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Ferulic acid photoprotective properties in association with UV filters: multifunctional sunscreen with improved SPF and UVA-PF

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ABSTRACT

Ultraviolet (UV) radiation stimulates several injurious biological effects on cutaneous tissue, causing, for instance, photocarcinogenesis. Sunscreens are topical products designed to protect the skin against these harmful effects and their use must be encouraged. The addition of antioxidants, as ferulic acid (FA), a phenolic compound from the class of the hydroxycinnamic acids, in sunscreens could improve their sun protection factor (SPF) and prevent inflammatory reactions. Here, the clinical safety and efficacy of an association of ethylhexyl triazone and bis-ethylhexyloxyphenol methoxyphenyl triazine (UV filters) with ferulic acid were assessed. Samples had good skin biocompatibility and presented satisfactory safety profile, even in a sun-exposed condition. A synergic effect between the natural polyphenol and the UV filters was evidenced, as well as, FA increased *in vivo* SPF in 37% and the UVA protection factor (UVA-PF) in 26%. The *in vivo* data indicated that FA reinforced the broad-spectrum characteristic of the photoprotective formulations. Additionally, according to the results from the *ex vivo* antioxidant test, it is plausible to recommend adjustments on the *ex vivo* protocol to explicitly determine the positive effects of topical antioxidant ingredients applied over the skin. These results provided a new perspective for the development of multifunctional bioactive sunscreens using FA as a new platform.

1. Introduction

The ferulic acid (FA) is a phenolic compound from the class of the hydroxycinnamic acids that can be found in several natural sources. This substance has proven results in the treatment of various diseases, such as cancer and diabetes, as well as antimicrobial action, anti-inflammatory and, mainly, antioxidant activity, responsible for its main benefits and applications [1,2].

FA exhibits marked antioxidant activity based on four structural features: (i) the hydroxyl group, electron donor, attached to the benzene ring, responsible for neutralizing the reactive oxygen species; (ii) the side vinyl chain, which connects the carboxyl group to benzene ring and increases the stability of the molecule; (iii) the methoxyl substituent capable of forming a hydrogen bond with the hydroxyl group and provide additional stability to the molecule; and (iv) the carboxylic group that provides protection against lipid peroxidation [2,3]. Furthermore, as may be seen in Fig. 1, the double bound in the side chain of FA is subjected to *cis-trans* isomerization [4]. *Cis*-FA is found as yellow oil, with maximum UV absorption at 316 nm, whereas *trans*-FA has two maximum absorption peaks at 284 and 307 nm. In previous research, we demonstrated, through *in vitro* estimated sun protection factor (SPF), critical wavelength (nm) and ultraviolet UV transmittances, that interactions occurred between FA and UV filters, being the FA an ingredient that positively affected the functional profile of the sunscreen system. Ferulic acid influenced the results for *in vitro* antioxidant activity, providing a 90% increase in the antioxidant potential. Conclusively, the analysis of the experimental design demonstrated the synergy between UV filters and FA [5].

Regarding the *in vivo* application, researchers have demonstrated the effectiveness of FA against the harmful effects of UV radiation, such as erythema, photoaging and skin cancer. In three similar *in vivo* studies, using a solar simulator to induce an inflammatory reaction, the FA incorporation in topical solutions containing vitamins increased the chemical stability of the vitamins and also enhanced the photoprotective effect, reducing the levels of erythema and apoptosis of corneocytes [1,6,7].

In the present study, we examined the association of ethylhexyl triazone and bis-ethylhexyloxyphenol methoxyphenyl triazine with

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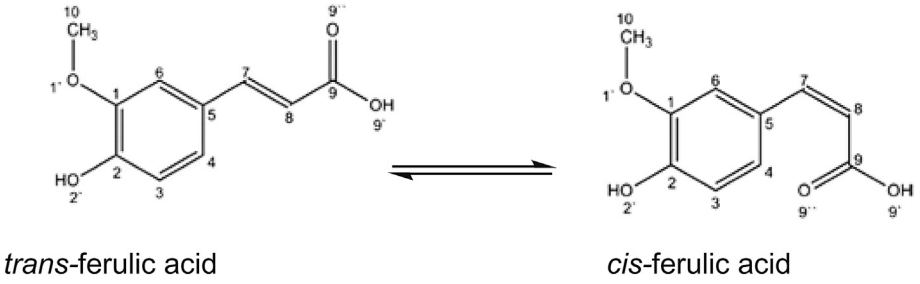

Fig. 1. Chemical structure of *trans*- and *cis*-ferulic acid [4].

ferulic acid, in order to obtain multifunctional sunscreens with antioxidant efficacy. Both UV filters are photostabilized molecules with low skin permeation and high efficacy at low concentrations, ideal characteristics for the preparation of photoprotectors. Here we investigated the clinical safety of the bioactive sunscreens and evaluated the effect of FA in improving the photoprotective and antioxidant efficacy of the samples.

