BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902023e23228

Article

In vitro approaches to antioxidant screening for the development of a sunscreen formulation

Bruna dos Santos Soares[1], Raiane Rosales Diniz[2], Juliana Patrão de Paiva[2], Marcelo de Pádula[2], Elisabete Pereira dos Santos[1], Mariana Sato de Souza de Bustamante Monteiro[1*]

[1]University Pharmacy, Department of Drugs and Medicines, Faculty of Pharmacy, Health Science Center, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Rio de Janeiro, Brazil, [2]Laboratory of Microbiology and Genotoxic Assessment, Department of Clinical and Toxicological Analysis, Faculty of Pharmacy, Health Science Center, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Rio de Janeiro, Brazil

The incorporation of antioxidants into sunscreens may provide additional skin photoprotection against the harmful photobiological effects of ultraviolet radiation. The present study evaluated the applicability of a screening approach to the assessment of the antioxidant and photoprotective properties of vitamin C, vitamin E, and coenzyme Q10 and then determined the performance of the most effective antioxidant in a sunscreen formulation. Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2`-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, and oxygen radical absorbance capacity (ORAC) assay, and the photoprotective potential was investigated by the yeast photoprotection assay. The antioxidant with the best effect was incorporated into sunscreen formulations which were evaluated for 120 days regarding their *in vitro* photoprotective parameters. Vitamin C showed high antioxidant capacity as well as a photoprotective potential against simulated solar irradiation applied for times longer than 1 h. Although the Sun Protection Factor, UVA/UVB ratio and critical wavelength did not differed significantly ($p < 0.05$) between the formulation blank and the formulations containing 0.5% or 1% vitamin C, formulations with vitamin C kept their photostability for 6 months. Consequently, the proposed screening approach seems to be promising for the development of an antisolar photostable formulation containing vitamin C as an antioxidant.

Keywords: Vitamin C. Vitamin E. Coenzyme Q10. Photoprotection. Emulsion.

INTRODUCTION

The skin is constantly exposed to environmental factors such as solar radiation, pollution, heat, and photo pollution, and such factors are associated with its aging (Krutmann *et al.,* 2017). The effect of solar radiation on the skin has been the external aggression most extensively studied to date; however, some recent studies have demonstrated a synergistic effect between pollution and ultraviolet (UV) radiation, called photo pollution. Photo pollution can cause increased lipid peroxidation, increased inflammatory mediators, depletion of endogenous antioxidants, and intensified premature skin aging (Krutmann *et al.,* 2017; Marrot, 2019).

A strategy to prevent the damage caused by photo pollution is the use of broad-spectrum sunscreens containing antioxidants with a sun protection factor (SPF) of 30 due to their high protection against UVB radiation. Moderate sun exposure, use of clothing and accessories with an SPF, reduction of direct exposure to pollution such as cigarette smoke, and use of topical antioxidants are also recommended (Krutmann *et al.,* 2017; Marrot, 2019).

***Correspondence:** M. S. de S. B. Monteiro. Farmácia Universitária. Departamento de Fármacos e Medicamentos. Faculdade de Farmácia. Centro de Ciências da Saúde. Universidade Federal do Rio de Janeiro (UFRJ). Avenida Carlos Chagas Filho, 373 - Campus Cidade Universitária. CEP: 21910-240, Ilha do Fundão, Rio de Janeiro, RJ, Brasil. Phone: +552139386625. Email: marianasato@pharma.ufrj.br. ORCID: https://orcid.org/0000-0002-2422-8048

Thus, the combination of skin's endogenous antioxidants such as vitamin C, vitamin E, and coenzyme Q10 in photoprotective products is a promising alternative for the improvement of sunscreen formulations (Afonso *et al.,* 2014; Addor, 2017) since these agents can neutralize the free radicals generated by sun exposure and pollution, restoring or maintaining a healthy skin barrier (Chen, Damian, Halliday, 2014).

