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Ex vivo penetration analysis and anti-inflammatory efficacy of the association of ferulic acid and UV filters

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ABSTRACT

Background: Unprotected chronic exposure to ultraviolet (UV) radiation generates many harmful effects to human skin and sunscreens are essential to health, however, traditional products do not provide enough protection against cutaneous oxidative stress, a process amplified by UV radiation. Therefore, the development of multifunctional photoprotective formulations seems to be a more efficacious approach, since these enable the absorption/reflection of UV radiation and maintain the cutaneous homeostasis.
Objectives: In the present study, ferulic acid (FA), a well-known antioxidant, has been combined with two UV filters, bemotrizinol and ethylhexyl triazone, and the safety and efficacy of this formulation has been assessed combining ex vivo and in vivo methods.
Methods: Skin permeation assays were performed by applying the formulation in the volar forearm of participants, after which consecutive samples of the stratum corneum were collected by tape stripping, and the quantification of FA, bemotrizinol and ethylhexyl triazone was performed by high-performance liquid chromatography (HPLC). Also, the FA anti-inflammatory action in combination with the UV filters was probed through a method employing Laser Doppler flowmetry to measure the vasodilatory response to methyl nicotinate topical application.
Results: Skin permeation assay was able to characterize the penetration depth of each substance. It should also be noted that a specific HPLC analytical method was developed in this study to enable the rapid simultaneous quantification of the three substances. Results from Laser Doppler flowmetry showed that the FA was able to mitigate the vasodilatory response.
Conclusions: FA proved to be a valuable resource in a multifunction sunscreen, not only providing an increase in the SPF of sunscreens, previously published, but also decreasing the extent of inflammation.

1. Introduction

Overexposure to sunlight causes adverse effects on our skin, being the development of cancer its most harmful effect, melanoma being one of the most prevalent types of cancers diagnosed worldwide IARC 2012, American Cancer, 2016). Ultraviolet (UV) radiation covers the wavelength range of 100 to 400 nm and is divided into UVC (100 to 290 nm), UVB (290 to 320 nm), UVA II (320 to 340 nm) and UVA I (340 to 400 nm) (Diffey et al., 2000). Since UVC rays are absorbed by the ozone layer in the atmosphere, they do not reach the Earth's surface, while ~ 5% of UVB rays and ~ 95% of UVA rays surpass this layer, inevitably reaching human skin at different depths and types of reactions (Lautenschlager et al., 2007; Young, 2009; de Oliveira et al., 2015). UVB radiation promotes direct photochemical damage to skin DNA, causing genetic mutations, while UVA radiation has indirect effects on DNA, mainly through the generation of reactive oxygen species (Lautenschlager et al., 2007). Both types of UV radiation can disrupt collagen and elastin fibers, accelerate skin aging, and increase the risk of skin cancer (Mead, 2008). Despite this, the human body has endogenous mechanisms of protection against UV, such as skin chromophores, such as melanin, tryptophan, tyrosine, nitrogen purine and pyrimidine bases (Jansen et al., 2013). In addition, other defense mechanisms like inhibition of free radicals by endogenous antioxidants, horny layer thickening, sweat, sebum and hair, also contribute to the UV-damage prevention (Jansen et al., 2013; Cestari et al., 2012). However, the endogenous protection is not sufficient to maintain

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cutaneous homeostasis at stressful conditions, and additional strategies are advised, such as protective clothes, promotion of educational habits regarding outdoor exposure, and use of photoprotective formulations (Cestari et al., 2012). Sunscreens contain substances that act on the skin surface, involving mainly two mechanisms: absorption (organic or chemical filters) or/ and reflection (inorganic or physical filters) of the UV radiation (Cestari et al., 2012).

Oxidative stress is amplified by UV radiation, being a biological condition responsible for several damages to the body caused by free radicals (Carocho and Ferreira, 2013). Free radicals that can be harmful to our organism are mainly classified as reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Phaniendra et al., 2015). However, ROS/RNS can also provide beneficial effects; at moderate/low levels they can be involved in immune function, mitogenic response, redox regulation and cellular signaling pathways (Valko et al., 2007; Nordberg and Arnér, 2001). Nevertheless, in excessive levels, they can lead to the disruption of the integrity of many biomolecules, like DNA, proteins and lipids (Craft et al., 2012; Lü et al., 2010). The examples of ROS can include superoxide ($O_2^{\bullet}$), oxygen radical ($O_2^{\bullet\bullet}$), hydroxyl ($OH^{\bullet}$) and peroxyl radical ($ROO^{\bullet}$); and, for NOS, nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$) (Halliwell, 2015).

Although traditional UV filters are the main form of protection against UV radiation in sunscreens, they are not completely adequate as a single source of prevention against oxidative stress (Zhang, 2010). Experiments conducted by Haywood et al. (2003), using electron spin spectroscopy to detect free radicals in human skin, have shown that sunscreens reduced free radical formation by only 55% after exposure to artificial UV radiation (Haywood et al., 2003). Consequently, there is a need for innovation and better elaboration of photoprotective formulations in order to contemplate a greater spectrum of protection, including aggregated benefits.

Ferulic acid (FA) (4-hydroxy-3-methoxy cinnamic acid), a natural phenolic compound, belongs to the class of hydroxycinnamic acids, being found mainly in rice, citrus fruits, wheat, corn, roasted coffee and several other vegetables (Gerin et al., 2016; Graf, 1992; Srinivasan et al., 2005). It is known that phenolic compounds have benefits to human health, mainly because of their antioxidant potential (Gerin et al., 2016). In addition to its antioxidant properties, the specialized literature has demonstrated that FA also has anti-inflammatory, hepatoprotective, anticarcinogenic, antimutagenic and neuroprotective potentials (Graf, 1992; Srinivasan et al., 2005; Murakami et al., 2002; Jin et al., 2005; Baskaran et al., 2010). The FA antioxidant activity occurs by the suppression of hydroxyl and superoxide radicals, nitric oxide and peroxynitrite (Graf, 1992). FA has cis-trans isomerism, being the trans form the most commonly found in nature. The cis form is presented as a yellowish oil with maximum UV absorption at 316 nm, whereas the trans form is presented as a crystal with two maximum absorption peaks in water, at 284 and 307 nm (Graf, 1992; Almeida et al., 2018). Topical administration is an efficacious strategy to increase the concentration of antioxidants in the skin and prevent endogenous molecules degradation, which increases their half-life and, at the same time, protects cells from negative effects caused by UV radiation (Chen et al., 2012). Therefore, FA is an excellent candidate for multifunction photoprotective formulations (Zduńska et al., 2018). It should also be highlighted that this antioxidant has good biocompatibility. Additionally, studies conducted by Peres et al., 2018, evaluated its photoprotection potential, and an increase was observed in the sun protection factor (SPF) by 37% and in the UVA protection factor (FP-UVA) by 26% when FA was combined with an UV filter system (Peres et al., 2018).

