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ORIGINAL ARTICLE

The Effect of Herbal Penetration Enhancers on the Skin Permeability of Mefenamic Acid Through Rat Skin

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ABSTRACT

Objectives: Mefenamic acid (MA) is a strong non-steroidal anti-inflammatory drug, but because of its limited oral bioavailability and the side effects that come with taking it systemically, it is better to apply it topically. The major goal of this study was to see how certain permeation enhancers affected MA is *in vitro* skin permeability. In manufactured Franz diffusion cells, MA permeability tests using rat skin pretreatment with several permeation enhancers such as corn oil, olive oil, clove oil, eucalyptus oil, and menthol were conducted and compared to hydrate rat skin as a control.

Materials and Methods: The steady-state flux (Jss), permeability coefficient (Kp), and diffusion coefficient are among the permeability metrics studied. The permeability enhancement mechanisms of the penetration enhancer were investigated using fourier transform infrared spectroscopy (FTIR) to compare changes in peak position and intensities of asymmetric and symmetric C-H stretching, C=O stretching, C=O stretching (amide I), and C-N stretching of keratin (amide II) absorbance, as well as differential scanning calorimetry (DSC) to compare mean transition temperature and their enthalpies.

Results: Clove oil, olive oil, and eucalyptus oil were the most effective enhancers, increasing flux by 7.91, 3.32, and 2.6 times, as well as diffusion coefficient by 3.25, 1.34, and 1.25, respectively, when compared to moist skin. FTIR and DSC data show that permeation enhancers caused lipid fluidization, extraction, disruption of lipid structures in the SC layer of skin, and long-term dehydration of proteins in this area of the skin.

Conclusion: According to the findings, the permeation enhancers used improved drug permeability through excised rat skin. The most plausible mechanisms for greater ERflux, ERD, and ERP ratios were lipid fluidization, disruption of the lipid structure, and intracellular keratin irreversible denaturation in the SC by eucalyptus oil, menthol, corn oil, olive oil, and clove oil.

Key words: Mefenemic acid, percutaneous absorption, natural enhancers, differential scanning calorimetry, fourier transform infrared spectroscopy

INTRODUCTION

Transdermal drug delivery, based on medication permeation *via* the skin, has a number of benefits, including controlled and continuous drug delivery, which is important for drugs with short biological half-lives and low therapeutic indice, first-pass intestinal and hepatic bypass; avoidance of gastrointestinal irritation, which is common with oral medication, and easier drug localization at the target site.[1]

Partitioning and diffusion across *stratum corneum* (SC) and viable epidermis, transit into the dermis, and eventually systemic absorption or penetration into deeper tissues are the two key phases in skin permeation. SC, the skin's outermost layer, is the most effective barrier against drug penetration. Many techniques have been employed to increase medication access into the lower skin layer and deeper tissues. Permeation enhancers, both chemical and physical, have been developed to help carry high medication concentrations over the skin and into the systemic circulation or deeper tissues. Types of enhancers employed and their mechanisms of action differ.[2] Penetration enhancers work by increasing drug diffusion in the skin, lipid fluidization in SC, and increasing drug thermodynamic activity in the skin and vehicles as well as influencing drug partition coefficient.

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Mefenamic acid (MA), an enolic acid-class non-steroidal anti-inflammatory medication, is often used to treat mild-to-moderate pain, such as headaches, tooth discomfort, dysmenorrhea, rheumatoid arthritis, osteoarthritis, and other joint problems. MA is classified as a class II biopharmaceutical, meaning it is highly permeable across biological membranes, but has poor water solubility.[3] Although oral administration of MA is widely used, it necessitates frequent dosing every 6 h to maintain steady-state plasma concentrations.[4] This route is associated with gastrointestinal side effects such as ulceration, bleeding or perforation of the stomach, small intestine or large intestine, which can be fatal; as a result, it is contraindicated in patients with active ulceration or chronic inflammation of the upper or lower gastrointestinal tract.[5] As a result, the only way to get MA through the skin is through a transdermal administration.