Several studies have been reported in the literature highlighting the use of photoprotective formulations with active antioxidant agents of natural or synthetic origin (He *et al*., 2021; Ruvolo *et al*., 2022; Valverde *et al*., 2023). According to Jesus *et al.* (2023), most sunscreen formulations sold in Portuguese pharmacies contain antioxidants, and vitamin E and its derivatives are the agents most frequently used in these formulations (Jesus *et al*., 2023).

Vitamin C (Vit. C) is a hydrophilic molecule known for its high antioxidant potential, collagen production regulation, and improvement of the changes induced by UV radiation in the skin's structure. Vit. C neutralizes oxidative stress by electron transfer or donation and has been reported to protect against photoaging, UV-induced immunosuppression, and photocarcinogenesis, besides having an anti-aging effect and an anti-pigmentary effect (Al-Niaimi, Chiang, Yi, 2017; Caritá *et al*., 2020).

Vitamin E (Vit. E) is a lipophilic antioxidant which has been in use for more than 50 years in dermatology (Thiele, Hsieh, Ekanayake-Mudiyanselage, 2005). It has a primary role in protecting cell membranes against oxidative stress and maintaining the collagen network of the skin. It is a free radical scavenger, and it can reduce DNA damage and keratinocyte death caused by the deleterious effects of solar radiation. Also, it can enhance stratum corneum hydration and reduce skin roughness, also exhibiting photoprotective properties (Keen, Hassan, 2016).

Coenzyme Q10 (Cq10) is an endogenously synthesized fat-soluble antioxidant. Endogenous levels of Cq10 in the skin decrease with age and are significantly reduced under the influence of external factors such as UV radiation and pollution (Barcelos, Haas, 2019). Topical treatment with Cq10 can replenish its levels in the skin and have antioxidant effects (Knott *et al.,* 2015).

Grether-Beck *et al.* (2014) studied the effect of incorporating antioxidants such as grape seed extract, Vit. E, Cq10, and Vit. C in a sunscreen formulation with SPF 30. These associations were found to be more effective in protecting the skin against the formation of matrix metalloproteinase (MMP-1), which is responsible for collagen degradation. Thus, the combination of photo protectors and antioxidants makes a formulation more effective (Grether-Beck *et al.,* 2014).

The development of new cosmetic formulations must involve extensive screening of components in the search for the most promising and efficient ones. Additionally, due to the current recommendation of methodologies that replace animal use in cosmetic efficacy and safety assessment, alternative and simplified approaches should be encouraged (Paiva *et al*., 2020).

The objective of the present study was to evaluate the use of a rational 3-step approach to the development of a photoprotective formulation with antioxidant potential. To test the applicability of the approach proposed in this work, three well-established antioxidants were used in an initial screening step of their antioxidant and photoprotective performance using different *in vitro* tests. After selecting the most promising antioxidant, a formulation combining the selected antioxidant with organic UV filters was developed and characterized for photoprotective parameters and photostability.

MATERIAL AND METHODS

Chemicals

Vit. C, Vit. E, and Cq10 were purchased from Henrifarma (Brazil), Sinochem Jiangs (China), and Fagron (Brazil), respectively. The chemical UV filters octylmethoxycinnamate (OMC), Diethylamino hydroxybenzoyl hexyl benzoate (DHHB), Octocrylene (OCT), and Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine (BEMT) were provided by Galena (Brazil), Fagron (Brazil), BASF (Brazil) and Valdequimica (Brazil), respectively. Carboxy polymethylene was purchased from Pharma Special (Brazil), Aminomethylpropanol 95% from Pharma Nostra (Brazil), Aluminium Starch Octenylsuccinate (Dry-

flo® pure) from Sarfam (Brazil), Caprylic/caprylic triglycerides from Infinity Pharma (Brazil), cetostearyl alcohol ethoxylates from Sarfam (Brazil), Stearic acid from Pharma Special (Brazil), Isoctyl stearate from Pharma Special (Brazil), methylisothiazolinone/phenoxyethanol solution (Conserve NovaMit MFTM) from Biovital (Brazil); Glyceryl monostearate from Pharma Nostra (Brazil), polysorbate 80 (TweenTM80) from Farmos (Brazil), glycerin from Fagron (Brazil), and propylene glycol from Pharma Nostra (Brazil). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2`-azino-bis (ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox™) from Sigma-Aldrich. Methanol was purchased from Vetec Quimica Fina (Brazil).