In this research, we further assessed the efficacy of a multifunctional sunscreen formulation containing ferulic acid (trans) and two UV filters (ethylhexyl triazone, bemotrizinol) using ex vivo and in vivo assays. The evaluation of the skin penetration of these molecules was determined by tape stripping using a developed high-performance liquid chromatography (HPLC) method. The antioxidant/anti-inflammatory activity of the photoprotective formulations was probed in vivo by a method that measures the response to the topical application of a vasodilator by Laser Doppler flowmetry, thus, the efficacy assessment is based on the ability of the samples to decrease the extent of an erythema-induced response.

2. Experimental

2.1. Chemicals, reagents and instrumentation

Trans-ferulic acid was purchased from Sino Lion (USA), and ethylhexyl triazone and bis-ethylhexyloxyphenol methoxyphenyl triazine (bemotrizinol) were purchased from BASF (Brazil). C_{12}-C_{15} alkyl benzoate and butilenoglicol cocoate were obtained from Brasquim (Brazil). Disodium EDTA, isopropyl miristate, phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben and cyclomethicone were bought from PharmaSpecial (Brazil). Cyclomethicone (and) dimethicone crosspolymer, glycerin and acrylates / C_{10-30} alkyl acrylate crosspolymer were purchased from Fagron (Brazil). Hydroxyethyl acrylate / sodium acryloyldimethyl taurate copolymer (and) isohexadecane (and) polysorbate 60 and triethanolamine were from, respectively, Seppic (Brazil) and Volp (Brazil). Sodium acetate buffer and methyl nicotinate were purchased from Sigma-Aldrich (St. Louis, USA). 1,4-Dioxane was purchased from Merck (Darmstadt, Germany). All reagents were of HPLC or analytical grade and were used as received, without any further purification. Purified water was used for all experiments, using Merck Millipore® Mili-Q® Simplicity UV.

Adhesive tapes were Tape Scotch® 3M Transparent 750 polypropylene; syringe filter was 0.22 μm, Millipore®, and the occlusive patch was Hill Top® Chamber occlusive patch (Hill Top® Research, Cincinnati, Ohio).

HPLC was Shimadzu® Prominence (Kyoto, Japan) composed of SPD-M20A diode array spectrophotometric detector and CTO-20A column oven. The column C18 (250 × 4.6 mm, 5 mm particle size, Shimadzu, Japan) was preceded by a pre-column (10 × 4.6 mm). Laser Doppler flowmetry equipment was PeriFlux® System 5000 (Perimed®, Sweden). Mechanical stirrer (IKA® RW 20n), analytical balance (Shimadzu® AUY 220), semi-analytical balances (Gehaka® BG 4000 and Ohaus® ARD 110), thermostatic bath (New Ethics® N480), ultrasonic bath (Unique® UltraCleaner 1600A), centrifuge (Hitachi® RX2) and single-channel micropipettes (Eppendorf® 100-1000 μL and 1.0-10.0 mL) were used.

2.2. Formulations

Emulsified oil-in-water (O/W) systems containing ethylhexyl triazone and bemotrizinol with or without ferulic acid were prepared according to Peres et al., 2018. The qualitative and quantitative composition (% w/w) of the samples is described in Table 1.

2.3. Ethical issues

The participants in this research were provided with the necessary information and clarification regarding the trial through oral and written informed consent. The study was developed in accordance with the principles in the Helsinki Declaration. The project was approved by the Ethics Committee by the Faculty of Pharmaceutical Sciences of USP, number CAAE: 31583814.0.0000.0067, protocol number 735.493. Participants (18-70 years old) with healthy skin participated in the study, with skin phototype II-V according to the Fitzpatrick classification. Participants were instructed not to apply cosmetic products for 24 hours before the study in the area to be tested.

Table 1
Qualitative and quantitative composition (% w/w) of photoprotective formulations with or without ferulic acid (F1 and F2) according to Peres et al. (2018).

	Ingredients (function)	Concentration (% w/w) F1	F2
Oil phase	Ethylhexyl triazone (UVB filter)	5.00	5.00
	Bis-ethylhexyloxyphenol methoxyphenyl triazine (broad spectrum filter)	10.00	10.00
	C12-C15 alkyl benzoate (emollient)	9.00	9.00
	Butylene glycol cocoate (emollient and stabilizer)	6.75	6.75
	Isopropyl myristate (emollient)	6.75	6.75
	Cyclomethicone (emollient and film-former)	1.75	1.75
	Cyclomethicone (and) dimethicone Crosspolymer (thickening agent and film-former)	1.25	1.25
	Hydroxyethyl acrylate (and) sodium acryloyldimethyl taurate copolymer (and) isohexadecane (and) polysorbate 60 (self-emulsifying agent)	4.00	4.00
Water phase	Glycerin (humectant)	5.00	5.00
	Phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) propylparaben (and) isobutylparaben (preservative)	0.75	0.75
	Disodium EDTA (chelating agent)	0.30	0.30
	Acrylates (and) C10–30 alkyl acrylate crosspolymer (film-former, thickening agent and co-emulsifier)	0.10	0.10
	Ferulic acid (antioxidant bioactive compound)	-	1.00
	Purified water	*	*
	Triethanolamine (pH value corrector)	**	**

3. *Ex vivo* test

3.1. Chromatographic conditions and analytical validation method

A single method was used to quantify the three bioactive compounds by HPLC, modified from Modi and Vukkum (2014). The mobile phase was composed in isocratic mode of sodium acetate buffer pH 4.2: 1,4-dioxane (20%: 80%) filtered through syringe filter, with a flow rate of 1.5 mL.min^{-1} for 25 minutes at 50°C, with 10 µL of sample injection amount. The diode detector was set at 311 nm for sample detection.

3.2. Ex vivo penetration assay

The study was conducted in the middle volar forearm of the 12 participants. The skin was cleansed with purified water, after which formulation F2 containing FA was applied (2.0 mg.cm^{-2}) in a 2.25 cm^2 site. After 2 hours, 20 consecutive samples of stratum corneum were collected by the tape stripping technique (de Oliveira et al., 2015; Benfeldt et al., 2007; Alonso et al., 2009; Peres, 2015).

