ORIGINAL ARTICLE

Effect of Terpenes on the Enhancement of Skin Permeation of Lipophilic Drugs: A Systematic Review

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ABSTRACT

Objectives. The stratum corneum (SC) remains an obstacle to the passage of drugs applied topically. Several investigations have focused on enhancing the penetration of drugs through the SC by integrating permeation enhancers (PE) into the drug formulation. Terpenes are among the PE utilized in formulations and are categorized by the regulatory bodies as generally recognized as safe (GRAS). This study aimed to comparatively analyze the skin permeation enhancing effect of terpenes on lipophilic drugs.

Methods. The present study reviewed the effects of terpenes on the permeation of lipophilic small-molecule drugs through the skin using original research published between 2000 - 2022 retrieved from PubMed®. The search phrase used was (lipophilic drug) AND (terpene) AND (permeation enhancer).

Results. Terpenes increase the percutaneous permeation of lipophilic small molecule drugs by 1.06 – 256.80-fold. Linear correlation analysis of terpenes' cLog *P* with enhancement ratio (ER) revealed moderate and strong positive correlations in pig skin (r = 0.21) and mouse skin (r = 0.27), and rat skin (r = 0.41) and human skin (r = 0.67), respectively. Drug cLog *P* is a poor (r = -0.06) predictor of permeation enhancement. Terpenes with cLog *P* higher than 2.40 had ER greater than 10. Higher ERs (>30) were recorded for nerolidol, carvacrol, borneol, terpineol, limonene, menthone, pulegone, and menthol among the terpene-chemical penetration enhancers.

Conclusion. cLog *P* of terpene-based chemical permeation enhancers (CPE) is strongly correlated with ER of lipophilic drugs across human skin. Non-polar groups in terpenes and hydrogen bond interactions by terpenes with SC lipid enhance cutaneous drug penetration of lipophilic drugs.

Keywords: terpenes, lipophilic drugs, permeation enhancer, skin, enhancement ratio

**Ms. Longgos and Ms. Pequiro shared first authorship.*

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INTRODUCTION

Topical and transdermal drug delivery systems are efficient methods to deliver drugs owing to their pharmacokinetic advantages, such as bypassing the hepatic first-pass metabolism and selective drug delivery to the desired site of action. Drugs that are administered by topical application or transdermal route permeate through the skin slowly.[1] As a result, a high drug concentration exists in the dermis and subcutaneous tissue.[1] This route is particularly advantageous for drugs that have short biological half-lives and those with narrow therapeutic indices.[2] Despite the advantages that these drug delivery systems offer, limitations still exist,

particularly, concerns about the permeation of drugs across the stratum corneum (SC).

The SC is the primary barrier for drugs to penetrate the skin. It exhibits selective permeability which only allows relatively lipophilic drugs to diffuse deep into the layers of the skin. Since the SC is composed of dead cells, studies show that the transport of solute is primarily by passive diffusion following a concentration gradient obeying Fick's law of diffusion.[3]

The lipid components of the SC include ceramide, cholesterol, and fatty acids. These lipid components are also responsible for the penetration of lipophilic drugs through the transcellular route by partitioning into the intercellular lipids of the SC. Lipophilic small-molecule drugs (molecular weight of fewer than 500 Daltons; positive log *P* (P < 1) are the ideal drugs that can be given through these drug delivery systems because these drugs can permeate through the SC passively. They represent a wide range of clinically important molecules used to treat and manage dermatological disorders, however, one of the major problems is the frequency of application required to deliver the desired drug concentration and elicit an effect. A relationship was elucidated between the lipophilicity of a drug and its ability to permeate across the skin. Highly lipophilic drugs exhibit low permeability and the optimal Log *P* value is approximately 2.[3]

An approach that is extensively studied to improve drug delivery through the skin is the use of permeation enhancers (PE). PEs enhance the passage of drugs through SC by overcoming the barrier properties of SC either by modifying or perturbing the lipid structures in the SC or by denaturing the proteins and keratin cells present in the layer.[4] Currently, more than 360 molecules have been observed to enhance the permeation of drugs across the SC. An ideal PE, however, must be pharmacologically inert, chemically compatible, and non-toxic. Several PEs have been reported to cause various problems when they are employed at concentrations needed for achieving its penetration enhancement effect. Sulfoxides and their derivatives have been reported to cause reversible denaturation of keratin, leading to keratolysis. Alcohols, which are also used as PE, can dissolve skin lipids and cause skin dryness.[4]

In the past few years, pharmaceutical formulators have directed development efforts in investigating the penetration enhancement effects of nature-derived and synthetic compounds, which are classified by the FDA as generally recognized as safe (GRAS). Among the widely investigated naturally occurring PEs include terpenes and fatty acid esters. Terpenes are naturally occurring volatile oils with a structure based on repeating units of isoprene. These compounds are effective PEs possessing a high percutaneous enhancement ability, less toxicity, low irritancy potential, and reversible effect on subcutaneous lipids.[5] Terpenes, being natural PEs, satisfy the growing movement and preference toward the use of natural pharmaceutical excipients. This strategy can be exploited to deliver an effective dose of a drug through the skin and promote adherence among patients. The interest, therefore, lies in developing dermatological formulations with desirable safety and permeation properties to support the delivery of drugs to and across the skin.

The available studies on terpenes are limited to describing their effect on both lipophilic and hydrophilic drugs. Molecular weights (MW) and lipophilicity values (e.g., Log *P*) of drugs are often used to predict their permeation across the skin. However, there are no studies that directly identify terpene-PEs across a wide variety of biological membranes with the ideal permeation-enhancing properties to support investigations aiming to develop terpene-type PEs-based formulations. The lack of guiding documents to identify terpenes with optimal permeation enhancing ability to deliver an effective dose of lipophilic small drugs topically and transdermally administered must be addressed. In this review, the comparative analysis of the degree of skin permeation enhancement effected by terpenes on lipophilic small-molecule drugs is conducted, an essential quality target profile in the development of dermatological formulations. Further, the evaluation of the relationship between an established physicochemical property of terpenes, cLog *P*, with permeation parameters (i.e., flux) of drugs is covered. This systematic review is a critical evaluation of the usefulness of terpenes in enhancing the penetration of lipophilic drugs through the skin.

METHODS

A systematic data search around the research question "What is the effect of terpenes on the skin permeation of lipophilic small-molecule drugs?" was conducted using PubMed® (https://pubmed.ncbi.nlm.nih.gov/) (accessed on 20 March 2022). The study utilized the recommendations prescribed in the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) 2020 statement[6] (Figure 1). A systematic review of all research articles retrieved from PubMed was critically assessed and evaluated in relation to the research question.