Investigation of the microstructure of intercellular or lipids in the SC layer of the skin is necessary to create transdermal medication delivery methods. Differential scanning calorimetry (DSC) and fourier transform infrared spectroscopy (FTIR) have been used in recent research to investigate the organization of lipids and skin microstructure. FTIR analysis of skin may be a useful method for researching the interaction of chemical enhancers with SC that produce bands with varied wave numbers.[6]

Molecular analyses of the entire rat skin were conducted using DSC and FTIR to determine the mechanism by which the characteristics of enhancers/retardants vary in a specific medium.[7] Several infrared spectral bands of the skin are attributed to vibration of protein and lipid molecules in SC.[8] Lipid vibration is a good way to look at the microstructure of lamellar lipids in the intercellular region of SC layer. Many of the skin's infrared spectral bands are caused by the vibration of protein and lipid molecules in SC. Lipid vibration is a good predictor of the architecture of lamellar lipids in the intercellular area of SC layer. SC stretching vibrations of C-H symmetric vibration (about 2850 cm^{-1}) and C-H asymmetric vibration (around 2920 cm^{-1}) have been recorded. The wave number and width of C-H stretching peaks increase, when the lipids in SC fluidize. If the shift is to a higher wavenumber (blue shift), it means that SC membrane (lipid bilayer) is fluidizing, which contributes to the breakdown of the barrier properties, allowing more material to pass through SC. Lipid groups, on the other hand, reorient, producing a change in wave number (*e.g.*, red shift) and strengthening of subcutaneous-barrier characteristics, which slows permeant transit through the skin. The phase transition of the lipids is illustrated by an increase or drop in the band position (wavenumber) of the signals at 2920, 2850, and about 1738 cm^{-1}, when the penetration modifier acts on the lipid pathway.[9-12] Thermal analysis methods such as DSC have been used to investigate thermal transitions in mammalian SC. Thermodynamic analysis techniques such as DSC have been used to study temperature (Tm) transitions in SC. The skin's barrier function is controlled by SC, which is the epidermis' outermost layer.[13]

DSC method is commonly used to study lipid melting, lipid bilayer phase transitions, and protein denaturation in SC layer. A DSC investigation was planned to learn more about the lipid components and protein conformational stability of the entire skin rat treated with enhancers.[14]

The thermotropic behavior of the treated skin was examined by comparing the mean transition Tm and enthalpies (H). Any decrease in Tm might be the result of lipid breakdown in the bilayer and irreversible protein denaturation in the SC. Enthalpy loss is often linked to lipid fluidization in lipid bilayers and protein-lipid interactions.[15]

MATERIALS AND METHODS

Ramopharmin pharmaceutical firm donated MA (Tehran, Iran). Barij Essence Iranian Company in Kashan (Iran) provided eucalyptus oil, olive oil, corn oil, clove oil, and menthol.

Animal experiments

For *in vitro* permeation investigation, male Wistar rats weighing 200-250 g were employed. The abdomen skin hair was meticulously cut using an electric clipper and razor after the animal was sacrificed under ether anesthesia. The skin was dissected, and any excess subcutaneous fat from the dermal surface was removed. The animals were cared for accordance with the guidelines for the care and use of laboratory animals, and the experiments were approved by the Ahvaz Jundishapur University of Medical Sciences' Ethical Committee (IR.AJUMS.REC.1396.295). The National Academy of Sciences issued recommendations, which were published by the National Institutes of Health (U.S. Department of Health and Human Services, Office of Laboratory Animal Welfare).[16,17]

Skin permeation experiments

Permeation tests were conducted using specifically built diffusion cells with an effective area of about 4.906 cm^2. In the donor phase, 2 mL of each natural permeation enhancer was applied to the surface of the skin for 2 or 4 h. After that, the donor and receptor compartments were rinsed and filled with 5.5 mL of MA suspension (1%, w/v) and 30 mL of phosphate buffer solution (PBS, pH 7.4), respectively. As a control, fully hydrated samples were used. On a magnetic stirrer with a heater, the diffusion cell was inserted and clamped in a water bath at 37 ± 0.05°C. A tiny magnetic bead was used to agitate the receptor medium at 200 rpm. At predefined time intervals (0.5, 1, 2, 3, 4, 5,6,7,8, and 24 h), 2 mL of the receptor medium was removed and replaced with an equivalent amount of fresh buffer. The quantity of MA was assessed using a ultraviolet spectroscopic technique at 289 nm, after the samples were filtered.[18,19]

