26), puerarin (PR, logK\(_{\text{o/w}}\) = -0.35), and geniposide (GP, logK\(_{\text{o/w}}\) = -1.01). The physicochemical parameters of these five model drugs are presented in Table 1. In addition, skin epidermal keratinocytes (HaCaT cells) and dermal fibroblasts (CCC-ESF-1 cells) were employed to monitor the cytotoxicity of mint oil.

Materials and methods

Drugs and chemicals

Osthole (OT), puerarin (PR) and geniposide (GP) were purchased from NCE Biomedical Co., Ltd. (Wuhan, China). Ferulic acids (FA), laurocapram (Azone), and i-menthol were supplied by Sinopharm Chemical reagent Co., Ltd. (Beijing, China). Menthone was purchased from Tokyo Chemical Industry Co., Ltd. (Shanghai, China). Tetramethylpyrazine (TMP), propylene glycol (PG), polyoxyethylene (20) oleyl ether (Brij-98), dimethyl sulfoxide (DMSO), C\(_8\)–C\(_{30}\) n-alkanes and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma–Aldrich (Shanghai, China). Acetonitrile of HPLC grade was purchased from Merck KGaA (Darmstadt, Germany). Acetic acid was supplied by Beijing Chemical Reagent Co., Ltd. All other chemicals used were of analytical grade.

Plant material and essential oil extraction

Aerial parts of Mint (Mentha haplocalyx Briq.) used in this work were supplied by Ben Cao Fang Yuan Pharmaceutical Co., Ltd. (Beijing, China), and identified by Associate Professor Peng Tan (Department of Chinese Pharmacy, Beijing University of Chinese Medicine, Beijing, China). A voucher specimen (#MH131007) was deposited in the Department of Chinese Pharmacy, Beijing University of Chinese Medicine. The dried herbs (2.0 kg) were subjected to hydrodistillation in 12 volumes of water (v/w) for 2 hours by using a Clevenger-type apparatus. The yellowish essential oil of mint (370.0 g) was extracted.

Cell line and culture

HaCaT (epidermal keratinocytes) and CCC-ESF-1 (dermal fibroblasts) cell lines were supplied by the Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Peking Union Medical College, China). The cells were grown in Minimum Essential Medium (MEM Eagles with Earle’s Balanced Salts) and Dulbecco’s Modified Eagle’s Medium (DME H-21 4.5 g/L glucose), supplemented with 10% (v/v) fetal bovine serum (FBS), 100 units/ml penicillin/ streptomycin at 37°C in a 5% CO\(_2\) incubator.

High performance liquid chromatography (HPLC) methods for determination of the model drugs

The HPLC system for analyzing drug concentrations was equipped with an SPD-20A variable-wavelength ultraviolet
absorbance detector, two LC-20AT pumps and computer integrating system (Shimadzu Corporation, Kyoto, Japan). Purospher STAR RP-18 (250 x 4.6 mm i.d., 5 μm particle size; Merck KGaA) reversed-phase column was used to determine the content of TMP. Alltima HP C18 AQ (250 x 4.6 mm i.d., 5 μm particle size; W.R. Grace & Co.-Conn, Columbia, MD, USA) column was utilized to measure the content of GP and PR, and Lichrospher RP-18e (250x4.6 mm i.d., 5 μm particle size; Merck KGaA) column was employed to analyze the content of OT and FA. HPLC conditions were as follows: mobile phase for OT was a mixture of acetonitrile and distilled water (80:20, v/v), wavelength was set at 251 nm, and retention time of OT was 5.8 minutes. Mobile phase for TMP consisted of acetonitrile and distilled water (60:40, v/v), wavelength was set at 282 nm, and retention time of TMP was 5.6 minutes. Mobile phase for FA consisted of acetonitrile and 1% acetic acid in distilled water (30:70, v/v), wavelength was set at 323 nm, and retention time of FA was 5.9 minutes. Mobile phase for PR was a mixture of acetonitrile and distilled water (20:80, v/v), wavelength was set at 240 nm, and retention time of PR was 5.5 minutes. Mobile phase for GP consisted of acetonitrile and distilled water (20:80, v/v), wavelength was set at 251 nm, and retention time of GP was 5.3 minutes. Flow rate under the above five conditions was 1.0 mL/min. Specificity, linearity, intra- and inter-day variability, and recovery of these methods were tested. Reproducibility, calculated as RSD of successive injection of six solutions carried out on 3 different days, was 0.34, 1.34, 0.24, 0.16, and 6.52% for OT, TMP, FA, PR and GP, respectively.