Medical Subject Headings (MeSH) and search terms derived around the research question were employed in searching for original articles archived in PubMed®, ScienceDirect, and MEDLINE in which the search phrase, lipophilic drug AND terpene AND permeation enhancer, was utilized. Systematic reviews and meta-analyses were excluded from this review. The systematic search was limited to articles published between 2000 - 2022. Calculated Log *P* (cLog *P*) values of the drugs and terpenes were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 25 May 2022).

Eligibility Criteria

The title of the research articles and their abstracts found in the database search were screened manually by the researchers to exclude unrelated studies. Duplicated articles

were excluded using the "Check for Duplicates" tool in Mendeley Reference Manager (version 2.57.0, Mendeley Ltd., Amsterdam, the Netherlands). Research articles were considered eligible if the permeation experiment was performed *in vitro* using Franz diffusion cells (FDC) and the cLog *P* values are available in PubChem. The specific inclusion-exclusion criteria are reflected in Figure 2.

The selection of the manuscripts involved four independent researchers who initially selected the articles based on the title (title screening); then abstract screening followed, and finally the analysis of the full-text publication. Any disagreement was resolved through a discussion and consensus between the investigators. Full-text articles were manually reviewed to identify and exclude articles that did not fit the stated criteria.

Data Analysis

The relationship between cLog *P* values of terpene - CPEs and the resulting ERs was determined through linear correlation analysis. The cLog *P* of terpene-CPEs were plotted against the ER for a particular drug reported in the article. The strength and direction between these variables were presented by the Pearson correlation coefficient (*r*) calculated using the Data Analysis ToolPak function in Microsoft® Excel Spreadsheet Software (version 16.64, Microsoft Corporation, Redmond, WA, USA).

RESULTS

Initial articles found using the search strategy were 1,292, and 56 were found to be duplicates (Figure 1). Evaluation of article titles and abstracts reduced the number of articles to 58. Full-text versions were then retrieved and evaluated. Of all the articles fully read, 35 were found to comply with eligibility criteria and were included in the final review.

Studies presented in the reviewed articles were performed under infinite dose conditions using biological membranes (n = 35). Artificial membranes (e.g., silicone membrane, Strat-M®) were not reported. All of the research articles collected employed an experimental research design (n = 35) to generate the permeation parameters. Enhancement ratios (ER; indicating the efficacy of drug permeation when co-administered with the enhancer) were reported in 11 papers while 24 reported fluxes (J_{flux}; the amount of permeant crossing the membrane per time in μg/cm^2 per hour). Data presented in the results section of these studies included cumulative drug receptor concentrations (Q24; n = 5) and drug skin concentrations (n = 4).

Terpenes reported as chemical permeation enhancers from the eligible research articles included monoterpenes (n = 18) and sesquiterpenes (n = 2) listed in Table 1.

MW and cLog *P* values of lipophilic small-molecule drugs were reported in the permeability studies and referenced from PubChem. cLog *P* values of the small-molecule drug penetrants ranged from 0.38 to 6.00 with MW ranging from

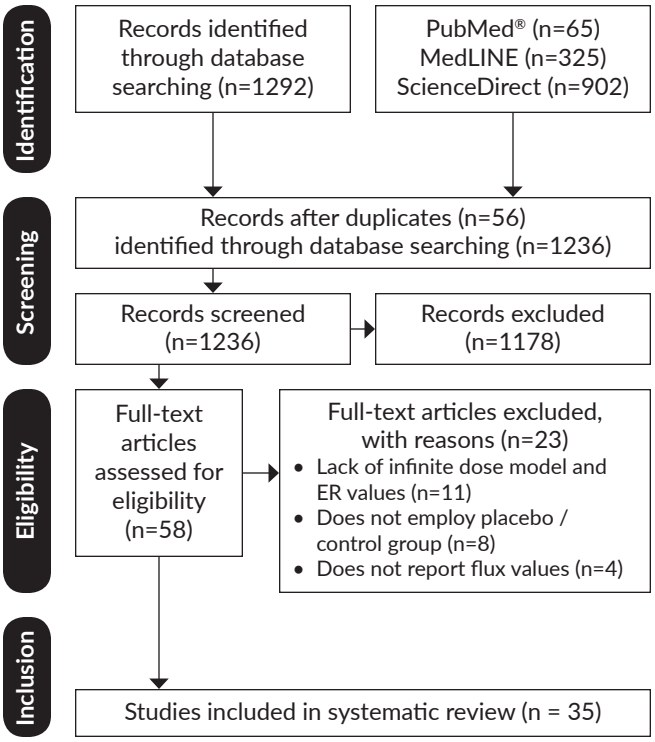

Figure 1. PRISMA flow diagram.

Inclusion Criteria:	*Exclusion Criteria:*
1. Experimental assay utilizing Franz Diffusion Cell apparatus.	1. Use of artificial membranes in the conduct of permeation experiments.
2. Permeant drug has MW <500 Daltons; positive (+) cLog *P*.	2. Systematic Reviews and Meta-analyses.
3. Studies published from 2000 to 2022.	3. Permeant drug >500 Daltons; negative (-) cLog *P*.
4. Results present infinite dose model and ER values.	4. Studies utilizing qualitative methods.
5. Studies published using the English language and available full-text.	5. Studies published exclusively in non-English language.

Figure 2. Inclusion and exclusion criteria of the study.

136.19 to 491.10. The MW and cLog *P* values of the drugs used are presented in Table 2.

The comparative analysis between the MW and cLog *P* values of the lipophilic small-molecule drugs and their respective ER values was conducted to establish the impact of size and lipophilicity on the permeation of drugs in terpene-PE-containing formulations through the SC. The MW, cLog *P*, and ER values were collated and plotted as shown in Figures 3 and 4. The results of the correlation analysis revealed that there is a weak negative relationship (r = -0.06) between cLog *P* and ER values (Figure 3). The molecular

weight and ER values resulted in a weak positive correlation (r = 0.12) in the analysis (Figure 4).