The adhesive tapes collected were analyzed as an aggregate of three regions: Region 1: composed by the first tape extracted, which represents the uppermost layer of the stratum corneum; Region 2: composed by tapes 2-10, representing intermediate layers of stratum corneum; and Region 3: composed by tapes 11 - 20, which represents the innermost layers of the stratum corneum. The tapes were transferred to a glass beaker and the stratum corneum was extracted by the addition of 10 mL sodium acetate buffer pH 4.2:1,4-dioxane (20:80%), in an ultrasonic bath for 15 minutes (Alonso et al., 2009; Peres, 2015). After mix in a vortex and filtration through a syringe filter, the samples were quantified by HPLC.

3.3. In vivo anti-inflammatory/ antioxidant assay

This study was conducted in 13 participants. Three areas of 2.25 cm^2 each were marked in the volar forearm of each of the participants. The determination of which forearm was selected (left or right) and the order of the sites were randomized (Gallin et al., 2017).One site was used as a negative control (purified water) and the other two sites were treated with the photoprotective formulation with (F2) or without FA (F1), using an occlusive patch. After 2 hours, the patches were removed and any formulation remaining on the skin was wiped with soft paper. Thirty minutes after patch removal, an erythema was induced in each site by the application for 60 s of a filter paper saturated with an aqueous solution of methyl nicotinate (0.5% w/v). Measurements of the cutaneous microcirculation in the individual test sites were then recorded continuously for fifteen minutes with a Laser Doppler flowmeter (de Oliveira et al., 2015). The mean perfusion unit values, maximum perfusion unit value, area under the curve, angular coefficient and onset time were obtained using the Laser Doppler flowmetry equipment and analyzed through PeriSoft® software, version 2.5.5.

3.4. Statistical treatment

Results were evaluated using t-test followed by Wilcoxon's test to make comparisons among the different parameters with the help of GraphPad Prism® software version 5.0 (GraphPad® Software, Inc.).

4. Results and discussion

4.1. Formulations

Sunscreens (products used mainly to prevent cutaneous erythema formation from outdoor exposure) are complex formulations containing several ingredients (adjuvants and actives) that must guarantee, besides safety and efficacy, physicochemical, functional and microbiological stability, some level of water resistance, low environmental risk and emolliency. Other cosmetic attributes can be achieved, such as moisturizing, antioxidant action and anti-aging prevention, enabling multi-aggregate benefits for the consumer. When these properties are combined in a single product it can be characterized as a multifunctional sunscreen (Balogh et al., 2011; Velasco et al., 2008;de Oliveira et al., 2015). The relevance of the most adequate selection of adjuvants is as important as the choice of the actives, among them, the UV filters.

Our samples were emulsified O/W systems, a less unctuous vehicle in comparison with other oil-based solutions or gels, W/O emulsions or wax-based matrixes. The sensorial properties of O/W emulsions are not only cosmetically acceptable, but also pleasurable, increasing the compliance for multiple re-applications, that are indispensable to keep sunscreen efficacy during outdoor exposure (Sarruf et al., 2020; Sarruf et al., 2020; Yap et al., 2017). The sunscreen vehicle was composed of emollients, film-formers, consistency/thickening agents, humectant, preservative, chelating agent, co-emulsifier, purified water and pH value correctors. Those substances were combined to obtain a stable topical product, with enough consistency to be adequately spread on the skin and to form a homogeneous film with water resistance, providing good sensorial properties and able to deliver the actives that protect against the harmful effects of UV radiation to the external layers of the epidermis.

4.2. Ex vivo penetration test

The surface of the human body is covered entirely by our biggest

organ, the skin, providing protection against the external environment, and being directly exposed to UV solar radiation (Di Domenico et al., 2009). Understanding the skin penetration of topically applied substances is crucial to optimize the safety and efficacy of cosmetics, especially sunscreens (Weigmann et al., 2005; Roussel et al., 2015). It is recommended that UV filters remain on the epidermal surface, preferably in the stratum corneum, thus avoiding a possible systemic absorption. However, studies on the penetration and permeation of sunscreens in/across the skin suggest that some ingredients can overcome the cutaneous barrier, reaching the systemic circulation (Hayden et al., 1997).

An ideal UV filter should be efficacious without penetrating the viable epidermis, dermis or appendages (Tampucci et al., 2018), since its mechanism of action is based in creating a “shield” that prevents the UV radiation from reaching the deeper layers of the skin. In contrast, a suitable topical antioxidant must penetrate the stratum corneum in order to prevent the deleterious action of the ROS/ RNS generated by UV radiation in the viable epidermis and dermis (Abla and Banga, 2013).

Several HPLC studies have been developed for the simultaneous analysis of UV filters, but these are usually applied for the quantification of ingredients in formulations and not in biological samples, like those obtained in this investigation (Chang et al., 2015; Wharton et al., 2011; Durand et al., 2009; Davies et al., 2017). Percutaneous studies can be conducted in vivo, in vitro and ex vivo (Monti et al., 2011). However, difficulties in obtaining human skin samples, as well as the increasing ban on animal testing of cosmetics around the world have steered researchers to develop new models for the evaluation of skin penetration/permeation, such as the tape stripping technique (Cândido et al., 2018).

The tape stripping technique can be used to investigate the distribution of UV filters in the skin layers by using analytical methods, such as HPLC, UV absorption spectroscopy, gas chromatography and mass spectrometry (Klinubol et al., 2008). This approach allows the noninvasive sampling of human skin (stratum corneum), increasing the precision and relevance of the experiment without causing discomfort to the participants. The technique is considered ex vivo, as it is based in the topical interaction of the sample with the volunteers´ skin, with subsequent removal of the stratum corneum by adhesive tapes, and the collected samples are also applicable in multiple types of tests (e.g. irradiation or chemical reactions) (Alonso et al., 2009).

The physicochemical properties are the main conditionings of the transcutaneous penetration and permeation behavior of UV filters (Tampucci et al., 2018), in particular their molecular weight and partition coefficient (log P) (Tampucci et al., 2018; Souza and Maia Campos, 2017).

Molecules that exhibit moderate hydrophilic and lipophilic properties are normally better candidates to permeate the skin (Tampucci et al., 2018). It is known that log P values between 1 and 3 may allow passive diffusion of the molecule into the skin; lower partition coefficients mean that the molecule is very soluble in water, thus, penetration across the stratum corneum will be more difficult. However, if the log P is too high, the molecule may be able to accumulate and form reservoirs within the stratum corneum lipid phases, and partition to the viable epidermis would be less favored (Tampucci et al., 2018; Durand et al., 2009). The log P values of the UV filters used in the present study, bemotrizinol and ethylhexyl triazone, are very high, therefore, these molecules tend to accumulate in the stratum corneum (Table. 2).