Gas chromatography–mass spectral analysis

Mint oil was subjected to gas chromatography–mass spectrometry (GC–MS) analysis on a Shimadzu QP-2010 system equipped with a NIST database and a Rxi-5Sil MS capillary column (30 m x 0.25 mm x 0.25 mm, Restek Corporation, Bellefonte, PA, USA). Helium (99.99%) was the carrier gas at a flow rate of 1.0 mL/min. Oven temperature was programmed from 50°C to 240°C at a rate of 3 °C/min. Injector temperature, MS transfer line, and ion source temperatures were set at 250°C, 280°C, and 250°C, respectively. Mass scanning was in the range of 35–500 amu. Split ratio was 1:50. An aliquot (1.0 μL) of the diluted samples (1/100 in ethyl acetate, v/v) was injected automatically.

The constituents of the essential oil were identified by matching their mass spectral fragmentation patterns with those in a NIST2008 mass spectra library and also by comparing their Kovats retention indices, which were determined by injection of the sample with a solution containing the homologous series of C8–C95 n-alkanes as reported previously. The major oil contributors were further confirmed by comparing the corresponding pure standard compounds. The percentage composition was computed by the normalization method from the GC peak areas.

Cytotoxicity

In vitro cellular toxicity of mint oil and associated enhancers was evaluated in dermal fibroblast and epidermal keratinocyte cultures using a MTT assay. Fibroblasts or keratinocytes were seeded into 96-well plates at a density of 7000 cells in 100 μL medium per well. After 24 hours, the cells were incubated with varying concentrations of enhancer solution in a culture medium with 1% DMSO (as a vehicle) for 24 hours at 37°C. Cells that were treated with culture medium containing 1% DMSO were used as control. Subsequently, the medium was replaced with fresh medium containing 20 μL MTT solution (5 mg/mL in phosphate buffer) and the cells were incubated for 4 hours. The medium was then replaced with 150 μL DMSO to dissolve the formazan crystals. The plate was incubated for 10 minutes while shaking. Absorbance was read at 490 nm using a Multiskan microplate spectrophotometer (Thermo Scientific, USA). The half maximal inhibitory concentrations (IC50) were calculated using SPSS software v16.0 as previously described.

Preparation of full thickness skin and stratum corneum (SC)

Male Sprague–Dawley rats (5 weeks of age, 200 ± 10 g) were purchased from Shibeifu Laboratory Animal Technology Co., Ltd. (Beijing, China). All experiments were carried out in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Beijing University of Chinese Medicine, China, and the experimental protocol was approved by the Committee on Animal Research of Beijing University of Chinese Medicine. After sacrificing the rats...

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Geniposide</th>
<th>Puerarin</th>
<th>Ferulic acid</th>
<th>Tetramethylpyrazine</th>
<th>Osthole</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW (g/mol)</td>
<td>388.37</td>
<td>432.38</td>
<td>194.18</td>
<td>136.20</td>
<td>244.34</td>
</tr>
<tr>
<td>logKow at 32 C</td>
<td>-1.01</td>
<td>-0.35</td>
<td>1.26</td>
<td>2.34</td>
<td>3.85</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>161–162</td>
<td>187–189</td>
<td>174</td>
<td>76–78</td>
<td>79.5–81.4</td>
</tr>
<tr>
<td>Solubility in water at 32°C (mg/mL)</td>
<td>481.87</td>
<td>3.64</td>
<td>0.54</td>
<td>4.72</td>
<td>1.32 × 10⁻³</td>
</tr>
<tr>
<td>pKa</td>
<td>12.80</td>
<td>6.46</td>
<td>4.58</td>
<td>3.20</td>
<td>—</td>
</tr>
</tbody>
</table>

a Molecular weight.

b N-octanol/water partition coefficient.

c Source = SciFinder database.
with excess ether inhalation, hair from the abdominal surface was shaved with caution so as not to injure the skin. The shaved skin was then excised from the animals. The subcutaneous tissue was removed surgically and the dermal surface was wiped with a cotton swab to remove adhered fat tissue. The full thickness skin was subsequently cleaned with phosphate buffer saline.

The excised rat skin was treated with 0.4% trypsin in PBS (pH 7.2) at room temperature for 10 hours. A cotton swab moistened with distilled water was used to separate the epidermis. SC sheets were thoroughly washed with water and dried in a vacuum desiccator until tested.