Antioxidant activity

DPPH Assay

The DPPH assay, based on 2,2-diphenyl-1-picryl-hydrazyl-hydrate, is an easy and rapid method for evaluating the antioxidant activity of a compound based on its electron-transfer capacity using spectrophotometry (Garcia *et al.,* 2012). The radical scavenger activity of the antioxidants Vit. C, Vit. E, and Cq10 was determined by the DPPH method and expressed as the requirement needed to obtain 50% of the antioxidant effect (EC_{50}).

A 0.1 mM DPPH solution using methanol was mixed with one volume of each sample solution. Absorbance at 515 nm was measured by spectrophotometry (V-630, Jasco, Pfungstadt, Germany) against a blank after 30 min using a quartz cuvette. This test was performed in triplicate, and the antioxidant activity was determined as shown in Equation 1:

$$Activity\ (\%) = \frac{Ac - At}{Ac\ x\ 100} \qquad \text{Equation 1}$$

Where: At is the absorbance of the samples and Ac the absorbance of the methanolic DPPH solution. All tests were carried out in triplicate and the results are reported as means ± standard deviation.

ABTS Assay

The ABTS assay evaluates the ability of substances to sequester the 2,2'-azino-bis (ethylbenzthiazolin-6-sulfonic acid) cation and was carried out as described by Re *et al.* (1999) and Arias (2012).

Initially, a 7 mM ABTS stock solution was mixed with 2.45 mM potassium persulfate in acetate buffer, pH 4.5, and left to stand in the dark at 7 °C for 24 hours. For the measurements the ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.20 at 734 nm. The photometric assay was performed with 190 µL of ABTS solution and 10 µL of each sample mixed for 2 min at 25 °C. Absorbance at 750 nm was measured after 60 min and Trolox® was used as a standard. Trolox® is a water- and ethanol-soluble substance derived from vitamin E, used as a standard antioxidant. The antioxidant activity of the samples was calculated by determining the decrease in absorbance at different concentrations using Equation 2:

$$ABTS\ value\ (\mu\ mol) = c\ x\ V\ x\ t/m \qquad \text{Equation 2}$$

Where: c is the Trolox® concentration (µmol/ml) of the corresponding standard curve of the diluted sample, V is the sample volume (ml), t is the dilution factor, and m is the weight of the sample (g)

The determinations were made using a Turner Biosystems Inc. microplate reader, Modulus ™ II Microplate Multimode Reader, (Sunnavale, CA, USA) with 96-well poly (styrene) microplates and an absorbance module. Each measurement was performed in triplicate, and the results are expressed as mean ± standard deviation.

ORAC Assay

Oxygen radical absorbing capacity (ORAC) was determined according to Ou *et al*. (2001) and Huang *et al.* (2005). Antioxidant activity was measured as the difference between the areas under the curve (AUC) of each sample compared to the standard. A 150 µL amount of fluorescein (FL) (8.16×10^{-5} mM) was added to the samples in phosphate buffer, pH 7.4, the mixture was

incubated at 37°C for 18 min, and 25 µL of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (153 mM) were added.

The decrease in fluorescence was recorded for 80 min at wavelengths λ = 490 nm for excitation and λ =510 nm, for emission. The amount of Trolox® (µmol), used as a standard, was calculated using a standard curve.

The measurements were made in triplicate using a Modulus™ II Multimode Microplate Reader (Turner Biosystems Inc., Sunnavale, CA, USA) with 96-well microplates and a fluorescence module. Results were expressed as mean ± standard deviation.

Assessment of in vitro photoprotective efficacy using *Saccharomyces cerevisiae*

Yeast Strain, Media, and Growth Conditions

The CD138 mutant *(ogg1::TRP1*) strain used in this work was from our laboratory stock. Yeast cultures were grown at 28°C in Yeast Peptone Dextrose (YPD) liquid medium (1% yeast extract, 1% bactopeptone, and 2% glucose). YPD solid medium (1% yeast extract, 1% bactopeptone, 2% glucose, and 2% agar) was used for photoprotection assessment after the treatments (Diniz *et al*., 2019).