Another strategy to reduce the cutaneous absorption of sunscreen components is the use of molecules of high molecular weight, which is also the case of bemotrizinol or ethylhexyl triazone (Table. 3). It is well established that most chemical compounds with molecular weight above 500 Da do not penetrate the skin in a high extent (Essendoubi et al., 2016). Thus, since both selected filters have molecular weights greater than 500 Da and are highly lipophilic, their penetration into/across the stratum corneum was expected to be irrelevant. Souza; Maia Campos, 2017, analyzed the penetration potential of bemotrizinol and ethylhexyl triazone, using porcine ear skin in a vertical diffusion cell during 24 hours, with analysis of the receptor fluid by HPLC. They used 16 adhesive tapes (tape stripping technique) in order to quantify the retained compounds in the epidermis. None of the UV filters was detected in the receptor fluid, thus remaining retained in the skin. More than 90% of these UV filters were detected in the stratum corneum (Souza and Maia Campos, 2017). Potard et al., 2000, applied a formulation with ethylhexyl triazone on excised human skin mounted in a diffusion cell, and removed the respective stratum corneum by tape stripping after 30 minutes and 16 hours of exposure, being the active quantified by HPLC. Ninety six percent of ethylhexyl triazone was found to be retained in the stratum corneum and the remainder penetrated into the viable epidermis without permeation to the dermis (diffusion cell receptor fluid) (Potard et al., 2000). Durand et al., 2009, also performed penetration experiments of bemotrizinol (aerosol formulation) in excised human skin through vertical diffusion cells, and were unable to find the UV filter in the receptor fluid after 24 hours (Durand et al., 2009). Thus, the results in the present work are in agreement with these in vitro studies, but, since the penetration of UV filters in the epidermis removed by ex vivo assay was investigated and quantified by a validated HPLC method, we further established the safety and reliability of the use of bemotrizinol and ethylhexyl triazone.

It is known that FA is soluble in hot water, being insoluble in oil (Souto et al., 2005). Zhang et al., 2010, through in vitro permeation tests in Franz cells (pig skin as membrane model), observed low FA deposition on the skin, possibly being insufficient to induce its antioxidant activity (Zhang, 2010). Peres, 2015, analyzed the antioxidant activity of the molecule in the corneous layers of the epidermis by a tape stripping technique, not observing differences among the adhesive tapes obtained, which was justified by the low degree of FA penetration in/across the skin, that was found mainly on the stratum corneum surface (Peres, 2015).

In the current study, tape stripping was used as an efficient method to investigate the penetration of cosmetic active ingredients (Lademann et al., 2009). In this method, stratum corneum cell layers are successively removed by adhesive films from the same skin area

Table 2
Physicochemical parameters of bemotrizinol, ethylhexyl triazone and ferulic acid (Zhang, 2010, Lifeng KYHTYKKJSLHPA-LK. 2014).

Ingredients (actives)	Molecular weight (DA)	Log P
Bemotrizinol	627.81	12.93
Ethylhexyl triazoneTRIAZONE	823.07	16.13
Ferulic acid	194.18	1.67

Table 3
Penetration test of ferulic acid, ethylhexyl triazone and bemotrizinol performed by removal of stratum corneum ex vivo.

Ferulic acid	Concentration (μg/mL)	Recovery (%)	Total recovery (%)
Region 1	3.43 ± 0.13	17.17	55.74
Region 2	6.34 ± 1.32	31.69	
Region 3	1.38 ± 2.56	6.88	
Ethylhexyl triazone	Concentration (μg/mL)	Recovery (%)	Total recovery (%)
Region 1	27.49 ± 0.13	27.49	56.34
Region 2	20.61 ± 0.42	20.61	
Region 3	8.24 ± 0.56	8.24	
Bemotrizinol	Concentration (μg/mL)	Recovery (%)	Total recovery (%)
Region 1	49.75 ± 0.09	24.88	46.84
Region 2	29.57 ± 0.83	14.79	
Region 3	14.35 ± 1.53	7.17	

Table 4
Laser Doppler flowmetry analysis of control, sunscreen and sunscreen with ferulic acid regions.

Parameters	Control (water)	Sunscreen	Sunscreen with ferulic acid
PERFUSION UNIT VALUES (PU)	$94.45^{A} \pm 48.72$	$73.75^{A} \pm 23.44$	$76.84^{A} \pm 23.47$
MAXIMUM PERFUSION UNIT VALUE (PU)	$176.2^{B} \pm 68.11$	$138.5^{B} \pm 28.38$	$139.3^{B} \pm 34.42$
AREA UNDER THE CURVE (UNIT.SECONDS)	$84490^{C} \pm 43340$	$66280^{C} \pm 21060$	$68650^{C} \pm 21220$
ANGULAR COEFFICIENT	$0.5670^{D} \pm 0.3003$	$0.3567^{E} \pm 0.1177$	$0.2861^{E} \pm 0.09646$
ONSET TIME OF PERFUSION UNIT INCREASE (UNIT.SECONDS(1)	$116.0^{F} \pm 155.0$	$136.3^{F} \pm 143.3$	$123.3^{F} \pm 145.6$

after topical application of the samples (Lademann et al., 2009). The adhesive tapes contain amounts of corneocytes and corresponding amounts of the penetrated ingredients, which can be quantified by analytical methods (Lademann et al., 2009). The stratum corneum thickness of the human skin is considered to be between 10-30 μm, having at least 16 layers of cells with a diameter of around 1 μm each (Böhling et al., 2014; Ya-Xian et al., 1999). Lademann et al., 2009, measured the amount of stratum corneum removed by the tape stripping technique and found that 5 tapes could remove about 3 μm of this layer. The first adhesive tapes removed contained a high concentration of corneocytes, whereas when increasing the consequential number of tapes, its concentration decreased significantly (Lademann et al., 2009). So, 20 adhesive tapes, as we used in the present research work, should not exceed 10 μm of thickness. The quantification by HPLC of the active compounds obtained in the ex vivo assay is shown in Table. 4

The formulation applied on the volar forearm of the participants had 20 μg/mL of FA, 100 μg/mL of ethylhexyl triazone and 200 μg/mL of bemotrizinol. FA showed to be more concentrated on Region 2, where its recovery was 31.69%, suggesting that this compound can penetrate easier than the UV filters, whereas ethylhexyl triazone and bemotrizinol were more concentrated on Region 1, with recoveries of 27.49 and 24.88%, respectively. However, only 55.74% of FA, 56.34% of ethylhexyl triazone and 46.84% of bemotrizinol were found in the removed stratum corneum of the ex vivo assay. It seems unlikely that the UV filters, with high molecular weight and logP could have a significant percutaneous penetration. On the other hand, as mentioned previously, despite having more favorable physicochemical properties, FA has been reported to have low permeation rates (Peres et al., 2018, Peres, 2015). Considering that the stratum corneum can have up to 30 μm thickness and the adhesive tapes used would not exceed 10 μm of removal, it is suggested that the remaining quantities of the active compounds could be in the deeper layers of the stratum corneum, and that none of these compounds would be reaching the viable epidermis in significant amounts. The lower amounts of actives found in Region 3 seem to corroborate this hypothesis.