Skin permeation studies

The modified Franz vertical diffusion cell system (Shanghai Kaikai Technology Trade Co., Ltd., Shanghai, China) was used, and each cell had a volume of 7 ml and an effective area of 1.77 cm². The epidermis was prepared sandwiched between the diffusion cells with the stratum corneum side up and the dermal side exposed to the receiver compartment. The donor compartment was filled with the drug solution (approx. 20 mg/mL) in propylene glycol:water (PG:water = 80:20). The receptor compartment was filled with the PBS (pH 7.4) for TMP, FA, PR, and GP, and 3% (w/v). Brij 98 in PBS was used as receiver solution for OT. The temperature and the stirring rate were maintained at 32°C and 300 rpm, respectively. The skin was then treated with 2 mL of various concentrations of the corresponding penetration enhancers. The control was treated with a vehicle only. An aliquot (1 mL) of the sample was withdrawn from the receptor chamber at a predetermined intervals (1, 2, 4, 6, 8, 10, 12, 22, and 24 hours), and replaced with the corresponding mobile phase before HPLC analysis.

Determination of saturation solubility of model drugs

To investigate the effect of mint oil on the saturation solubility of the model drugs, excess drug was added to the known volumes of the vehicle with or without varying concentrations of mint oil, vortexed for 5 minutes followed by sonication for 15 minutes to dissolve the drug and then equilibrated at 32°C for more than 24 hours. Finally, the saturated solution was centrifuged at 10,000 rpm for 10 minutes and aliquots of supernatant were filtered through 0.22 μm nylon filter and diluted by the corresponding mobile phase before HPLC analysis.

Measurement of SC/vehicle partition coefficient of model drugs

The partition coefficient of model drugs into stratum corneum with or without various concentrations of mint oil treatment was measured as reported previously. SC samples were pulverized using mortar and pestle. One milliliter of varying concentrations of mint oil in propylene glycol:water (PG:water = 80:20 v/v) containing 10% (w/v) of model drug was added to 10 mg ground SC with frequent vortexing. The control was treated with a vehicle only. The mixture was equilibrated for 12 hours at 32°C. The supernatant solution was obtained by centrifuging at 10,000 rpm for 10 minutes and then analyzing for the drug content. The amount of drug bound to the SC was calculated by subtracting the amount of the drug in the supernatant from the initial drug concentration. All partition studies were conducted in triplicate. The partition coefficient (K) of the model drug was obtained using the following equation: 

\[ K = \text{drug concentration in SC/drug concentration in vehicle} \]

Calculation of physicochemical parameters of model drugs

Five model drugs were chosen based on their lipophilicity represented as logkow. The melting point and pKa values of model drugs were obtained from the SciFinder database. The solubility in water at 32°C of model drugs was measured with the method described above under Determination of saturation solubility of model drugs. The logkow values were determined with reference to the previously reported method.

Fourier transform infrared spectroscopy (FTIR) studies

The dried SC sheet was cut into approximately 1 cm² pieces and incubated for 12 hours in 5 mL of the respective solutions at room temperature, where the solution was 1%, 3%, 5% mint oil, solvent (propylene glycol: Water = 80:20, used as the control), respectively. Meanwhile, normal SC without any treatment was used as the blank group. The SC was then cleaned carefully with distilled water to remove the residual solvent on the SC surface and placed in a vacuum desiccator at 37°C overnight for complete dehydration. The spectral measurements of all pieces were made with a NEXUS FTIR spectrometer (Thermo Nicolet, USA) equipped with an attenuated total reflectance (ATR) attachment with the following parameters: resolution of 2 cm⁻¹, scanning times of 100, and scanning range of 650–4000 cm⁻¹. The FTIR spectral curves in each group were recorded using OMNIC 6.2 program attached to the IR instrument itself.

Data and statistical analysis

Parameters for the skin permeation studies were calculated by plotting the cumulative amount of drug permeated across the skin against time (h). Steady state flux (Jss) was calculated as the slope of the linear portion of the plot (between 8 and 14 hours). Lag time (Tlag) was determined by extrapolating the linear portion of the curve to the X-axis. The cumulative drug amount in the receptor chamber after 24 hours (Q24) and diffusion parameter (D/h²) were calculated using the following equations:

\[ Q_{24} = V_{r}C_{t} + \sum_{i=0}^{t-1} V_{r}C_{i} \]  \hspace{1cm} (1)

\[ D/h^2 = 1/(6T_{lag}) \]  \hspace{1cm} (2)

Where C_i is the drug concentration of the receiver solution at each sampling time, C_t is the drug concentration of the...
sample in the receiver, \( V_s \) and \( V_r \) are the volumes of the sampling solution and the receiver solution, respectively. \( D \) is the diffusion coefficient within the skin, \( h \) is the diffusional path length.