Statistical analysis

The total quantity of MA that penetrated into the receptor *via* each unit area of the diffusion surface was determined and displayed as a function of time. Linear component of the permeation curve's slope was used to compute the steady state flux (mg/cm^2.h). The permeability coefficient (K_p, cm/h) of MA through the skin was calculated using equation 1:

$$K_p = \frac{Jss}{c_v} \quad \text{..... (equation 1)}$$

Where J_{ss} and C_v are the steady-state flux and initial concentration of MA in receptor compartment, respectively. Also, the lag time (T_{lag}) and clear diffusivity coefficient (D_{app}) parameters were calculated. Since h does not represent the actual length of the pathway, D calculated from this formula is also clear to D. The value of D_{app} is calculated from equation 2:

$$D_{app} = h^2/6T_{lag} \quad \text{..... (equation 2)}$$

Enhancement ratios (ER) were calculated from equation 3:[20,21]

$$ER = \frac{\text{permeability parameter after treatment}}{\text{permeability parameter before treatment}} \quad \text{..... (equation 3)}$$

Statistical comparison was made using One-Way ANOVA, and $p<0.05$ was considered statistically significant.

T_{lag} of the drug obtained from the skin along the line of equilibrium to the axis of time in the cumulative curve of the drug. The value of D is calculated from equation 2: $D = h^2/ 6 T_{lag}$. Since h does not represent the actual length of the pathway, D calculated from this formula is also clear to D. Seeing that all calculations are based on the steady-state region, the cumulative flow rate of the drug is determined, hence, the establishment of sink conditions is indispensable for the citation of these parameters. In this work, the maximum concentration established in the receptor phase was less than 10% of the saturation solubility of the drug in the receptor phase, and therefore, a steady concentration gradient was established during the experiments, and with these conditions, a steady state flux was computed.

The differential scanning calorimeter

Using a DSC (Mettler-Toledo DSC[1] system) equipped, the changes in the structure of the entire skin caused by permeation enhancers were investigated. The skin samples were submerged in each natural permeation enhancer for 4 h before being blotted clean. In hermetically sealed aluminum pans, about 6-10 mg of treated skin samples were deposited. Simultaneously, an empty pan served as a reference. Skin samples were regularly subjected to heat between 20 and 200˚C at a rate of 5 degrees *per* min. At least three times, each experiment was conducted. DSC analyzer was calibrated and verified using an indium standard to assure data accuracy and reproducibility.[12]

FTIR experiments

To eliminate evidence of the permeation enhancer, the excised rat skin samples were treated for 4 h with olive oil, corn oil, clove oil, menthol, and eucalyptus oil, then vacuum dried (650 mmHg, 25 ± 1˚C) for 30 minutes and kept in desiccators. An FTIR facility was used to scan the skin samples in the 4000 to 500 cm^{-1} range (Uker, Vertex70, and Germany).[12]

RESULT AND DISCUSSION

Effect of herbal penetration enhancers on MA permeability

Tables 1, 2, and Figures 1, 2 show the permeability parameters following skin pretreatment with natural enhancers for 2 and 4 h compared to control as well as the quantity of MA penetrated through the rat abdomen skin from different enhancers. Table 1 demonstrates the impact of natural enhancers' pretreatment for 2 h on MA permeability compared to control as ERflux (drug flux ratio after and before skin pretreatment with enhancer) and ERD (drug flux ratio after and before skin pretreatment with enhancer) (drug diffusion coefficient after and before skin pretreatment with enhancer).[12] According to the findings, eucalyptus oil, olive oil, corn oil, clove oil, and menthol substantially improved MA flux and diffusion coefficient, according to the findings. Clove oil increased MA flux the most after a 2 hour skin pretreatment, increasing it by up to 7.91 fold compared to control, followed by eucalyptus oil (3.32 fold), olive oil (2.6 fold), corn oil (1.119 fold), and menthol (1.13 fold). Except for corn oil ($p>0.05$), all of the natural penetration enhancers had a significant influence on the diffusion coefficient ($p<0.05$), with clove oil having the largest enhancement effect compared to control.