Nevertheless, further studies should be conducted to further establish the epidermal and dermal penetration of FA, since even though only approximately 50% was found in the tape stripped stratum corneum of the participants, evidence of its efficacy was observed, not only in the current research work, that will be discussed further, but also in the study by Peres et al., 2018, that showed that FA enhanced in vivo UV protection.

4.3. In vivo anti-inflammatory/ antioxidant assay

Laser Doppler flowmetry has been used to non-invasively assess skin blood flow in clinical and experimental studies and measure tissue perfusion (Vertuani et al., 2003). This equipment offers an excellent temporal resolution of local skin blood flow changes caused by diverse variables (Petersen, 2013). Its functioning is based in emitted light (formed by a Helion-Neon laser) which is dispersed and partially absorbed by the moving blood cells, suffering changes in the wavelength (Doppler shift), while the light that reaches the static objects is not altered (Fullerton et al., 2002). The magnitude and frequency distribution of these changes in wavelength are directly related to the number and velocity of blood cells in the sample volume. The information is captured by a feedback fiber, converted into an electronic signal and analyzed by a software, where blood perfusion is shown in real time throughout the procedure, expressed in arbitrary perfusion units (PU). The physiological response from inflammatory mediators can be quantitatively measured by blood flow instruments, non-invasively and in real time (Henricson et al., 2007; Cracowski et al., 2006).

In the present study, three sites were analyzed on the forearm of participants: two were treated for 2 hours with sunscreens with and without FA, and the remainder was a control region (purified water). After this period, a solution containing 0.5% of methyl nicotinate was applied to the skin, to stimulate a vasodilatory reaction, after which blood perfusion measurements were recorded continuously for 15 minutes. The topical application of methyl nicotinate stimulates a fast vasodilatation of the peripheral blood capillaries of the connective tissue bellow the epidermis (Jumbelic et al., 2006; Elsner and Maibach, 1991). Five parameters were considered in this study: (1) mean perfusion unit values; (2) maximum perfusion unit value; (3) area under the curve; (4) angular coefficient; and (5) onset time of perfusion increase. The comparison among the three regions can be seen in Table .

The angular coefficient of both formulations had a significantly decreased value compared to that observed in the negative control site. It is known that water can be a penetration enhancer to the skin, and raising the stratum corneum hydration is a strategy that can potentiate drugs locally applied to the cutaneous tissue (Bond and Barry, 1988; Lundborg et al., 2018; Merwe and Ackermann, 1987). Thus, the higher angular coefficient of the control (purified water) region could be explained by its permeation enhancement. In other words, the vasodilatory effect of the methyl nicotinate solution applied on the skin was amplified. It should also be noted that, although no significant differences were found, the onset time was quicker in the control and the remaining parameters were also higher. To minimize the effect of inter-individual variability, which is a well-known conditioning of in vivo studies, results were also analysed as the ratio between the values obtained at each sample site and the respective control values. Significant differences in the angular coefficient were established between formulations F1 and F2, as seen in

Fig. 1, that can be attributed to the FA presence.

The results obtained in this study showed that the presence of FA decreased the intensity of the erythema caused by methyl nicotinate when compared to the formulation that contained only the ethylhexyl triazone and bemotrizinol. This is an original result in the literature, being reported for the first time the FA influence with a method employing Laser Doppler flowmetry in research and development applied to sunscreens. It is well established that methyl nicotinate raises prostaglandin synthesis in skin cells, acting in the peripheral blood capillaries to cause vasodilatation (Katzman et al., 2003; Wilkin et al., 1985). The prostaglandin synthesis is a subproduct of COX enzymes in our body, which are responsible for the formation of important biological mediators, called prostanoids (like prostaglandins, prostacyclin and thromboxane) (Resler et al., 2014). Studies indicated that the FA could inhibit the COX-2 enzyme activity, which triggers a pro-inflammatory activity (Nile et al., 2016;Jayaprakasam et al., 2006 ; Hosoda et al., 2002). As seen in

Fig. 2 if the COX pathway is blocked, the synthesis of prostaglandins

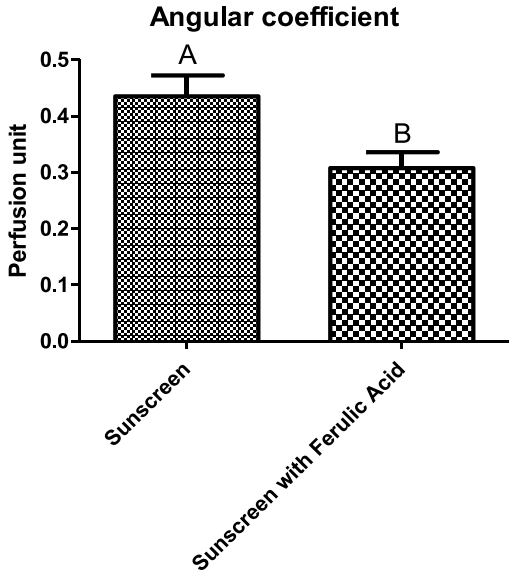

Fig. 1. Angular coefficient of sunscreens with or without ferulic acid analyzed by the Laser Doppler flowmetry equipment. The parameters were examined by Mann-Whitney U test (*$p < 0.05$, $n = 13$). Different letters mean statistical difference found.

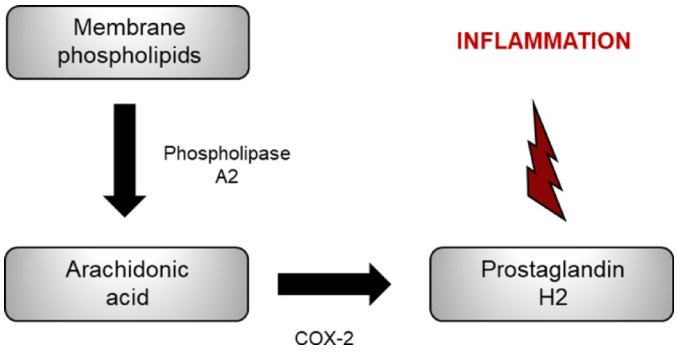

Fig. 2. Inflammatory pathway adapted from Resler et al. (2014), Martel-Pelletier et al. (2003).

is reduced, consequently affecting inflammatory responses. Therefore, it could be hypothesized that the formulation with FA had an anti-inflammatory effect detected by this in vivo assay due to its potential to decrease the COX-2 enzyme. Considering that an inflammation response can occur after UV exposure and can cause tissue damage (Lin et al., 2017), modulating this phenomenon, to maintain skin homeostasis, using a bioactive compound with efficacy at a low concentration of 1.0% can be considered a valuable strategy to develop new sunscreens with multi-aggregate benefits.