Simulated Solar Light (SSL)

SSL irradiation was performed using a solar simulator (Oriel Model 91192–1000, Newport Corp., USA) emitting 21.7 J/m^2/s UVA and 1.58 J/m^2/s UVB. The atmospheric attenuators AMO and 87066 were used, resulting in a final UVB/UVA ratio emission of 1/16 (93.21% UVA and 6.79% UVB), which corresponds to the mean measurements for summer in Rio de Janeiro, Brazil (-22.9 latitude and longitude -43.17) (Diniz *et al*., 2019). Dosimetry at 1000 W/m^2 was measured using a VLX-3-W dosimeter (Vilber Lourmat, Marne-la-Vallée, France) with appropriate photocells for UVA (CX-365) and for UVB (CX-312). The dose rates adopted for UVA and UVB irradiation upon SSL were 20 J/m^2/s and 1.2 J/m^2/s, respectively. The SSL irradiation was conducted using different irradiation times and doses as described by Diniz *et al*. (2019).

Assessment of the in vitro photoprotective efficacy of Vit. C, Vit. E and Cq10 using the CD138 (ogg1) yeast strain

Yeast cultures (10 ml) of the CD138 strain (*ogg1*) were grown to a cell density of 1×10^8 cells/ml at 28ºC with agitation for 48 h (stationary phase). After this pre-cultivation, cells were harvested by centrifugation, washed twice, and resuspended in distilled and deionized sterile water. The cell suspension was adjusted to 1×10^7 cells/ml and transferred to 5.0 cm diameter glass Petri dishes with a final volume of 12 ml. The yeast suspension was exposed to increasing SSL doses under agitation in the presence or absence of 100 µg/ml of either Vit. C, Vit. E or Cq10, a dose established as the maximal nontoxic antioxidant concentration when yeast cells were incubated under agitation in the dark (data not shown).

After each dose and irradiation time, aliquots were taken, properly diluted in sterile water, plated on YPD medium, and incubated at 28°C for 3 days. Surviving colonies were counted and plotted on a survival graph. All survival experiments were independently performed at least four times and are expressed as mean ± standard deviation (Silva *et al*., 2019; Diniz *et al*., 2019)

Topical sunscreen formulation

Three emulsions formulations were used: formulation 1 or blank, without Vit. C; formulation 2 with 0.5% Vit. C; formulation 3 with 1% Vit. C (Table I).

TABLE I - Composition of the formulations developed

Components (INCI name)	Formulation 1 (w/w%)	Formulation 2 (w/w%)	Formulation 3 (w/w%)
Phase A			
Glycerin	5%	5%	5%
95% Aminomethyl propanol	1%	1%	1%
Carboxy polymethylene	0.2%	0.2%	0.2%
Water	q.s 100.0	q.s 100.0	q.s 100.0
Phase B			
Polysorbate 80	0.6%	0.6%	0.6%
Octyl methoxycinnamate	8%	8%	8%
Octocrylene	8%	8%	8%
Diethylamino Hydroxybenzoyl Hexyl Benzoate	4%	4%	4%
Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine	3%	3%	3%
Phase C			
Propylene glycol	10%	10%	10%
Aluminum Starch Octenylsuccinate	5 %	5%	5%
Phase D			
Caprylic/caprylic acid triglycerides	3%	3%	3%
Ethoxylate-cetostearyl acid	5%	5%	5%
Stearic acid	4%	4%	4%
Isoctyl stearate	7%	7%	7%
Glyceryl monostearate	2%	2%	2%
Methylisothiazolinone/phenoxyethanol solution	0.3%	0.3%	0.3%
Phase E			
Vitamin C	-	0.5%	1%

Legend: w/w%: weight by weight proportion; q.s: quantum satis (the amount which is enough)

The formulations (Table I) were prepared as an emulsion, an oil-in-water disperse system. Phase A consisted of glycerin, 95% aminomethyl propanol, carboxy polymethylene, and distilled water. Phase B consisted of polysorbate 80, octyl methoxycinnamate, octocrylene, diethylamino hydroxybenzoyl hexyl benzoate, and bis-ethylhexyloxyphenol methoxyphenyl triazine. Phase C consisted of propylene glycol and aluminum starch octenylsuccinate. Phase D consisted of caprylic/caprylic acid triglycerides, ethoxylate-cetostearyl acid, stearic acid, isoctyl stearate, glyceryl monostearate, and methylisothiazolinone/phenoxyethanol solution. Phase E consisted of ascorbic acid.