Table 2 illustrates the impact of natural penetration enhancers' pretreatment for 4 h on MA permeability as ERflux and ERD compared to control. According to the data, eucalyptus oil, olive oil, corn oil, clove oil, and menthol substantially enhance MA flux and diffusion coefficient. After a 4 hour skin pretreatment, clove oil increased MA flow the most, up to 18.65 fold, compared

Table 1. Permeability parameters after 2 hours pretreatment with permeation enhancers compared with control (mean ± SD, n: 3)

Enhancer	J_{ss} (mg/cm².h)	D_{app} (cm²/h)	*p* (cm/h)	T_{lag} (h)	ERflux	ERD	ERP
Control	0.0060 ± 0.00010	0.1090 ± 0.17000	0.0006 ± 0.00001	5.62 ± 0.10	-	-	-
Menthol	0.0067 ± 0.00010	0.0835 ± 0.00200	0.0007 ± 0.00010	4.20 ± 0.22	1.13 ± 0.28	1.12 ± 0.35	1.13 ± 0.28
Eucalyptus oil	0.0190 ± 0.00100	0.0550 ± 0.00600	0.0019 ± 0.00010	2.60 ± 0.62	3.32 ± 1.26	1.51 ± 1.03	3.31 ± 0.26
Olive oil	0.0145 ± 0.00100	0.1405 ± 0.00100	0.0014 ± 0.00020	3.85 ± 0.86	2.60 ± 1.57	3.91 ± 0.95	2.60 ± 0.57
Corn oil	0.0069 ± 0.00100	0.0826 ± 0.01100	0.0007 ± 0.00020	3.30 ± 0.60	1.199 ± 0.35	1.46 ± 0.70	1.20 ± 0.35
Clove oil	0.0460 ± 0.0003	0.1103 ± 0.00800	0.0046 ± 0.00030	1.93 ± 0.01	7.91 ± 0.80	6.24 ± 0.59	7.90 ± 0.06

J_{ss}: Steady-state flux, D_{app}: Diffusivity coefficient, T_{lag}: Lag time, SD: Standard deviation

to control, followed by eucalyptus oil (3.57 fold), olive oil (3.57 flod), corn oil (2.65 flod), and menthol (2.65 flod) (2.17 fold). Except for corn oil (p>0.05), all of the penetration enhancers had a significant impact on the diffusion coefficient, with clove oil having the biggest enhancing effect on the diffusion coefficient compared to the control.

1,8-Cineole makes up about 75% of eucalyptus oil. Cineole is a cyclic terpene that makes liquid pools in SC and changes SC's lipid structure. This makes it easier for polar and non-polar medicines to get through the membrane.[20]

Wang et al.[21] investigated the impact of corn oil, olive oil, and jojoba oil variations on the increase in skin permeability of aminophylline *via* the human skin. The data revealed that vegetable oils had a larger role in increasing drug permeability with jojoba oil having the most significant impact.[21] Salimi and Fouladi[22] looked at how different penetration enhancers affected meloxicam's skin permeability. Transcutol oil, eucalyptus oil, and oleic acid had the greatest impact on skin flux increase.[22] Salimi et al.[23] looked at how different penetration enhancers affected adapalene skin permeability. Clove oil and eucalyptus oil had the greatest impact on skin flux and partition coefficient increase.[23]

Differential scanning calorimetry

Thermotropic behavior of the treated skin was assessed using mean transition Tm and corresponding enthalpies (H). Transition Tm and enthalpies are shown in Table 3, Tm1 and Tm2 from hydrated rat skin were 67.5°C and 112°C, which means that the lipids in the skin had melted and the keratin in the skin had been broken down irreversibly. Any decrease in Tm might be the result of lipid breakdown in the bilayer and irreversible protein denaturation in the SC. While lipid fluidization in lipid bilayers and protein-lipid complexes is often linked to a decrease in enthalpy, this is not always the case.[16] In human dermal DSC graphs, Kaushik and Michniak-Kohn[15] found three endothermic transition peaks at Tm of 59-63°C (Tm1), 75-82°C (Tm2), and 99.5-120°C (Tm3). They proposed that Tm1 relates to the change of lipid forms from a lamellar to a disordered state, Tm2 to protein-lipid or the rupture of polar head groups of lipids, and Tm3 to irreversible denaturation of proteins, respectively.[15]

When compared to hydrate rat skin, the thermograms of skin treated with menthol show reduced Tm2 and H1 and H2. Tm1 was also eliminated by menthol. In this study, menthol was found to change the structure of SC layer in a number of ways, including making lipids more fluid in the intercellular area, breaking down lipids in the bilayer, and permanently breaking down proteins.