Skin Permeation Enhancement Effect of Terpenes

In this review, permeation profiles of lipophilic drugs from terpene-CPE-based formulations were compared to that of the control formulations (without terpene-CPE) consisting of the vehicle and the drug. Data collated from the results of the eligible research articles consist of the values representing the fluxes, ERs, and cumulative drug receptor concentrations or skin drug concentrations after 24 hours. Some ER values were derived from the reported fluxes by dividing the flux of the drug with the permeation enhancer over the flux of the drug without the permeation enhancer (control). The drug permeation enhancement ratio by terpenes using human skin, mouse skin, pig skin, and rat skin

Table 1. Class and cLog *P* Values of Terpenes Utilized in Included Studies

Class	Terpene
Sesquiterpene	Bisabolol
	Nerolidol
Monoterpene	1,8- cineole
	α-terpineol
	Borneol
	Camphor
	Carvone
	Cymene
	Carvacrol
	d-limonene
	Fenchone
	Geraniol
	Linalool
	Menthol
	Menthone
	p-menthane-3,8-diol
	Pulegone
	Terpinen-4-ol
	Thymol
	Verbenone

Table 2. Calculated Log *P* and Molecular Weight of Lipophilic Small-Molecule Drugs

Drug	cLog *P*	MW	Drug	cLog *P*	MW
Aceclofenac	2.17	354.20	***Ketoprofen***	3.12	254.28
Alprazolam	2.12	308.80	***Lidocaine***	2.44	234.34
Antipyrine	0.38	188.32	***Lomerizine***	4.90	468.50
Aspirin	1.19	180.16	***Melatonin***	0.80	232.28
Buspirone HCl	2.63	422.00	***Memantine HCl***	3.31	215.76
Carbamazepine	2.77	236.27	***Nicardipine HCl***	3.82	479.50
Clobetasol propionate	3.50	467.00	***Osthole***	3.80	244.28
Diclofenac potassium	4.98	334.20	***Piroxicam***	3.10	331.30
Etodolac	2.80	287.35	***Propranolol HCl***	3.03	295.80
Ferulic acid	1.51	194.18	***Puerarin***	0.48	416.40
Flurbiprofen	4.20	244.26	***Quercitin***	1.81	302.23
Genistein	3.04	270.24	***Salicylic acid***	2.26	138.12
Haloperidol	4.30	375.90	***Tamoxifen***	5.93	371.50
Hydrocortisone	1.61	362.50	***Terbinafine***	6.00	291.40
Ibuprofen	3.97	206.28	***Tetramethylpyrazine***	1.28	136.19
Imipramine HCl	4.53	316.90	***Verapamil HCl***	5.23	491.10
Indomethacin	4.30	357.80	***Zidovudine***	0.05	267.24

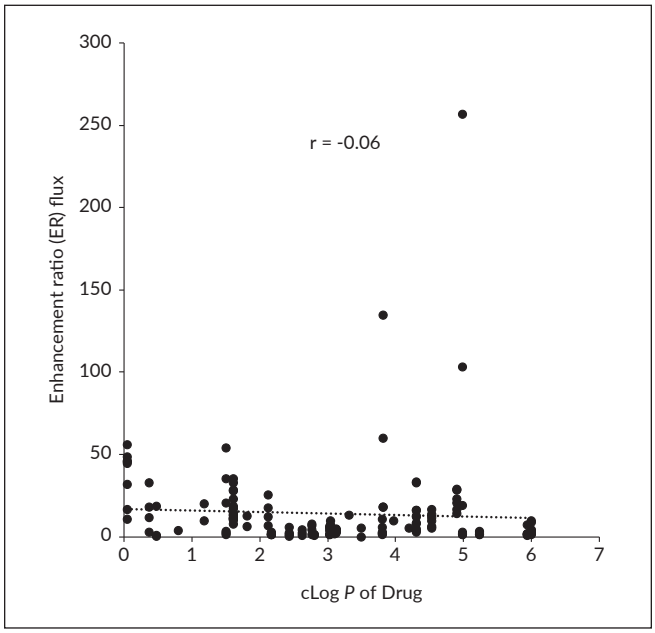

Figure 3. The correlation between the cLog *P* of the drug and the enhancement ratio.

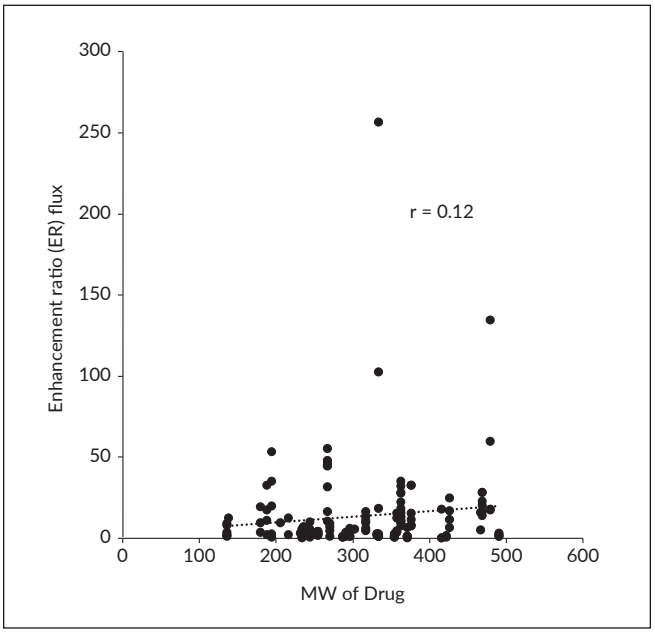

Figure 4. The correlation between the MW of the drug and the enhancement ratio.

as membranes together with their respective cLog *P* values are presented in Table 3.

Overall, terpenes increased the permeation of lipophilic small-molecule drugs by 14-fold when used as PE in comparison to control formulations (Table 3). Most of the terpene enhancers evaluated had significantly higher permeability effects on the drugs relative to the control. Monoterpenes, a class of terpenes, were the most extensively studied terpene class as permeation enhancers in the experiments listed.

The biological membranes used in the skin permeation experiment were rat skin (n = 15), pig skin (n = 10), human skin (n = 7), and mouse skin (n = 4). Analysis of drug permeant in samples was either quantified through techniques such as UV-VIS spectrophotometry, high-performance liquid chromatography (HPLC), or liquid scintillation counting (LSC).

The cLog *P* of the terpenes referenced from PubChem and the ERs from the *in vitro* drug permeation parameters of drugs in different research articles were collated and