In phase A, carboxy polymethylene was dispersed in water under continuous stirring with a mechanical stirrer (Fisaton, 713 D) at 25°C and 1,000 rpm.

Glycerin and 95% aminomethyl propanol were then added to the system under continuous stirring. All the components of phase B were heated to 70ºC ± 2 ºC and homogenized. In phase C, the dry-flo was added gradually to the glycerin under stirring. Phase D was also heated to 60ºC ± 2 ºC with constant agitation at 60 rpm for homogenization. Phase A was added to phase B, phase C was added next, and phase D was the last one added to the system. All phases were homogenized with a mechanical stirrer at 40 rpm until the system reached room temperature (25ºC).

Phase E consists of the incorporation of Vit. C in the emulsion. After the development of the emulsion, Vit. C was incorporated at two concentrations, i.e., 0.5% w/w and 1% w/w, into the final system to complete 100 g of formulation, and its influence on the *in vitro* photoprotective efficacy and photostability of the preparation was determined. Vit. C concentrations were chosen according to Afonso *et al.* (2014), who observed that higher concentrations of antioxidants did not increase the photostabilization effect (Afonso *et al.*, 2014).

Characterization of the emulsions

The macroscopic characteristics of the emulsions such as color, aspect, or instabilities (creaming, coalescence, or separation of phases) were evaluated by visual inspection after 30, 60, 90, and 120 days. Creaming is defined as oil droplets in the layer at the top of the emulsions; coalescence as emulsified droplets joining to form larger droplets that will eventually be separated from the external phase; separation of phases is defined as the complete separation of the aqueous and oily phases of the emulsion (Alves *et al.*, 2020).

The mean droplet diameter and polydispersity index (PDI) of the emulsions were measured with a dynamic light scattering (DLS) instrument (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) at 25ºC, with the laser incidence angle about the sample being 173°, using a 5-ml quartz cuvette. The emulsions were diluted 1:10 in distilled water before the analysis. All values are reported as mean ± standard deviation (SD) of three measurements for each formulation.

The viscosity of the emulsions was analyzed using a rotary digital viscometer (DV-II model, Brookfield®, USA), with spindle number 94 and varying the rotation speed from 0.3 to 6 rpm, at 25ºC. The rheogram was constructed by the correlation of viscosity versus speed.

Assessment of the photoprotective parameters of the formulations

The *in vitro* photoprotective parameters of the formulations, such as SPF, critical wavelength (λc), and UVA/UVB ratio were evaluated with a UV transmittance analyzer (Labsphere® UV-2000S). Fifty mg (2 mg/cm^2) of the formulations were deposited and homogeneously spread on Quartz plates of 25 cm^2 with a rough surface covered with Transpore™ tapes until a uniform layer was obtained. Three quartz plates were used for each sample. Glycerin was used as a reference for 100% transmission. All analyses were performed in triplicate and nine points were measured per plate (Silva *et al.*, 2019; Coutinho *et al.*, 2015).

To determine the stability of the photoprotective parameters, all emulsions were stored for 3 months at 25ºC ± 2, with SPF, UVA/UVB ratio, and λc values being measured at 0, 30, 60, 90, and 120 days.