2. Material and Methods

2.1. Reagents, Solvents and Active Ingredients

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Brazil). Analytical grade methyl alcohol was acquired from Merck (São Paulo, Brazil). *Trans*- Ferulic acid was purchased from Henrifarma (Brazil). Ethylhexyl triazone was acquired from D'Altomare (Brazil) and bis-ethylhexyloxyphenol methoxyphenyl triazine was purchased from Brasquim (Brazil). All materials were used as received, without any further purification. Purified water was used for all experiments.

2.2. Formulations

Oil-in-water emulsions associating or not FA and the UV filters were developed based on an anionic self-emulsifying agent. Table 1 describes the qualitative and quantitative composition (% w/w) of the samples.

2.3. Clinical Assays

Procedures were in accordance with the ethical standards on human experimentation and with the Helsinki Declaration. All protocols were approved by the Human Experimentation Committee of the School of Pharmaceutical Sciences of the University of São Paulo (protocol number: 735.493). For all subjects, oral informed and written consent were previously provided.

2.4. Human Repeat Insult Patch Test (HRIPT) and Phototoxic/Photosensitivity Potential

A six-week HRIPT assay was performed in 55 male and female volunteers. Subjects were 18–60 years old with skin phototypes of II to IV. Epicutaneous semi-oclusive patches were applied to volunteers´ backs for 48 h, three times a week. Each patch had three chambers, two that contained the sunscreen samples (F1 and F2) and, another, containing purified water, as negative control. The skin was scored 30 min later and new material was applied for two more weeks. The next two weeks were the rest period (no samples applied) and, after that, new patches with the samples and negative control were applied for one last week, *i.e.*, the challenge phase. The scores used were 0 for no erythema, 1 for well-defined erythema, 2 for erythema and induration, and 3 for vesiculation and bullous reaction [8–10].

A phototoxic and photosensitivity potential assays were performed in 27 male and female volunteers aged between 18 and 52 years old and with skin phototypes of II to IV. Epicutaneous patches were applied to the volunteers´ backs for 48 h, twice a week (patches contained F1, F2 and purified water in separate chambers). After 48 h, sites were exposed to an UVA simulated irradiation dose of 4.0 J/cm^2 for 7 min. The skin was scored 30 min later, aiming to evaluate any phototoxic reaction. The formulations were, then, reapplied and the sites were irradiated for one more week. The next two weeks were the rest period and, subsequently, new patches with the samples were applied and irradiated for the challenge phase, targeting to evaluate photosensitivity reactions. The scores used were: 0 for no erythema; 1 for well-defined erythema; 2 for erythema and induration; 3 for vesiculation and bullous reaction [10,11].

Table 1
Qualitative and quantitative (% *w*/w) composition of the sunscreen samples.

Ingredients		Concentration (% w/w)	
		F1	F2
Oil phase	Ethylhexyl triazone	5.0	5.0
	Bis-ethylhexyloxyphenol methoxyphenyl triazine	10.0	10.0
	C12-C15 alkyl benzoate	9.0	9.0
	Butylene glycol cocoate	6.75	6.75
	Isopropyl myristate	6.75	6.75
	Hydroxyethyl acrylate (and) sodium acryloyldimethyl taurate copolymer (and) isohexadecane (and) polysorbate 60	4.00	4.00
	Cyclomethicone	1.75	1.75
	Cyclomethicone (and) dimethicone crosspolymer	1.25	1.25
Water phase	Glycerin	5.00	5.00
	Phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) propylparaben (and) isobutylparaben	0.75	0.75
	Disodium EDTA	0.30	0.30
	Acrylates (and) C10–30 alkyl acrylate crosspolymer	0.10	0.10
	Ferulic acid	–	1.0
	Triethanolamine	*	*
	Purified water	**	**

* Sufficient to adjust the pH value.
** Sufficient to complete to 100%.

2.5. Ex vivo Antioxidant Activity Assay

F1 and F2 (2.0 mg/cm^2) were applied over the volar forearm of 10 subjects in randomized areas of 9.0 cm^2 previously outlined. Two consecutive applications of each sample were carried out with an interval of 2 h between them [12]. Two hours after the last application, a tape stripping technique was performed. Twenty tapes (2.0 × 2.0 cm, 3 M®) were taken for each area and, after, the tapes were exposed for 2 h in a solar simulator (Suntest® CPS +, Atlas, Germany) equipped with a xenon lamp, an optical filter to cut off wavelengths shorter than