Peres et al., 2018, in earlier in vivo studies from our research group, found that FA, in a sunscreen formulation, amplified the SPF in about 37% and UVA-PF in 26% (Peres et al., 2018). The in vivo SPF evaluation is measured by the UVB energy required to produce a minimal erythemal dose (MED) on the protected skin of participants, divided by the UVB energy required to produce a MED on unprotected skin. Substances that delay the formation of erythema on the skin, like anti-inflammatory or antioxidant molecules, could significantly improve UV photoprotection, acting by a non-sunscreen mechanism. In this study, The results from the present investigation provided further insights into the mechanism of action of FA, establishing its anti-inflammatory activity in vivo, which seems to indicate that this is a promising molecule to be added to multifunctional sunscreen formulations.

5. Conclusions

The evaluation of skin penetration of ferulic acid, ethylhexyl triazone and bemotrizinol by an ex vivo tape stripping method was performed with an original HPLC method. Almost 50% of all active compounds were found in the sampled adhesive tapes, suggesting that the remaining amounts penetrated deeper into the stratum corneum. Using a method employing Laser Doppler flowmetry to measure the vasodilatory response to the topical application of methyl nicotinate, ferulic acid was shown to behave as an anti-inflammatory substance, providing a superior performance when compared to the sunscreen formulation without this compound. This is an interesting result, as this anti-inflammatory activity may explain the previously reported increase in the in vivo SPF of a formulation combining ferulic acid and UV filters.

Declaration of Competing Interest

None.

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References

Abla, M.J., Banga, AK., 2013. Quantification of skin penetration of antioxidants of varying lipophilicity. Int. J. Cosmet. Sci. 35, 19–26.
Almeida, D.D., Daud, F., Areias, C., Oliveira, D., 2018. Ferulic acid photoprotective properties in association with UV fi lters : multifunctional sunscreen with improved SPF and UVA-PF. J. Photochem. Photobiol. B Biol. 185, 46–49 March.
Alonso, C., Barba, C., Rubio, L., et al., 2009. An ex vivo methodology to assess the lipid peroxidation in stratum corneum. J. Photochem. Photobiol. B 97, 71–76. https://doi.org/10.1016/j.jphotobiol.2009.08.003.
Balogh, T.S., Velasco, M.V.R., Pedriali, C.A., Kaneko, T.M., Baby, A.R., 2011. Proteção à radiação ultravioleta: recursos disponíveis na atualidade em fotoproteção. An. Bras. Dermatol. 86, 732–734. https://doi.org/10.1590/S0365-05962011000400016.
Baskaran, N., Manoharan, S., Balakrishnan, S., Pugalendhi, P., 2010. Chemopreventive potential of ferulic acid in 7,12-dimethylbenz[a]anthracene-induced mammary carcinogenesis in Sprague-Dawley rats. Eur. J. Pharmacol. 637, 22–29.
Benfeldt, E., Hansen, S.H., Vølund, A., Menné, T., Shah, V.P., 2007. Bioequivalence of topical formulations in humans: evaluation by dermal microdialysis sampling and the dermatopharmacokinetic method. J. Invest. Dermatol. 127, 170–178.
Böhling, A., Bielfeldt, S., Himmelmann, A., Keskin, M., Wilhelm, K.-P., 2014. Comparison of the stratum corneum thickness measured in vivo with confocal Raman spectroscopy and confocal reflectance microscopy. Ski Res. Technol. 20, 50–57.
Bond, J.R., Barry, BW., 1988. Limitations of hairless mouse skin as a model for in vitro permeation studies through human skin: hydration damage. J. Invest. Dermatol. 90, 486–489.
Cândido, T.M., de Oliveira, C.A., Ariede, M.B., Velasco, M.V.R., Rosado, C., Baby, A.R., 2018. Safety and Antioxidant Efficacy Profiles of Rutin-Loaded Ethosomes for Topical Application. AAPS PharmSciTech. 19 (4), 1773–1780. https://doi.org/10.1208/s12249-018-0994-3.
Carocho, M., Ferreira, I., 2013. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol. 51, 15–25. https://doi.org/10.1016/j.fct.2012.09.021.
Cestari, T.F., de, O.F.B., Boza, J.C., 2012. Considerations on photoprotection and skin disorders. Ann. Dermatol. Venereol. 139, S135–S143 Suppl.
Chang, N.I., Yoo, M.Y., Lee, S.H., 2015. Determination of fourteen sunscreen agents in cosmetics using high-performance liquid chromatography. Int. J. Cosmet. Sci. 37, 175–180.
Chen, L., Hu, J.Y., Wang, S.Q., 2012. The role of antioxidants in photoprotection: a critical review. J. Am. Acad. Dermatol. 67, 1013–1024. https://doi.org/10.1016/j.jaad.2012.02.009.
Cracowski, J.-L., Minson, C.T., Salvat-Melis, M., Halliwill, J.R., 2006. Methodological issues in the assessment of skin microvascular endothelial function in humans. Trends Pharmacol. Sci. 27, 503–508.
Craft, B.D., Kerrihard, A.L., Amarowicz, R., Pegg, R.B., 2012. Phenol-based antioxidants and the in vitro methods used for their assessment. Compr. Rev. Food Sci. Food Saf.