Table 3. Summary of Drug Permeation Enhancement Ratio by Terpenes

Terpene	cLog *P*	Drug	ER	Formulation	Membrane	Reference
Anethole	3.3	Etodolac	1.52**	Carboxymethylcellulose gel	Rat skin	Tas et al., 2007[7]
Bisabolol	3.8	Propranolol HCl	6.28**	Ethanol	Rat skin	Cui et al., 2011[8]
Borneol	2.70	Propranolol HCl	5.01**	Ethanol	Rat skin	Cui et al., 2011[8]
		Aspirin	19.81*	Propylene glycol	Rat skin	Yi et al., 2015[9]
		Antipyrine	32.84*	Propylene glycol	Rat skin	Yi et al., 2015[9]
		Ibuprofen	9.78	Propylene glycol	Rat skin	Yi et al., 2015[9]
		Salicylic acid	12.76*	Propylene glycol	Rat skin	Yi et al., 2015[9]
Camphor	2.20	Propranolol HCl	3.67**	Ethanol	Rat skin	Cui et al., 2011[8]
		Aspirin	9.82*	Propylene glycol	Rat skin	Xie et al., 2016[10]
		Antipyrine	17.80*	Propylene glycol	Rat skin	Xie et al., 2016[10]
		Indomethacin	3.97*	Propylene glycol	Rat skin	Xie et al., 2016[10]
		Lidocaine	5.68*	Propylene glycol	Rat skin	Xie et al., 2016[10]
Carvacrol	3.49	Haloperidol	33.16**	Ethanol	Human skin	Vaddi et al., 2002[11]
		Etodolac	0.95**	Carboxymethylcellulose gel	Rat skin	Tas et al., 2007[7]
Carvone	2.40	Genistein	4.78**	Ethanol, methocel gel	Human skin	Chadha et al., 2011[12]
		Hydrocortisone	13.1*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Zidovudine	32.02**	Ethanol	Rat skin	Narishetty & Panchagnula, 2004[14]
		Diclofenac *(2.5%)	18.85**	Sodium carboxymethylcellulose	Rat skin	Nokhodchi et al., 2007[15]
		Aceclofenac	1.44**	Microemulsion	Rat skin	Lee et al., 2005[16]
		Imipramine HCl	6.27*	Ethanol	Rat skin	Jain et al., 2002[17]
Cineole (Eucalyptol)	2.50	Buspirone HCl	2.2*	Ethanol	Human skin	Meidan et al., 2003[18]
		Alprazolam	6.9**	Propylene glycol	Human skin	Boix et al., 2005[19]
		Haloperidol	8.1**	Propylene glycol	Human skin	Lim et al., 2006[20]
		Genistein	7.41**	Ethanol, methocel gel	Human skin	Chadha et al., 2011[12]
		Zidovudine	16.65**	Ethanol	Human skin	Narishetty & Panchagnula, 2005[21]
		Zidovudine	55.8**	Ethanol	Rat skin	Narishetty & Panchagnula, 2004[14]
		Hydrocortisone	14.5*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Lidocaine	2.05**	Hydrogel	Mouse skin	Song et al., 2009[22]
		Lomerizine	28.8**	Propylene glycol	Mouse skin	Furuishi et al., 2013[23]
		Tamoxifen	7.03*	Borage oil, ethanol	Pig skin	Ho et al., 2004[24]
		Piroxicam	1.99**	Carbopol gel	Pig skin	Doliwa et al., 2001[25]
		Osthole	2.27*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Ferulic acid	1.22*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Trimethylpyrazine	2.16*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Puerarin	0.60*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Memantine	2.58**	Ethanol	Pig skin	del Rio-Sancho et al., 2012[27]
		Aceclofenac	1.13**	Microemulsion	Rat skin	Lee et al., 2005[16]
		Verapamil	1.4*	Propylene glycol	Rat skin	Güngür et al., 2008[28]
		Imipramine HCl	13.6*1	Ethanol	Rat skin	Jain et al., 2002[17]
		Propranolol HCl	1.48**	Ethyl cellulose, polyvinyl pyrrolidone film	Rat skin	Amnuaikit et al., 2005[29]
Cymene	4.10	Hydrocortisone	22.9*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
Farnesol	4.80	Diclofenac	102.91**	Sodium carboxymethylcellulose	Rat skin	Nokhodchi et al., 2007[15]

Table 3. Summary of Drug Permeation Enhancement Ratio by Terpenes *(continued)*

Terpene	cLog *P*	Drug	ER	Formulation	Membrane	Reference
Fenchone	2.30	Ketoprofen	3.7*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2000a[2]
		Ketoprofen	4.4*	Pluronic F-127 gel	Mouse skin	El-Kattan et al., 2000a[2]
		Hydrocortisone	10.1*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Hydrocortisone	7.8*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Nicardipine HCl	17.9*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Carbamazepine	1.5*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Tamoxifen	0.6*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Aceclofenac	1.23**	Microemulsion	Rat skin	Lee et al., 2005[16]
Geraniol	2.90	Hydrocortisone	16.9	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Diclofenac	1.58**	Hyrdroxyethyl cellulose gel	Rat skin	Arunkumar et al., 2018[31]
Limonene	3.40	Haloperidol	32.75**	Propylene glycol	Human skin	Lim et al., 2006[18]
		Genistein	1.73**	Ethanol, methocel gel	Human skin	Chadha et al. 20111[2]
		Alprazolam	25.3**	Propylene glycol	Human skin	Boix et al., 2005[19]
		Ketoprofen	4.5*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2000a[2]
		Ketoprofen	3.1*	Pluronic F-127 gel	Mouse skin	El-Kattan et al., 2000a[2]
		Hydrocortisone	28.4*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Hydrocortisone	28.0*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Nicardipine HCl	60.0*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Carbamazepine	6.6*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Tamoxifen	1.6*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Lidocaine	0.42*	Hydrogel	Mouse skin	Song et al., 2009[20]
		Clobetasol propionate	5.43**	Chitosan gel	Pig skin	Senyigit et al., 2009[32]
		Terbinafine	1.36**	Carbopol, ethanol gel	Pig skin	Erdal et al., 2013[33]
		Memantine	12.9**	Ethanol	Pig skin	del Rio-Sancho et al., 2012[25]
		Ketoprofen	2.03**	Nanoemulsion	Rat skin	Sakeena et al., 2010[34]
		Diclofenac	1.92**	Sodium carboxymethylcellulose	Rat skin	Nokhodchi et al., 2007[15]
		Osthole	10.55*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Ferulic acid	53.78*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Trimethylpyrazine	9.61*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Puerarin	18.40*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Aceclofenac	3.0**	Microemulsion	Rat skin	Lee et al., 2005[16]
		Verapamil	3.3*	Propylene glycol	Rat skin	Güngür et al., 2008[28]
		Flurbiprofen	5.36**	Sodium carboxymethylcellulose gel	Rat skin	Fang et al., 2003a[35]
		Indomethacin	4.80**	Sodium carboxymethylcellulose gel	Rat skin	Fang et al., 2003b[36]
Linalool	2.70	Haloperidol	12.0**	Ethanol	Human skin	Vaddi et al., 2002[11]
		Haloperidol	8.3**	Propylene glycol	Human skin	Lim et al., 2006[18]
		Lomerizine	16.6**	Propylene glycol	Mouse skin	Furuishi et al., 2013[23]
Menthol	3.0	Genistein	9.59**	Ethanol, methocel gel	Human skin	Chadha et al., 2011[19]
		Alprazolam	12.0**	Propylene glycol	Human skin	Boix et al., 2005[19]
		Zidovudine	10.77**	Ethanol	Human skin	Narishetty & Panchagnula, 2005[19]
		Zidovudine	48.46**	Ethanol	Rat skin	Narishetty & Panchagnula, 2004[14]
		Lidocaine	1.31**	Hydrogel	Mouse skin	Song et al., 2009[22]
		Lomerizine	28.4**	Propylene glycol	Mouse skin	Furuishi et al., 2013[23]
		Antipyrine	11.49**	Ethanol	Yucatan pig skin	Fujii et al., 2004[37]
		Indomethacin	12.73**	Ethanol	Yucatan pig skin	Fujii et al., 2004[37]
		Quercetin	6.07**	Carbopol gel	Pig skin	Olivella et al., 2007[38]
		Etodolac	1.06**	Carboxymethylcellulose gel	Rat skin	Tas et al., 2007[7]
		Osthole	1.21*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Ferulic acid	35.32*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Trimethylpyrazine	3.92*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Aceclofenac	1.47*	Microemulsion	Rat skin	Lee et al., 2005[16]
		Verapamil	1.79*	Propylene glycol	Rat skin	Güngür et al., 2008[27]
		Imipramine HCl	16.69*	Ethanol	Rat skin	Jain et al., 2002[17]
		Diclofenac	1.5**	Hyrdroxyethyl cellulose gel	Rat skin	Arunkumar et al., 2018[31]
		Propranolol HCl	1.36**	Ethyl cellulose, polyvinyl pyrrolidone film	Rat skin	Amnuaikit et al., 2005[29]