In order to compare the permeation enhancement capacity of different concentrations of mint oil, the enhancement ratio (ER) for flux was calculated using the following equation: \( ER = \frac{\text{flux for skin treated with essential oil}}{\text{flux for control (exposed to only vehicle)}} \).

Data were expressed as the mean value ± SD and the number of replicates (n) was given in the pertinent figures. A two-tailed Student’s t-test was used when comparing two different conditions. In all cases, \( P < 0.05 \) was considered significant.

### Results and discussion

#### Chemical compositions of mint oil

Principal compounds were identified from mint oil, accounting for 92.84% of the total oil (Table 2). Two major oil contributors (menthol and menthone, which represented 57.11% of the total oil) were further confirmed using the corresponding pure compounds. Based on the results of GC–MS analysis, mint oil contained high contents of oxygenated monoterpenes, and the principal components were menthol (51.00%), 2-isopropylidenecyclohexanone (11.49%), menthone (6.11%), cinerolone (3.19%) and eucarvone (2.58%). Generally, the biologic properties of the
essential oil depended directly on its principal components, and some terpenes, such as menthol\textsuperscript{10} and menthone,\textsuperscript{11} from the essential oil could facilitate the transdermal permeation of drugs, suggesting these major oil contributors play an important role in the enhancement activity of mint oil as penetration enhancer.

**Cytotoxicity of M. haplocalyx oil on skin cells**

Although many penetration enhancers exhibit satisfactory performance in facilitating transdermal permeation absorption of drug molecules, few have been used in clinical application on account of their skin irritation or toxic properties. Thus, the cytotoxicity on skin cells of mint oil was measured with a MTT assay. The well-established and standard penetration enhancer Azone was selected to compare and better evaluate skin cytotoxicity of the essential oil. The examined enhancers induced roughly dose-dependent reductions in cellular viability (Fig. 1). The IC\textsubscript{50} values (namely the concentration of drugs inducing a 50\% decrease in cell viability) of mint oil were significantly higher in both HaCaT keratinocytes and CCC-ESF-1 fibroblasts in comparison to those of Azone (Table 3), indicating that mint oil probably possesses low skin irritation potential.

**Effect of mint oil on percutaneous permeation of model drugs**

To investigate the permeation enhancement activity of mint oil through rat skin, low, medium, and high concentrations (1\%, 3\%, and 5\% v/v) of the oil were prepared by dissolving corresponding content of the essential oil in a mixture of PG: water (80:20, v/v). PG:water was selected as a base solvent due to its ability to solubilize all tested components, including OT, mint oil and Azone. Moreover, considering the poor water solubility of OT, 3\% (w/v) Brij 98 was added into the receiver fluid to improve its solubility, resulting in the sink condition for the skin permeation experiment of OT.\textsuperscript{12}

The permeation profile and permeation parameters of OT (flux, \(T_{lag}\), \(Q_{24}\), and ER) through rat skin are presented in Fig. 2 and Table 4, respectively. Mint oil increased the steady state flux and \(Q_{24}\) value of OT in a concentration-dependent manner, and the \(Q_{24}\) value of 5\% mint oil (457.07 ± 52.47 \(\mu\)g/cm\(^2\)) was increased by 6.76-fold compared with the control. \(T_{lag}\) value of the 10\% oil group was significantly higher than that of the control (\(P < 0.05\)), while the other groups exhibited no obvious difference.

The effect of mint oil on the percutaneous profile and permeation parameters of TMP (flux, \(T_{lag}\), \(Q_{24}\), and ER) through the rat skin are shown in Fig. 2 and Table 4, respectively. All examined enhancers had promoting effect on the percutaneous permeation of TMP (\(P < 0.05\)) in comparison with the control. The steady state flux, \(T_{lag}\), and \(Q_{24}\) values of three different concentrations of oil significantly increased with the increase in oil concentration. The 5\% oil group exhibited (ER\textsubscript{flux} = 15.14) better effect on the percutaneous permeation of TMP compared with the 1\% oil group (ER\textsubscript{flux} = 1.58) and 3\% oil group (ER\textsubscript{flux} = 2.36).