11, 148–173.
Davies, D.J., Heylings, J.R., Gayes, H., McCarthy, T.J., Mack, M.C., 2017. Further development of an in vitro model for studying the penetration of chemicals through compromised skin. Toxicol. Vitr. 38, 101–107.
de Oliveira, C.A., Dario, M.F., Sarruf, F.D., Mariz, I.F.A., Velasco, M.V.R., Rosado, C., Baby, A.R., 2015. Safety and efficacy evaluation of gelatin-based nanoparticles associated with UV filters. Colloids Surf. B Biointerfaces 140, 531–537. https://doi.org/10.1016/j.colsurfb.2015.11.031.
de Oliveira, C.A., Peres, D.D., Rugno, C.M., et al., 2015. Functional photostability and cutaneous compatibility of bioactive UVA sun care products. J. Photochem. Photobiol. B Biol. 148, 154–159 doi:10.1016/j.jphotobiol.2015.04.007.
Di Domenico, F., Perluigi, M., Foppoli, C., et al., 2009. Protective effect of ferulic acid ethyl ester against oxidative stress mediated by UVB irradiation in human epidermal melanocytes. Free Radic. Res. 43, 365–375.
Diffey, B.L., Tanner, P.R., Matts, P.J., Nash, J.F., 2000. In vitro assessment of the broad-spectrum ultraviolet protection of sunscreen products. J. Am. Acad. Dermatol. 43, 1024–1035.
Durand, L., Habran, N., Henschel, V., Amighi, K., 2009. In vitro evaluation of the cutaneous penetration of sprayable sunscreen emulsions with high concentrations of UV filters. Int. J. Cosmet. Sci. 31, 279–292.
Elsner, P., Maibach, HI., 1991. Cutaneous responses to topical methyl nicotinate in human forearm and vulvar skin. J. Dermatol. Sci. 2, 341–345.
Essendoubi, M., Gobinet, C., Reynaud, R., Angiboust, J.F., Manfait, M., Piot, O., 2016. Human skin penetration of hyaluronic acid of different molecular weights as probed by Raman spectroscopy. Ski Res. Technol. 22, 55–62.
Fullerton, A., Stücker, M., Wilhelm, K.-.P., et al., 2002. Guidelines for visualization of cutaneous blood flow by laser Doppler perfusion imaging. A report from the standardization group of the European society of contact dermatitis based upon the HIRELADO European community project. Contact Dermatitis 46, 129–140.
American Cancer Society. 2016.
Gallin, J.I., Ognibene, F.P., Johnson, L.L.. Principles and practice of clinical research. 2017.
Gerin, F., Erman, H., Erboga, M., et al., 2016. The effects of ferulic acid against oxidative stress and inflammation in formaldehyde-induced hepatotoxicity. Inflammation 39, 1377–1386.
Graf, E., 1992. Antioxidant potential of ferulic acid. Free Radic. Biol. Med. 13, 435–448.
Halliwell, B., 2015. Free Radicals and other Reactive Species in Disease. In: eLS. John Wiley & Sons, Ltd, Chichester, UK, pp. 1–9.
Hayden, C.G.J., Roberts, M.S., Benson, H.A.E., 1997. Systemic absorption of sunscreen after topical application. Lancet 350, 863–864.
Haywood, R., Wardman, P., Sanders, R., Linge, C., 2003. Sunscreens inadequately protect against ultraviolet-a-induced free radicals in skin: implications for skin aging and melanoma. J. Invest. Dermatol. 121, 862–868.
Henricson, J., Tesselaar, E., Persson, K., Nilsson, G., Sjöberg, F., 2007. Assessment of microvascular function by study of the dose–response effects of iontophoretically applied drugs (acetylcholine and sodium nitroprusside)—methods and comparison with in vitro studies. Microvasc. Res. 73, 143–149.
Hosoda, A., Ozaki, Y., Kashiwada, A., et al., 2002. Syntheses of ferulic acid derivatives and their suppressive effects on cyclooxygenase-2 promoter activity. Bioorg. Med. Chem. 10, 1189–1196.
IARC. A review of human carcinogens. 2012;100:35–101.
Jansen, R., Wang, S.Q., Burnett, M., Osterwalder, U., Lim, H.W., 2013. Continuing photoprotection part I. Photoprotection by naturally occurring, physical, and systemic agents. J. Am. Acad. Dermatol. 69, 853–866.
Jayaprakasam, B., Vanisree, M., Zhang, Y., Dewitt, D.L., Nair, M.G., 2006. Impact of alkyl esters of caffeic and ferulic acids on tumor cell proliferation, cyclooxygenase enzyme, and lipid peroxidation. J. Agric. Food Chem. 54 (15), 5375–5381. https://doi.org/10.1021/jf060899p.
Jin, Y., Yan, E.Z., Fan, Y., Zong, Z.H., Qi, Z.M., Li, Z., 2005. Sodium ferulate prevents amyloid-beta-induced neurotoxicity through suppression of p38 MAPK and upregulation of ERK-1/2 and Akt/protein kinase B in rat hippocampus. Acta Pharmacol. Sin. 26, 943–951.
Jumbelic, L.C., Liebel, F.T., Southall, M.D., 2006. Establishing a minimal erythema concentration of methyl nicotinate for optimum evaluation of anti-inflammatories. Skin Pharmacol. Physiol. 19, 147–152.
Katzman, M., Cornacchi, S., Coonerty-Femiano, A., et al., 2003. Methyl nicotinate-induced vasodilation in generalized social phobia. Neuropsychopharmacology 28, 1846–1851.
Klinubol, P., Asawanonda, P., Wanichwecharungruang, S.P., 2008. Transdermal penetration of UV filters. Skin Pharmacol. Physiol. 21, 23–29.
Lademann, J., Jacobi, U., Surber, C., Weigmann, H.J., Fluhr, J.W., 2009. The tape stripping procedure - evaluation of some critical parameters. Eur. J. Pharm. Biopharm. 72, 317–323. https://doi.org/10.1016/j.ejpb.2008.08.008.
Lautenschlager, S., Wulf, H.C., Pittelkow, M.R., 2007. Photoprotection. Lancet 370, 528–537.
Lifeng KYHTYKKJSLHPA-LK., 2014. Handbook of Cosmeceutical Excipients and their Safeties 1st Edition. Woodhead Publishing.
Lin, T.-.K., Zhong, L., Santiago, J., 2017. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. Int. J. Mol. Sci. 19, 70. https://doi.org/10.3390/ijms19010070.
Lü, J.M., Lin, P.H., Yao, Q., Chen, C., 2010. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. J. Cell Mol. Med. 14, 840–860.
Lundborg, M., Wennberg, C.L., Narangifard, A., Lindahl, E., Norlén, L., 2018. Predicting drug permeability through skin using molecular dynamics simulation. J. Control Release 283, 269–279.
Martel-Pelletier, J., Lajeunesse, D., Reboul, P., Pelletier, J.-.P., 2003. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. Ann. Rheum. Dis. 62, 501–509.