Table 3. Summary of Drug Permeation Enhancement Ratio by Terpenes *(continued)*

Terpene	cLog *P*	Drug	ER	Formulation	Membrane	Reference
Menthone	2.70	Buspirone HCl	4.04**	Ethanol	Human skin	Meidan et al., 2003[18]
		Alprazolam	17.4**	Propylene glycol	Human skin	Boix et al., 2005[19]
		Hydrocortisone	18.7*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Zidovudine	46.09**	Ethanol	Rat skin	Narishetty & Panchagnula, 2004[14]
		Lomerizine	20.6**	Propylene glycol	Mouse skin	Furuishi et al., 2013[23]
		Piroxicam	2.84**	Carbopol gel	Pig skin	Doliwa et al., 2001[25]
		Melatonin	3.5**	Ethanol	Pig skin	Godavarthy et al., 2009[39]
		Diclofenac	2.81**	Sodium carboxymethylcellulose	Rat skin	Nokhodchi et al., 2007[15]
		Osthole	5.82*	Propylene glycol	Rat skin	Lan et al., 2015[40]
		Ferulic acid	20.42*	Propylene glycol	Rat skin	Lan et al., 2015[40]
		Trimethylpyrazine	8.54*	Propylene glycol	Rat skin	Lan et al., 2015[40]
		Verapamil	1.37*	Propylene glycol	Rat skin	Güngür et al., 2008[28]
		Imipramine HCl	9.80*	Ethanol	Rat skin	Jain et al., 2002[17]
p-menthane-3,8-diol	2.20	Antipyrine	2.67**	Ethanol	Yucatan pig skin	Fujii et al., 2004[37]
		Indomethacin	15.8**	Ethanol	Yucatan pig skin	Fujii et al., 2004[37]
Nerolidol	4.60	Ketoprofen	4.4*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2000a[2]
		Ketoprofen	3.1*	Pluronic F-127 gel	Mouse skin	El-Kattan et al., 2000a[2]
		Hydrocortisone	35.3*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Hydrocortisone	32.7*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Nicardipine HCl	134.8*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Carbamazepine	7.5*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Tamoxifen	1.7*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Lomerizine	14.2**	Propylene glycol	Mouse skin	Furuishi et al., 2013[23]
		Clobetasol propionate	16.16**	Chitosan gel	Pig skin	Senyigit et al., 2009[32]
		Terbinafine	4.13**	Carbopol, ethanol gel	Pig skin	Erdal et al., 2013[33]
		Diclofenac	256.80**	Sodium carboxymethylcellulose	Rat skin	Nokhodchi et al., 2007[15]
		Aceclofenac	1.77**	Microemulsion	Rat skin	Lee et al., 2005[16]
		Verapamil	3.42*	Propylene glycol	Rat skin	Güngür et al., 2008[28]
Pinene oxide	2.10	Lomerizine	23.1**	Propylene glycol	Mouse skin	Furuishi et al., 2013[23]
Pulegone	2.80	Zidovudine	45.34**	Ethanol	Rat skin	Narishetty & Panchagnula, 2004[14]
		Osthole	2.87*	Propylene glycol	Rat skin	Lan et al., 2015[40]
		Ferulic acid	3.07*	Propylene glycol	Rat skin	Lan et al., 2015[40]
		Trimethylpyrazine	2.67*	Propylene glycol	Rat skin	Lan et al., 2015[40]
		Imipramine HCl	5.03*	Ethanol	Rat skin	Jain et al., 2002[17]
Terpineol	2.98	Haloperidol	15.80**	Ethanol	Human skin	Vaddi et al., 2002[11]
		Hydrocortisone	13.3*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Zidovudine	44.56**	Ethanol	Rat skin	Narishetty & Panchagnula, 2004[14]
		Imipramine HCl	11.58**	Ethanol	Rat skin	Jain et al., 2002[17]
Terpinene-4-ol	2.20	Hydrocortisone	11.3*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Osthole	1.90*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Ferulic acid	2.02*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Trimethylpyrazine	1.64*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Puerarin	0.40*	Propylene glycol	Rat skin	Lan et al., 2014[26]
		Buspirone HCl	0.80*	Ethanol	Human skin	Meidan et al., 2003[16]
Thymol	3.30	Ketoprofen	3.1*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2000a[2]
		Ketoprofen	3.1*	Pluronic F-127 gel	Mouse skin	El-Kattan et al., 2000a[2]
		Hydrocortisone	11.0*	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]
		Hydrocortisone	10.5*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Nicardipine HCl	18.2*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Carbamazepine	4.2*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Tamoxifen	1.4*	Hydroxypropyl cellulose gel	Mouse skin	El-Kattan et al., 2001[30]
		Piroxicam	3.05**	Carbopol gel	Pig skin	Doliwa et al., 2001[25]
		Diclofenac	1.44**	Hyrdroxyethyl cellulose gel	Rat skin	Arunkumar et al., 2018[31]
Verbenone	1.60	Hydrocortisone	11.5**	Hydroxypropylmethyl cellulose gel	Mouse skin	El-Kattan et al., 2000b[13]

*ER (enhancement ratio) = $J_{PE}/J_{control}$; *retrieved ER; ** computed ER*

plotted as shown in Figures 5, 6, 7 and 8. The linear correlation analysis shows a moderate to strong positive correlation in CPEs tested in pig skin (r = 0.21), mouse skin (r = 0.27), rat skin (r = 0.41), and human skin (r = 0.67) between the two variables.