The permeation profile and permeation parameters of FA (flux, \(T_{lag}\), \(Q_{24}\), and ER) through rat skin are displayed in Fig. 2 and Table 5, respectively. All examined enhancers had promoting effect on the percutaneous permeation of FA (\(P < 0.05\)) in comparison with the control. The steady state flux and \(T_{lag}\) values of three concentrations of oil significantly increased with the increase in oil concentration. The 5\% oil group exhibited (ER\textsubscript{flux} = 206.90) better effect on the percutaneous permeation of FA compared with the 1\% oil group (ER\textsubscript{flux} = 1.46) or 3\% oil group (ER\textsubscript{flux} = 5.06).

The permeation profile and permeation parameters of GP (flux, \(T_{lag}\), \(Q_{24}\), and ER) through rat skin are displayed in Fig. 2 and Table 6, respectively. Apart from the 5\% mint oil, 1\% or 3\% mint oil had no promoting effect on the permeation of GP (\(P > 0.05\)).
percutaneous permeation of GP ($P > 0.05$) in comparison with the control. The increase in steady state flux, $T_{lag}$, and $Q_{24}$ was observed with incorporation of 5% mint oil ($P < 0.05$).

Based on the results of the skin penetration studies, mint oil could enhance the percutaneous permeation of those model drugs in a concentration-dependent manner, and the $T_{lag}$ values of all model drugs increased by incorporation of different concentrations of essential oil. Among the three different concentration groups, 5% mint oil exhibited significantly higher penetration enhancement activity than the other two concentrations, suggesting that better percutaneous enhancement effect in TCM external preparations could be obtained when the concentration of mint oil reached up to 5%. Although 1% mint oil exhibited negligible enhancement in the percutaneous permeation of those model drugs, except for TMP, compared with the control, mint oil at proper concentration could effectively facilitate percutaneous permeation of drugs with different lipophilicity, including extremely lipophilic and hydrophilic drugs.

According to the relationship between log $K_{o/w}$ of the model drugs and their log $ER_{flux}$ (Fig. 3), it was found that the relationship tended to exhibit as a parabolic curve, and

<table>
<thead>
<tr>
<th>Enhancer</th>
<th>IC$_{50}$ (mg/mL)$^a$</th>
<th>HaCaT</th>
<th>CCC-ESF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azone</td>
<td>0.047 ± 0.002</td>
<td>0.048 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>Mint oil</td>
<td>1.255 ± 0.016$^b$</td>
<td>2.461 ± 0.074$^b$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Values expressed as mean ± SD ($n = 6$). $^b$ $P < 0.05$.

Figure 2  Permeation profiles of five model drugs through excised rat skin. Values are expressed as mean ± SD ($n = 5$).
Table 4  Percutaneous permeation parameters of OT and TMP through excised rat skin.

<table>
<thead>
<tr>
<th>Enhancer</th>
<th>OT</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>TMP</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solubility (mg/mL)</td>
<td>Flux (µg/cm²/h)</td>
<td>Tlag (h)</td>
<td>Q₂₄ (µg/cm²)</td>
<td>ER₉₅₆₄ (logER)</td>
<td>Solubility (mg/mL)</td>
<td>Flux (µg/cm²/h)</td>
<td>Tlag (h)</td>
<td>Q₂₄ (µg/cm²)</td>
</tr>
<tr>
<td>Control</td>
<td>9.14 ± 0.36</td>
<td>1.68 ± 0.14</td>
<td>2.79 ± 0.61</td>
<td>67.58 ± 7.22</td>
<td>—</td>
<td>268.97 ± 0.92</td>
<td>20.10 ± 2.36</td>
<td>2.10 ± 0.39</td>
<td>929.89 ± 84.00</td>
</tr>
<tr>
<td>1% mint oil</td>
<td>12.15 ± 0.39</td>
<td>2.20 ± 0.49</td>
<td>3.13 ± 0.51</td>
<td>88.02 ± 15.19</td>
<td>1.31 (0.12)</td>
<td>278.61 ± 4.98</td>
<td>31.66 ± 6.29</td>
<td>2.69 ± 0.34</td>
<td>1561.44 ± 307.12</td>
</tr>
<tr>
<td>3% mint oil</td>
<td>14.42 ± 1.25</td>
<td>3.35 ± 0.15</td>
<td>2.94 ± 0.97</td>
<td>177.24 ± 27.65</td>
<td>1.99 (0.30)</td>
<td>290.76 ± 5.99</td>
<td>47.36 ± 8.62</td>
<td>3.08 ± 0.18</td>
<td>2163.20 ± 329.74</td>
</tr>
<tr>
<td>5% mint oil</td>
<td>14.01 ± 0.98</td>
<td>13.49 ± 2.60</td>
<td>4.31 ± 0.63</td>
<td>457.07 ± 52.47</td>
<td>8.03 (0.90)</td>
<td>291.68 ± 3.09</td>
<td>304.32 ± 88.99</td>
<td>8.62 ± 0.34</td>
<td>1561.44 ± 329.74</td>
</tr>
</tbody>
</table>