DSC findings from skin pretreatment with eucalyptus oil revealed reduced Tm1 and H1 as well as decreased H2 and Tm2. This means that eucalyptus oil may make the skin more permeable by causing lipids in the bilayer to break down and irreversible protein destabilization in SC layer.

Tm1 changed to lower melting points and Tm2 rose in skin

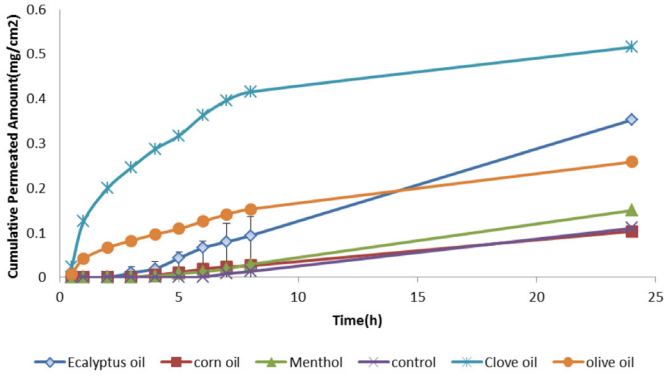

Figure 1. The amount of mefenamic acid permeated after 2 hours pretreatment rat skins with various herbal penetration enhancers

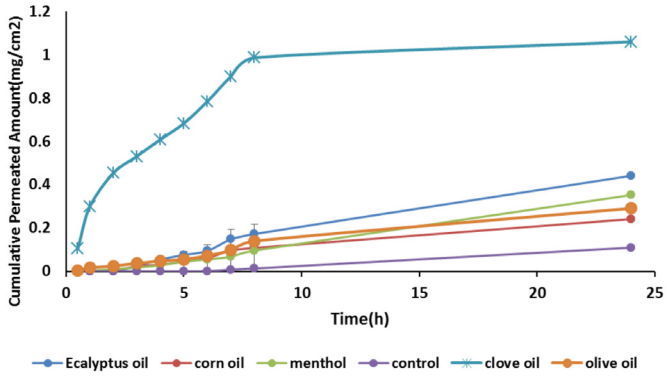

Figure 2. The amount of mefenamic acid permeated after 4 hours pretreatment rat skins with various herbal penetration enhancers

Table 2. Permeability parameters after 4 hours pretreatment with permeation enhancers compared with control (mean ± SD, n: 3)

Enhancer	J_{ss} (mg/cm².h)	D_{app} (cm²/h)	p (cm/h)	T_{lag} (h)	ERflux	ERD	ERP
Control	0.0060 ± 0.0001	0.1093 ± 0.017	0.0006 ± 0.00001	5.61 ± 0.10	-	-	-
Menthol	0.0126 ± 0.0001	0.4095 ± 0.002	0.0012 ± 0.00010	1.42 ± 0.50	2.17 ± 0.68	36.11 ± 0.68	2.17 ± 0.68
Eucalyptus oil	0.0208 ± 0.0010	0.1808 ± 0.002	0.0020 ± 0.00100	2.85 ± 0.70	3.57 ± 0.64	5.78 ± 0.06	3.57 ± 0.64
Olive oil	0.0206 ± 0.0020	0.4976 ± 0.030	0.0020 ± 0.000100	0.30 ± 0.03	3.63 ± 0.56	49.83 ± 0.64	3.63 ± 0.56
Corn oil	0.0158 ± 0.0010	0.0627 ± 0.004	0.0015 ± 0.00100	1.74 ± 0.60	2.65 ± 0.20	2.82 ± 0.66	2.65 ± 0.20
Clove oil	0.1098 ± 0.0500	0.0337 ± 0.003	0.0110 ± 0.00200	4.1 ± 0.90	18.35 ± 0.92	1.55 ± 0.05	18.35 ± 0.40

J_{ss}: Steady-state flux, D_{app}: Diffusivity coefficient, T_{lag}: Lag time, SD: Standard deviation

pretreated with olive oil thermograms, whereas H1 and H2 were reduced compared to controls. This means that olive oil may make the skin more permeable by causing lipids in the bilayer to break down and irreversible protein destabilization in SC layer.