DISCUSSION

The effectiveness of transdermal drug preparations relies on the ability of its active components to permeate through the skin.[41] The primary goal of these formulations is to achieve maximum flux across the layers of the skin. While the SC

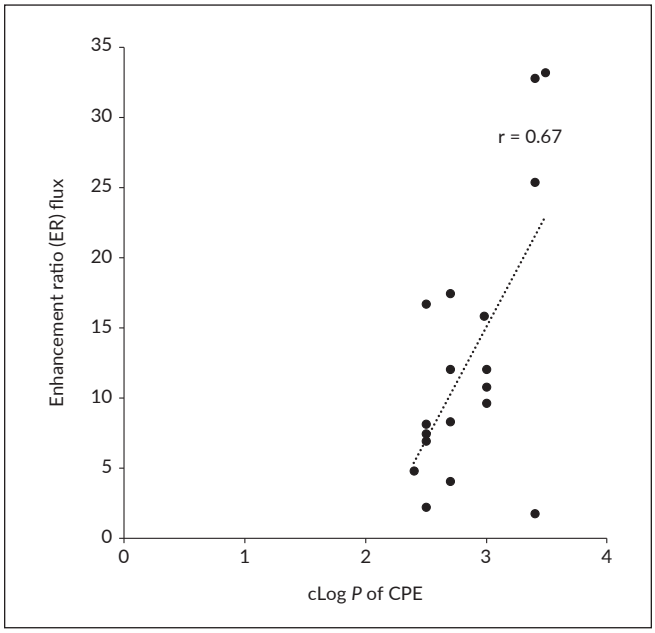

Figure 5. The correlation between the cLog *P* of the terpene enhancers and the enhancement ratio in human skin.

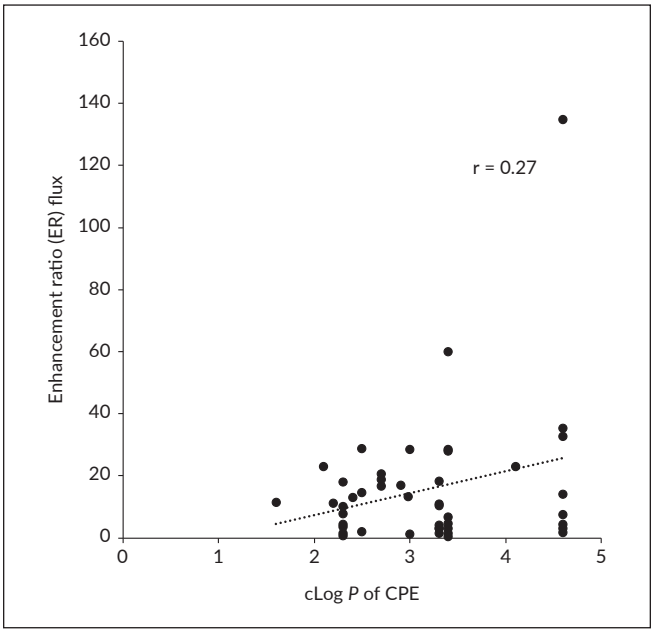

Figure 6. The correlation between the cLog *P* of the terpene enhancers and the enhancement ratio in mouse skin.

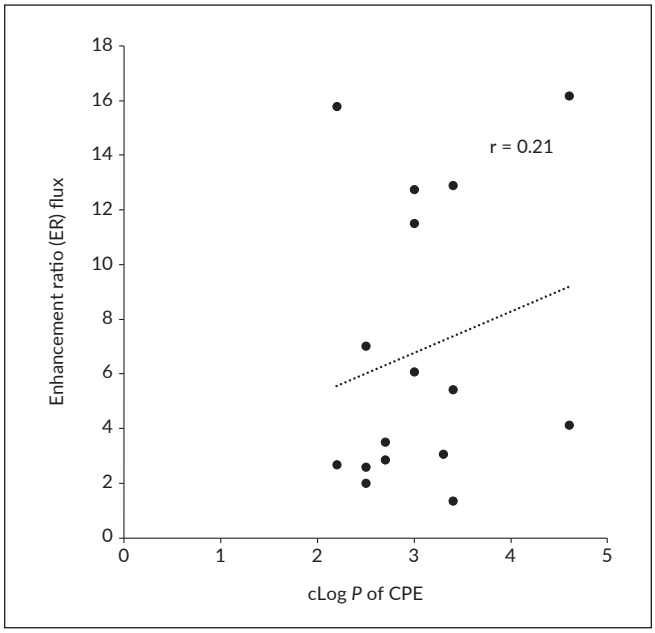

Figure 7. The correlation between the cLog *P* of the terpene enhancers and the enhancement ratio in pig skin.

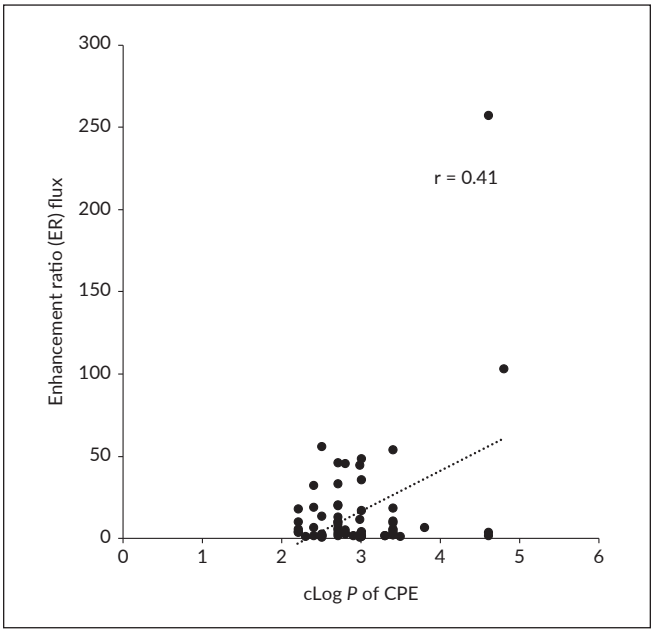

Figure 8. The correlation between the cLog *P* of the terpene enhancers and enhancement ratio in rat skin.

forms a primarily rate-limiting barrier against the permeation of drugs, lipophilic small-molecule drugs are regarded to have a more favorable fate in terms of skin permeation. The same was found in this study where MW was found to have a positive relationship with ER. An interesting finding in our analysis, however, demonstrated that cLog *P* of lipophilic drugs was inversely related to ER (Figure 4). The cLog *P* of terpenes, however, was moderately and strongly correlated with the enhancement of drug permeation in human and rat skin, and mouse and pig skin, respectively (Figure 5, 6, 7 and 8). Terpene-based permeation enhancers increased drug transport across the skin by 1.06 - 256.80-fold (Table 3).