Table 5  Percutaneous permeation parameters of FA and PR through excised rat skin.

<table>
<thead>
<tr>
<th>Enhancer</th>
<th>FA</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>PR</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solubility (mg/mL)</td>
<td>Flux (µg/cm²/h)</td>
<td>Tlag (h)</td>
<td>Q₂₄ (µg/cm²)</td>
<td>ER₉₅₆₄ (logER)</td>
<td>Solubility (mg/mL)</td>
<td>Flux (µg/cm²/h)</td>
<td>Tlag (h)</td>
<td>Q₂₄ (µg/cm²)</td>
</tr>
<tr>
<td>Control</td>
<td>53.51 ± 0.16</td>
<td>1.63 ± 1.10</td>
<td>2.61 ± 1.09</td>
<td>71.02 ± 44.80</td>
<td>—</td>
<td>126.60 ± 0.52</td>
<td>0.05 ± 0.04</td>
<td>2.59 ± 0.61</td>
<td>2.19 ± 1.86</td>
</tr>
<tr>
<td>1% *M.*haplocalyx oil</td>
<td>61.06 ± 0.89</td>
<td>2.38 ± 1.73</td>
<td>2.66 ± 0.93</td>
<td>125.95 ± 91.77</td>
<td>1.46 (0.16)</td>
<td>131.37 ± 1.84</td>
<td>0.03 ± 0.02</td>
<td>2.97 ± 0.87</td>
<td>2.79 ± 1.36</td>
</tr>
<tr>
<td>3% *M.*haplocalyx oil</td>
<td>67.54 ± 1.16</td>
<td>8.25 ± 4.11</td>
<td>4.46 ± 0.61</td>
<td>938.40 ± 629.09</td>
<td>5.06 (0.70)</td>
<td>130.74 ± 3.95</td>
<td>0.77 ± 0.15</td>
<td>5.40 ± 0.46</td>
<td>83.54 ± 39.85</td>
</tr>
<tr>
<td>5% *M.*haplocalyx oil</td>
<td>74.76 ± 2.14</td>
<td>621.43 ± 73.90</td>
<td>6.10 ± 0.24</td>
<td>12293.80 ± 1859.65</td>
<td>206.90 (2.32)</td>
<td>135.77 ± 1.86</td>
<td>16.56 ± 91.61</td>
<td>7.03 ± 0.24</td>
<td>7127.66 ± 505.97</td>
</tr>
</tbody>
</table>

*ER₉₅₆₄ is the enhancement ratio calculated as follows: ER = Flux (with enhancer)/Flux (without enhancer); logER is natural logarithm value of the corresponding ER₉₅₆₄. Each value represents the mean ± SD (n = 5), except for ER₉₅₆₄.*

*P < 0.05, statistically significant difference between enhancers and control.*
Possible penetration enhancement mechanisms of mint oil

Although the mechanism of drug diffusion across skin appears complex in many similar studies, their enhancement activities have been suggested as follows: (1) change in thermodynamic activity of drug molecules causing the penetrants to readily permeate the skin; (2) increasing partitioning of the drug molecules into the SC; (3) interaction with lipids or keratin of the SC resulting in disorganization of the highly ordered structures thus enhancing delivery of drug molecules across the SC. Certainly, other potential mechanisms for explaining the activity probably exist. Thus, the effect of mint oil on drug saturation solubility, SC/vehicle partition coefficient of model drugs, and secondary structure changes of SC were investigated in an attempt to explain the penetration enhancement mechanisms of mint oil.