When the skin was treated with corn oil, the melting points of Tm1 and Tm2 changed lower and higher, respectively, compared to the control. Additionally, compared to the control, H1 and H2 shifted to lower levels. This suggests that corn oil increase skin permeability by breaking down lipids in the bilayer and irreversibly destabilizing proteins in SC layer.

As the skin was treated with clove oil, the melting points of Tm1 and Tm2 changed lower and higher, respectively, when compared to the control. Additionally, as compared to the control, H1 and H2 shifted to lower levels. A study found that clove oil may make the skin more permeable because it breaks down lipids in the bilayer and permanently changes proteins in SC layer, which makes the skin more permeable.

FTIR spectroscopy

Tables 4-6 provide spectrum analysis of samples, showing peak position and intensity variations from 4000 cm^{-1} to 500 cm^{-1}. If the wave number increases (blue shift), it indicates that SC membrane (lipid bilayer) is becoming more fluid, making it simpler for drugs to enter the body through SC.[24] On the other hand, lipid groups reorient, changing a lower wave number (*e.g.*, red shift) and strengthening of subcutaneous-barrier qualities, resulting in a slowing down of permeant passage through the skin.[11]

The spectra of menthol-treated rat skin showed changes in peak height and wave numbers. Red shifts were noticed in the skin treated with menthol at wave numbers 2838.63cm^{-1} and 2747.53cm^{-1}. This shows that lipid groups have been altered, resulting in a stronger SC barrier. There was a relative red shift in 1728.58 cm^{-1} band was observed in the skin pretreated with menthol, indicating the formation of strong hydrogen bonds within the lipid structures. Pretreating skin rats with menthol had 75.17% reduction in the mean peak height of C-N stretching (amide I) absorbance, showing that it interacts mostly with proteins in SC layer.

Significant reductions in the height of peaks in the 2981.66, 2915.86, 1690.2, and 1642.65 cm^{-1} wave numbers were seen in the FTIR spectra of skin pretreated with clove oil. According to the findings, clove oil interacts mostly with lipids and proteins in SC layer. The findings of the permeability parameters after clove oil pretreatment correlated with FTIR and DSC measurements.

Changes in peak height and wave numbers are seen in the spectra of rat skin prepared with eucalyptus oil. At wave numbers 2848.59 cm^{-1} and 2741.57 cm^{-1}, a red shift was seen in the skin prepared with eucalyptus oil, suggesting lipid reorientation that causes SC barrier characteristics to be strengthened. The skin prepared with eucalyptus oil showed a red shift in which the peak number's height (1654.93 cm^{-1}) increased. The wave number (1791.51cm^{-1}) decreases in peak height, causing blue shifts.

Changes in peak height and wave numbers may be seen in the spectra of corn oil-treated rat skin. The skin treated with corn oil showed a blue shift in peak number heights (2981.64, 2919.9, 1733.58, and 1561.21 cm^{-1}), suggesting the denaturation of proteins and lipids in SC layer.

The spectra of rat skin pretreated with olive oil reveal changes in peak height and wave numbers. This implies that when olive oil was used to treat skin, the peak number shifted blue (2981.64, 1733.85 cm^{-1}). This shows that the olive oil broke down the proteins and lipids in SC layer.

CONCLUSION

According to the findings, the permeation enhancers used improved drug permeability through excised rat skin. The most plausible mechanisms for greater ERflux, ERD, and ERP ratios were lipid fluidization, disruption of the lipid structure, and intracellular keratin irreversible denaturation in the SC by eucalyptus oil, menthol, corn oil, olive oil, and clove oil.

Table 3. Effect of permeation enhancer on the thermal properties of excised rat skin (mean ± SD, n: 3)

Enhancer	Transition temperature (°C)		Transition enthalpy (mj/mg)	
	Tm1	Tm2	H1	H2
Water (control)	67.5 ± 2.1	112.0 ± 6.6	7.010 ± 0.4	552.4 ± 9.0
Menthol	0	124.0 ± 0.1	0	2.7 ± 0.3
Eucalyptus oil	31.0 ± 0.9	127.5 ± 1.1	2.672 ± 0.1	8.9 ± 0.8
Olive oil	37.7 ± 0.9	115.0 ± 0.9	5.844 ± 0.6	6.2 ± 0.2
Corn oil	0	118.1 ± 1.1	0	99.9 ± 2.1
Clove oil	36.0 ± 0.2	116.0 ± 0.5	0.900 ± 0.2	2.2 ± 0.1