FDC permeability experiments are widely used method to evaluate *in vitro* drug permeation and derive permeation parameters (e.g., flux, diffusivity).[42] The enhancement ratio (ER) as presented in Table 3 were derived from the fluxes of the drug formulation containing the permeation enhancer and the control formulation. ERs are practical expressions to denote the increase in drug permeation through the skin from dermatological formulations. Thus, in comparison to drug flux or skin concentration alone, ERs are more appropriate in quantifying the permeation enhancing the ability of terpenes-PE.

The skin's outermost layer, SC, is primarily composed of dead cells filled with keratin, which are embedded in a lipid matrix. The lipids present in the intercellular matrix are called ceramides. They are packed tightly in the lipid bilayer, thus inciting the protective barrier of the skin. This barrier effect is attributed to the hydrogen bonds that hold together different lipids, providing stability and strength to the SC.[8] It is the lipophilicity of a drug that determines its movement across the SC. A relationship exists between skin permeation and the lipophilicity (i.e., cLog *P*) of the drug in which an optimal log P of 2 indicates efficient permeation across the SC. Drugs with extremely low (highly hydrophilic) or high (highly lipophilic) cLog *P* values tend to have decreased permeability due to insolubility with the lipid components of the SC or accumulation of the drug in the SC/non-passage due to low aqueous solubility in the viable epidermis layer, respectively. The desirable log *P* value for a drug to penetrate the SC is within the range of 1 and 3. Drugs for dermal and transdermal preparations should be lipophilic enough to ensure partitioning into the lipid matrix of the SC and at the same time have sufficient solubility in their solvent to be in solution to facilitate absorption.[43] Another important factor in drug permeation across the skin is the molecular weight of the drug. Drugs with MW greater than 500 Daltons do not permeate easily as the arrangement of lipids in the SC are tightly packed and thus large molecules cannot easily be partitioned into.[43] These circumstances compel the addition of PE in formulations containing lipophilic small molecular weight drugs.[3] Interestingly, a linear correlation analysis of ERs against the reported lipophilicity and MW of the drugs revealed weak negative and positive relationships, respectively. This posits that physicochemical characteristics of the drug, even in the case of lipophilic ones, and the chemical permeation enhancer play a predictive role in the permeation of the drug.

Terpenes as CPE

Terpenes are a large class of chemical permeation enhancers employed among dermatologicals that are abundant in nature. The commonly proposed mechanism of action of its permeation enhancement effect is through the disruption of the SC. This molecular mechanism is based upon a three-step process. These steps include (1) the partial disordering that terpenes cause in the lipid alkyl chains of the SC; (2) the disruption of the hydrogen bonds by preferential binding of terpenes with the ceramide head groups of the SC; and (3) the excessive hydration of ceramides brought about by the previous steps thus establishing new polar pathways.[44-47] The mechanism behind the permeation enhancement capacity of the terpenes is thought to be dependent on their structures or lipophilicity. Hydrocarbon and nonpolar terpenes are postulated to mainly act on step one of the processes, while oxygen-containing and relatively polar terpenes influence the second and third steps of the process. The effects of terpenes were observed to be reversible as studies demonstrated that the enhancing effect is rather brief and that the SC recovers from the disruption.

This paper provides insights into the effect of terpenes on the permeation of lipophilic drugs across a variety of skin models. There are a total of 25 terpenes evaluated in this review. Among these compounds, the most commonly employed are cineol (n = 20; ER_{Ave} = 8.89), menthol (n = 18; ER_{Ave} = 11.40), menthone (n = 13; ER_{Ave} = 12.46), and nerolidol (n = 13; ER_{Ave} = 39.69). All of these terpenes have exhibited permeation enhancing ability, as evidenced by their respective enhancement ratios (>1).

Penetration enhancers, in general, may act in one or more of three main mechanisms: (1) disruption of the highly ordered lipid structures in the SC, (2) interaction with intracellular protein, and (3) increased drug partitioning. Among the studies included in this review, the most commonly proposed mechanism of the permeation-enhancing ability of terpenes is through the disruption of the highly ordered organization of lipids in the SC, as well as increasing the partition of the drug molecule. In a molecular dynamics simulation, geraniol has been shown to fully partition from the formulation into the SC and disperses into the interiors of the lipid layer. They then forge interactions with the head groups of ceramides and cholesterol avoiding cluster formations on top and within the SC, thus aiding its diffusion across the skin.[44]

Factors affecting ER of Terpene CPE

Lipophilicity

Terpene chemical enhancers, based on previous reports, have established that their optimum lipophilicity varies dependently on the lipophilicity of the drug employed.[25]

In one study, bisabolol provided the most permeation-enhancing effect when employed in a formulation containing propranolol HCl. It must be noted that bisabolol is relatively a lipophilic terpene and propranolol is also lipophilic (drug).[8] Terpenes with relatively high lipophilic index values were shown to provide significant enhancement effects. For hydrophilic drugs, terpenes are thought to increase their diffusion coefficient, thus increasing their permeation into the skin. A similar study reported lipophilicity of the terpene-CPE and the permeating drug as the main factors causing the permeation enhancement effect.[45] This is consistent with the results of the linear correlation analyses conducted between the cLog *P* of the terpene-enhancers and the ERs in various membranes (Figures 5, 6, 7 and 8). However, as the lipophilicity of the terpene increases, this results in lower enhancement activity. The decrease in the thermodynamic activity of the permeating drug with terpene was linked as the cause of lower permeation at very high lipophilicity.