The effect of mint oil on the saturation solubility of five model drugs is summarized in Tables 4—6. The saturation solubility of the four model drugs (OT, TMP, FA, and PR) gradually increased as oil concentration increased, suggesting that the thermodynamic activity of these model drugs could be changed to some extent in the presence of the essential oil. Mint oil did not significantly alter (P > 0.05) saturation solubility of GP, indicating that the oil had no obvious effect on the thermodynamic activity of GP. Mint oil tended to increase saturation solubility of lipophilic drugs due to its lipophilicity properties, but contributed negligibly to that of hydrophilic drugs, which was the partial reason why the skin permeability of these lipophilic drugs was improved with incorporation of mint oil.

The results of SC/vehicle partition coefficient of the five model drugs with and without different concentrations of mint oil are listed in Table 7. Mint oil did not significantly increase (P > 0.05) SC/vehicle partition coefficient of OT, except for its 1% concentration, compared with the control. Meanwhile, SC/vehicle coefficient of TMP actually

### Table 6  Percutaneous permeation parameters of GP through excised rat skin.

<table>
<thead>
<tr>
<th>Enhancer</th>
<th>Solubility (Mg/mL)</th>
<th>Flux (μg/cm²/h)</th>
<th>Tlog (h)</th>
<th>Q24 (μg/cm²)</th>
<th>ERflux (logER)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>340.78 ± 4.59</td>
<td>0.10 ± 0.01</td>
<td>0.80 ± 0.39</td>
<td>5.42 ± 1.88</td>
<td></td>
</tr>
<tr>
<td>1% mint oil</td>
<td>339.28 ± 0.56</td>
<td>0.11 ± 0.06</td>
<td>1.05 ± 0.74</td>
<td>5.85 ± 2.49</td>
<td>1.10 (0.04)</td>
</tr>
<tr>
<td>3% mint oil</td>
<td>340.12 ± 13.56</td>
<td>0.18 ± 0.05</td>
<td>1.89 ± 0.57</td>
<td>9.19 ± 6.77</td>
<td>1.80 (0.26)</td>
</tr>
<tr>
<td>5% mint oil</td>
<td>343.15 ± 7.73</td>
<td>80.96 ± 51.08</td>
<td>6.55 ± 0.64</td>
<td>8048.94 ± 1183.64</td>
<td>809.60 (2.91)</td>
</tr>
</tbody>
</table>

P < 0.05, statistically significant difference between enhancers and control.  

a Values expressed as mean ± SD (n = 5), except for ERflux.  
b ERflux is the enhancement ratio calculated as follows: ER = Flux (with enhancer)/Flux (without enhancer); logER is natural logarithm value of the corresponding ERflux.

### Table 7  Partition coefficients of model drugs treated with different concentrations of mint oil.

<table>
<thead>
<tr>
<th>Enhancer</th>
<th>OT</th>
<th>TMP</th>
<th>FA</th>
<th>PR</th>
<th>GP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K⁺</td>
<td>PER</td>
<td>K⁺</td>
<td>PER</td>
<td>K⁺</td>
</tr>
<tr>
<td>Control</td>
<td>0.020 ± 0.004</td>
<td>0.047 ± 0.006</td>
<td>0.092 ± 0.022</td>
<td>0.052 ± 0.016</td>
<td>0.072 ± 0.017</td>
</tr>
<tr>
<td>1% mint oil</td>
<td>0.055 ± 0.009</td>
<td>2.75 ± 0.015</td>
<td>0.010 ± 0.001</td>
<td>0.010 ± 0.014</td>
<td>1.42 ± 0.025</td>
</tr>
<tr>
<td>3% mint oil</td>
<td>0.043 ± 0.011</td>
<td>2.16 ± 0.016</td>
<td>0.012 ± 0.001</td>
<td>0.34 ± 0.016</td>
<td>1.81 ± 0.025</td>
</tr>
<tr>
<td>5% mint oil</td>
<td>0.053 ± 0.032</td>
<td>2.64 ± 0.049</td>
<td>0.022 ± 1.05</td>
<td>1.87 ± 0.009</td>
<td>2.03 ± 0.151</td>
</tr>
</tbody>
</table>

P < 0.05, statistically significant difference between enhancers and control.  

Note: K: partition coefficient; PER: penetration enhancement ratio, with PER = (partition coefficient with essential oil treatment)/ (partition coefficient without essential oil treatment).  

* K values are expressed as mean ± SD (n = 3).
decreased in the presence of mint oil. However, SC/vehicle partition coefficients of FA, PR, and GP gradually increased as oil concentration increased, and the variation trend was similar to their permeation enhancement activity. This implied that mint oil could readily increase the partition of hydrophilic drugs into the SC, likely resulting in enhanced skin permeability of hydrophilic drugs.