Tm1: Mean transition temperature of lipids, SC Tm2: Mean transition temperature of irreversible denaturation of intracellular, SC keratin, H1: Transition enthalpy of lipid phase, SC H2: Transition enthalpy of keratin phase SC, SD: Standard deviation

Table 4. FTIR peak wave numbers (cm^{-1}) changes compared to control (untreated skin) and abdominal hydrated whole skin rat following treatment with different enhancers (mean SD, n: 3)

Enhancer	C-H stretching Asy	C-H stretching Sym	C=O stretching of lipid ester	Amide I	Amide II
Water	2981.77 ± 0.16	2856.34 ± 0.16	1731.68 ± 0.14	1667.04 ± 0.12	1547.67 ± 0.11
Menthol	2838.63 ± 0.15	2747.53 ± 0.13	1728.58 ± 0.11	1603.87 ± 0.16	1538.91 ± 0.20
Eucalyptus oil	2848.59 ± 0.12	2741.57 ± 0.15	1791.51 ± 0.13	1654.93 ± 0.18	1565.24 ± 0.12
Olive oil	2990.78 ± 0.12	2887.85 ± 0.14	1743.31 ± 0.18	1626.18 ± 0.18	1549.92 ± 0.15
Corn oil	2981.64 ± 0.21	2919.9 ± 0.14	1733.85 ± 0.19	1647.25 ± 0.13	1561.27 ± 0.14
Clove oil	2981.66 ± 0.15	2915.86 ± 0.11	-	1690.2 ± 0.21	1642.65 ± 0.17

SD: Standard deviation

Table 5. Decrease in mean peak height (± SD), compared with control (hydrated skin) of C=O stretching (amide I) and C-N stretching of keratin (amide II) absorbance of abdominal hydrated whole skin rat following treatment with different enhancers (mean ± SD, n: 3)

Enhancer	Asymmetric C-H stretching		Symmetric C-H stretching		C=O stretching of lipid ester	
	Peak height	%D	Peak height	%D	Peak height	%D
Water	1.8355 ± 0.008	-	1.95 ± 0.005	-	2.061 ± 0.001	-
Menthol	0.527 ± 0.050	71.29	0.512 ± 0.003	73.74	2 ± 0.01	2.96
Eucalyptus oil	0.517 ± 0.010	71.83	0.436 ± 0.008	77.64	0.775 ± 0.006	62.40
Olive oil	0.381 ± 0.001	79.24	0.207 ± 0.010	89.38	0.425 ± 0.002	79.38
Corn oil	2.272 ± 0.005	N.D	2.323 ± 0.007	N.D	2.079 ± 0.005	N.D
Clove oil	1.227 ± 0.004	33.15	0.464 ± 0.010	76.21	0	100.00

% Decrease in peak height (%D) = (peak height from untreated whole skin-peak height from solvent treated whole skin)/peak height from untreated whole skin x 100. N.D: No decrease in peak height, SD: Standard deviation

Table 6. Decrease in mean peak height (± SD), compared with control (hydrated skin) of C=O stretching (amide I) and C-N stretching of keratin (amide II) absorbance of abdominal hydrated whole skin rat following treatment with different enhancers (mean ± SD, n: 3)

Enhancer	Amide I stretching of keratin		Amide II stretching of keratin	
	Peak height	%D	Peak height	%D
Water	2.111 ± 0.006	-	2.151 ± 0.005	-
Menthol	1.2 ± 0.009	43.15	0.534 ± 0.009	75.17
Eucalyptus oil	0.727 ± 0.005	65.56	0.727 ± 0.007	66.20
Olive oil	0.479 ± 0.010	77.31	0.611 ± 0.010	71.59
Corn oil	2.1 ± 0.006	0.52	2.04 ± 0.011	5.16
Clove oil	1.889 ± 0.020	10.52	1.94 ± 0.006	9.81

SD: Standard deviation

Ethics

Ethics Committee Approval: Ahvaz Jundishapur University of Medical Sciences' Ethical Committee (IR.AJUMS.REC.1396.295).

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.S., Design: A.S., Data Collection or Processing:S.S., Analysis or Interpretation: A.S., Literature Search: S.S., Writing: A.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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