Mead, MN., 2008. Benefits of sunlight: a bright spot for human health. Environ. Health Perspect. 116, A160–A167.
Modi, P.B., Vukkum, B.A stability indicating RP-HPLC method for simultaneous estimation of ethylhexyl triazone and bemotrizinol in sun care formulations. 2014;4February.
Merwe, E., Ackermann, C., 1987. Physical changes in hydrated skin. Int. J. Cosmet. Sci. 9, 237–247.
Monti, D., Tampucci, S., Chetoni, P., et al., 2011. Permeation and distribution of ferulic acid and its α-cyclodextrin complex from different formulations in hairless rat skin. AAPS PharmSciTech 12, 514–520.
Murakami, A., Nakamura, Y., Koshimizu, K., et al., 2002. FA15, a hydrophobic derivative of ferulic acid, suppresses inflammatory responses and skin tumor promotion: Comparison with ferulic acid. Cancer Lett. 180, 121–129.
Nile, S.H., Ko, E.Y., Kim, D.H., et al., 2016. Screening of ferulic acid related compounds as inhibitors of xanthine oxidase and cyclooxygenase-2 with anti-inflammatory activity. Rev. Bras. Farmacogn. 26, 50–55.
Nordberg, J., Arnér, E.S., 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic. Biol. Med. 31, 1287–1312.
Peres, D.D., 2015. Ácido Ferúlico em Protetores Solares: Desenvolvimento e Eficácia Multifuncional in vitro, ex vivo e in vivo. Ácido Ferúlico em Protetores Solares: Desenvolvimento e Eficácia Multifuncional in vitro, ex vivo e in vivo. University of São Paulo, São Paulo, SP, Brazil.
Peres, D.D.A., Sarruf, F.D., de Oliveira, C.A., Velasco, M.V.R., Baby, A.R., 2018. Ferulic acid photoprotective properties in association with UV filters: multifunctional sunscreen with improved SPF and UVA-PF. J. Photochem. Photobiol. B Biol. 185, 46–49. https://doi.org/10.1016/j.jphotobiol.2018.05.026. May.
Petersen, L.J., 2013. Direct comparison of laser Doppler flowmetry and laser Doppler imaging for assessment of experimentally-induced inflammation in human skin. Inflamm. Res. 62, 1073–1078.
Phaniendra, A., Jestadi, D.B., Periyasamy, L., 2015. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J. Clin. Biochem. 30, 11–26.
Potard, G., Laugel, C., Schaefer, H., Marty, J.-.P., 2000. The stripping technique: in vitro absorption and penetration of five UV filters on excised fresh human skin. Skin Pharmacol. Physiol. 13, 336–344.
Resler, A.J., Makar, K.W., Heath, L., et al., 2014. Genetic variation in prostaglandin synthesis and related pathways, NSAID use and colorectal cancer risk in the Colon Cancer Family Registry. Carcinogenesis 35, 2121–2126.
Roussel, L., Gilbert, E., Salmon, D., et al., 2015. Measurement, analysis and prediction of topical UV filter bioavailability. Int. J. Pharm. 478, 804–810.
Sarruf, F.D., Cândido, T.M., Oliveira, C.A., Bou-Chacra, N.A., Robles Velasco M, V., Baby, A.R., 2020. Influence of Shea (Butyrospermum parkii) butter, TiO_2 and ethylhexyl methoxycinnamate on physical parameters and in vitro photoprotective efficacy. J. Cosmet. Dermatol. 19, 2076–2085. https://doi.org/10.1111/jocd.13238.
Sarruf, F.D., Sauce, R., Candido, T.M., et al., 2020. Butyrospermum parkii butter increased the photostability and in vivo SPF of a molded sunscreen system. J. Cosmet. Dermatol In press.
Souto, E.B., Anselmi, C., Centini, M., Müller, R.H., 2005. Preparation and characterization of n-dodecyl-ferulate-loaded solid lipid nanoparticles (SLN®). Int. J. Pharm. 295, 261–268.
Souza, C., Maia Campos, P., 2017. Development of a HPLC method for determination of four UV filters in sunscreen and its application to skin penetration studies. Biomed. Chromatogr. 31.
Srinivasan, M., Rukkumani, R., Sudheer, A.R., Menon, V.P., 2005. Ferulic acid, a natural protector against carbon tetrachloride-induced toxicity. Fundam. Clin. Pharmacol. 19, 491–496.
Tampucci, S., Burgalassi, S., Chetoni, P., Monti, D., 2018. Cutaneous permeation and penetration of sunscreens: formulation strategies and in vitro methods. Cosmetics 5 (1), 1. https://doi.org/10.3390/cosmetics5010001.
Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44–84.
Velasco, M.V.R., Sarruf, F.D., Salgado-Santos, I.M.N., Haroutiounian-Filho, C.A., Kaneko, T.M., Baby, A.R., 2008. Broad spectrum bioactive sunscreens. Int. J. Pharm. 363, 50–57. https://doi.org/10.1016/j.ijpharm.2008.06.031.
Vertuani, S., Ziosi, P., Solaroli, N., et al., 2003. Determination of antioxidant efficacy of cosmetic formulations by non-invasive measurements. Skin Res. Technol. 9, 245–253.
Weigmann, H.-.J., Jacobi, U., Antoniou, C., et al., 2005. Determination of penetration profiles of topically applied substances by means of tape stripping and optical spectroscopy: UV filter substance in sunscreens. J. Biomed. Opt. 10, 014009.
Wharton, M., Geary, M., O'Connor, N., Murphy, B., 2011. A rapid High Performance Liquid Chromatographic (HPLC) method for the simultaneous determination of seven UV filters found in sunscreen and cosmetics. Int. J. Cosmet. Sci. 33, 164–170.
Wilkin, J.K., Fortner, G., Reinhardt, L.A., Vogt Flowers, O., Kilpatrick, S.J., Streeter, W.C., 1985. Prostaglandins and nicotinate-provoked increase in cutaneous blood flow. Clin. Pharmacol. Ther. 38, 273–277.
Yap, F.H.X., Chua, H.C., Tait, C.P., 2017. Active sunscreen ingredients in Australia. Australas J. Dermatol. 58, e160–e170.
Ya-Xian, Z., Suetake, T., Tagami, H., 1999. Number of cell layers of the stratum corneum in normal skin - relationship to the anatomical location on the body, age, sex and physical parameters. Arch. Dermatol. Res. 291, 555–559.
Young, C., 2009. Solar ultraviolet radiation and skin cancer. Occup. Med. (Chic Ill) 59, 82–88.
Zduńska, K., Dana, A., Kolodziejczak, A., Rotsztejn, H., 2018. Antioxidant properties of ferulic acid and its possible application. Skin Pharmacol. Physiol. 31, 332–336.
Zhang, L.W., Al-Suwayeh, S.A., Hsieh, P.W., Fang, J.Y., 2010. A comparison of skin delivery of ferulic acid and its derivatives: evaluation of their efficacy and safety. Int. J. Pharm. 399, 44–51. https://doi.org/10.1016/j.ijpharm.2010.07.054.