Among all the studies evaluated in this review, nerolidol had the highest permeation enhancement ability (ER = 256) among all terpenes. Nerolidol, an amphiphilic terpene, is capable of disrupting the tight packing of the SC, and its hydrophilic tail facilitates the ease of passage of the solubilized drug across the hydrophilic viable epidermis and dermis layer. This characteristic afforded nerolidol the ability to fluidize the intercellular lipid bilayers. Extraction of SC lipids by nerolidol and other highly lipophilic terpenes (high log P) also takes place. Terpenes such as nerolidol can act as 'spacers' by interacting with membrane lipid chains and promoting lipid extraction.[46] Furthermore, nerolidol was found to alter the orthorhombic and hexagonal lipid structures of the SC. Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR) measurements revealed disordering of lateral packing by nerolidol through disruption of the lattices of lipid structures leading to an increase in fluidity. Nerolidol alone increases lipid dynamics causing membrane fluidity. The 2.8-fold higher ER observed in nerolidol over limonene and other terpenes is consistent with the lipid probe studies where the interaction of nerolidol with SC intercellular membranes resulted in greater membrane fluidity.[47] Similarly, menthol disrupts the same orthorhombic and hexagonal lipid structures by crystallization. The application of menthol as CPE promotes the fluidity of the SC's intercellular lipids. In these cases, both nerolidol and menthol appear to cause disorders in the lipid structures.[47]

Structure of Terpene-CPEs

Several studies have established a relationship between the functional groups present in the terpene's structure and its permeation enhancement effect. Lipophilicity or cLog *P* of chemicals determined by their molecular structures and straightforward assumptions based on these functional groups in terpenes such as hydrocarbon class have been described to enhance the permeation of lipophilic drugs. In contrast, polar group-containing terpenes enhance the permeation of hydrophilic drugs.[30] The magnitude of a terpene's effect on the permeation of a drug is influenced by the composition of the terpene. Previous studies have observed that terpenes primarily composed of hydrocarbon or nonpolar groups produced a better enhancing effect than significantly polar terpenes when employed for lipophilic drugs. Majority of the terpene PEs identified in this review, except borneol, camphor, menthol, and menthone, share these characteristics making them suitable for enhancing the cutaneous absorption of lipophilic small-molecule drugs as supported by data where ERs are reported to be greater than 1 (Table 3). The complex formation between the penetrant and terpene enhancers such as nerolidol (ER = 256.80) and farnesol (ER = 102.9) which exhibited a high permeation enhancing effect is proposed as the reason behind the higher enhancement activity.[15]

On the contrary, polar group-containing terpenes are better at enhancing the permeation of hydrophilic drugs.[30] A study conducted by El-Kattan et al. investigated the permeation enhancement effects of limonene, thymol, fenchone, and nerolidol on the permeability of ketoprofen used as the model compound.[30] Limonene, a hydrocarbon and nonpolar terpene, was found to be the most effective enhancer for ketoprofen, a lipophilic drug. It was further concluded that the proposed mechanism of action by which limonene increases the permeation of ketoprofen was due to its ability to disrupt the lipids in the SC.[34]

The degree of saturation present in the chemical structure of terpenes also plays a role in their ability to enhance drug permeation. Terpenes with a minimum degree of unsaturation were observed to exhibit good permeation enhancement effects in polar and hydrophilic drugs. The proposed mechanism of action is the disruption of the hydrogen bonds present in the ceramides of the SC.[28] Terpenes preferentially form hydrogen bonds with the head portions of the ceramides in the lipid bilayer; thus, destroying the existing bonds between these lipid molecules, and ultimately disrupting the skin barrier property.

Terpenes with ketone functional groups were shown to be more effective compared to terpenes with either alcohols or ethers as functional groups.[45] This effectiveness was attributed to the slight variations in the interactions of the lipid bilayer at the molecular level, generating a greater extent of skin permeability.

Many factors affect the ER and there is not a single and straightforward reason that exists to explain this phenomenon. Readily available physicochemical parameters are often used in predicting the ability of CPEs to enhance skin permeation of molecules across skin, with studies often suggesting the use of these parameters as important factors in identifying ideal CPEs when designing formulations.[48] These factors include the diversity of the formulations used in the articles reviewed, physicochemical properties of the vehicles utilized, physical properties of the drugs (e.g., molecular weight and Log *P* values), physical properties of the terpenes (i.e., solubility in the formulation and/or

vehicle), chemical structure or class (e.g., monoterpenes, sesquiterpenes, etc.) and type (monocyclic, bicyclic, tricyclic, etc.) of terpenes, and the membranes used in the skin permeation experiments. Studies covered in this review point out the fact that molecular weight and lipophilicities are poor predictors of drug permeability in the skin. It has become apparent in this study that ERs are more practical parameters to employ in selecting CPE in the interest of dermatological formulation development.

The present review has its strengths and weaknesses. The articles gathered in this review were similar in terms of the method by which the permeation experiment was conducted, allowing direct comparison of fluxes. The selection of drugs included in the present has clinical advantages and is of great importance in the development of terpene-CPE-based topical and transdermal drug preparations. Published reviews on terpenes neglect the impact of permeation experimental conditions (i.e., dose conditions) which are central in interpreting permeation parameters (e.g., flux). Article-derived data in this study were conducted under infinite dose conditions (i.e., non-depleting conditions) which alone would provide a favorable environment for passive diffusion for any permeating drug. The current review presents the values of the factors which dictate the ER of a drug, such as the cLog *P* of terpene-CPE and the molecular weight of the lipophilic drugs.

There are confounding factors that were encountered in the making of this review. The articles included in this review employed varied formulations, vehicles, and concentrations of the drug and the terpenes and thus, affect the comparability of data. The membranes (i.e., rat skin, pig skin, mouse skin, human skin) and receptor media used in the FDC also vary in each article included.

The synthesis derived from this study is pivotal in the development of dermal and transdermal preparations of lipophilic drugs possessing narrow therapeutic indices and those with shorter biological half-lives. It allows for the determination of the appropriate terpene-CPE to be employed as a permeation enhancer relative to the MW, lipophilicity, and pharmaceutical excipients used in the formulation. This is useful in the development of dermatological formulations that aim to improve topical and transdermal delivery of drugs with the use of terpene-CPEs.

CONCLUSION

Terpenes as PE significantly increase the percutaneous permeation of lipophilic small-molecule drugs through intact skin by 1.06 – 256.80-fold when co-administered. Higher ER was recorded for nerolidol, carvacrol, borneol, terpineol, limonene, menthone, linalool, pulegone, and menthol in decreasing order among the surveyed terpene-CPEs. Terpenes with cLog *P* higher than 2.40 had ERs greater than 10. The lipophilicity of the terpenes had a moderate to strong positive correlation with ER. Chemical properties (i.e., non-polar groups) and the ability to interact with the hydrogen bonds of the lipid structures in the SC are factors that enhance skin permeation of drugs by terpenes. Non-polar groups in terpenes and hydrogen bond interactions by terpenes with SC lipid promote penetration of lipophilic drugs. cLog *P* of terpene-CPEs is strongly correlated with ER of lipophilic drugs across human skin.

As a means to further ease the comparability of data, it is recommended that investigations on the effects of formulations and terpene concentrations be conducted using harmonized experimental conditions (e.g., the membrane of choice, finite dose conditions) to determine the efficacy of a class of terpenes in dermatological formulations.

Statement of Authorship

All authors contributed to the conceptualization of the study, data gathering, analysis, interpretation, writing, and preparation of the manuscript.

Author Disclosure

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