$T_{lag}$ can reflect changes in the length of the permeation path ($h$) and diffusion coefficient ($D$) according to Eq. (2) as described above, and the diffusivity can be affected by structural alteration of lipid and keratin in the SC by penetration enhancers. As evident from Tables 4–6, $T_{lag}$ values of most examined drugs were gradually increased with increasing oil concentration, indicating that mint oil probably interacted with the SC lipids or keratin to change its barrier properties. FTIR was thus employed to investigate the biophysical changes of skin SC on account of its advantages in obtaining information about the lipid and protein conformation.

A representative spectrum of normal untreated skin SC showed absorption peaks around 2850 and 2918 cm$^{-1}$ due to symmetric and asymmetric C–H stretching vibration of lipid alkyl chains,$^{16}$ C–O peak of ester at 1740 cm$^{-1}$ region,$^{11}$ and two strong amide absorption peaks in the range of 1500–1700 cm$^{-1}$, namely amide I ($\sim$1650 cm$^{-1}$) and amide II ($\sim$1550 cm$^{-1}$).$^{17,18}$ The shift to a higher frequency occurred when CH$_2$ groups along the alkyl chain of lipids changed from trans to gauche conformation, and indicated that the SC lipid was disturbed,$^{13}$ and the change in the amide I and amide II absorption peaks was observed, indicating alterations in the keratin conformation under the effect of penetration enhancers.$^{9,19}$ In addition, frequencies of fatty acid carbonyl stretching (–C=O peak of ester) modes were sensitive to hydrogen bonding and provided information on head-group interactions in SC lipids.$^{18}$ FTIR absorption spectra and peak positions of lipids and amides in rat SC treated with different concentrations of mint oil are illustrated in Fig. 4 and Table 8, respectively.

FTIR spectra of rat stratum corneum after treatment with different agents for 12 hours.

### Figure 4

![FTIR spectra of rat stratum corneum after treatment with different agents for 12 hours.](image)

### Table 8

<table>
<thead>
<tr>
<th>Enhancer</th>
<th>Peak positions of lipid (cm$^{-1}$)</th>
<th>Peak positions of amide (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asymmetric C–H stretching</td>
<td>Symmetric C–H stretching</td>
</tr>
<tr>
<td>Blank</td>
<td>2917.85</td>
<td>2849.82</td>
</tr>
<tr>
<td>Control</td>
<td>2918.10</td>
<td>2849.93</td>
</tr>
<tr>
<td>1% mint oil</td>
<td>2919.23</td>
<td>2850.34</td>
</tr>
<tr>
<td>3% mint oil</td>
<td>2918.95</td>
<td>2850.24</td>
</tr>
<tr>
<td>5% mint oil</td>
<td>2920.81</td>
<td>2850.78</td>
</tr>
</tbody>
</table>

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resulted in a shift of approximately 3 cm$^{-1}$ in asymmetric C–H stretching peak. Meanwhile, the wavelength movements of amide I and amide II bands were narrow, indicating mint oil changed SC barrier mainly by perturbing SC lipids rather than affecting SC proteins. In addition, the –C=O peak of fatty acid gradually disappeared as oil concentration increased, suggesting that alteration in the hydrogen bond of the polar regions of the lipid bilayers occurred.

Conclusions

Results of this study appeared to show that mint (Mentha haplocalyx Briq.) oil possessed lower cytotoxicity compared with the standard and well-established penetration enhancer Azone, and effectively facilitated the percutaneous permeation of five model drugs with different lipophilicity in a concentration-dependent manner. The relationship between the log$K_{o/w}$ of the model drugs and their log$ER_{\text{flux}}$ tended to be in parabolic curve, indicating that mint oil probably exhibited higher efficiency on the percutaneous penetration of moderate hydrophilic drugs in Chinese herbal external preparations. Saturation solubility and SC/vehicle partition coefficient studies revealed that mint oil was prone to increase saturation solubility of lipophilic drugs due to its lipophilicity properties and improve the partition of hydrophilic drugs into skin stratum corneum. FTIR studies exhibited that mint oil probably changed the barrier function of the stratum corneum mainly by perturbing stratum corneum lipids.

In conclusion, mint oil at proper concentration appeared to effectively facilitate percutaneous permeation of both hydrophilic and lipophilic drugs in Chinese herbal external preparations, and exhibited higher efficiency for the percutaneous permeation of moderate hydrophilic drugs.

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References