Photostability Assay

The SPF, UVA/UVB ratio, and λc values of the emulsions were evaluated after exposure to SSL irradiation using a solar simulator (Oriel Model 91192–1000, Newport Corp., USA) according to the following procedure: a 50 mg (2 mg/cm^2) amount of each formulation was homogeneously distributed on quartz plates with a 25 cm^2 area; the plates were then exposed to an SSL simulator and SPF, UVA/UVB ratio and λc were determined at nine points on the plates by Integration Sphere Transmittance Spectrophotometry (Labsphere UV-2000S). Irradiation conditions were as previously described, but with a 2 h exposure, following the recommendation to reapply sunscreen products every two hours (Coutinho *et al.*, 2015; FDA, 2012; Teixeira *et al.*, 2019).

Statistical analysis

Data are reported as mean ± standard deviation. The non-parametric Mann-Whitney test and Kruskal-Wallis test followed by Dunn's multiple comparisons test were used to determine the photoprotective effect on the *S. cerevisiae* formulations using GraphPad Prism software (La Jolla California USA), with the level of significance set at $p < 0.05$. *In vitro* photoprotective efficacy data were analyzed by one-way Analysis of Variance (ANOVA) using GraphPad Prism version 6.00 for Windows, with the level of significance set at $p < 0.05$.

An illustrative scheme of the rational 3-step approach for the development of a photoprotective formulation with antioxidant potential is presented in Figure 1.

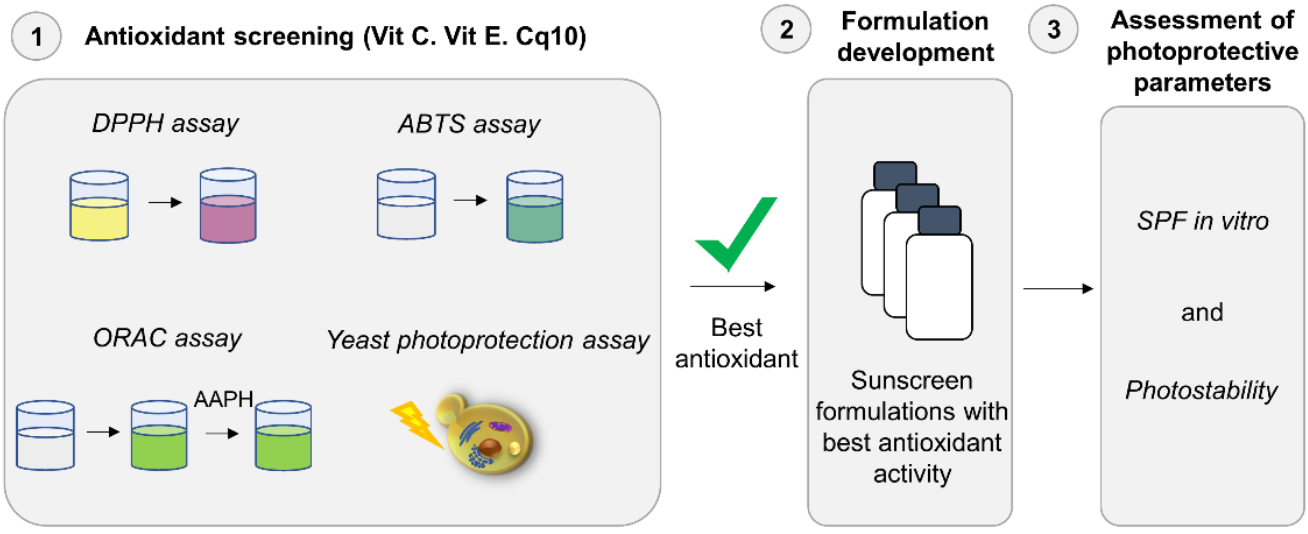

FIGURE 1 - Scheme of the rational 3-step approach for the development of a photoprotective formulation with antioxidant potential. The first step consisted of evaluating the *in vitro* antioxidant and photoprotective performance of the three antioxidants Vit. C (Vitamin C), Vit. E (Vitamin E) and Cq10 (Coenzyme Q10) by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, ABTS (2,2`-azino-bis (ethylbenzthiazoline-6-sulfonic acid)) assay, ORAC (oxygen radical absorbance capacity) assay with azo-radical initiator AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride), and by the yeast photoprotection assay. The second step consisted of the formulation of photoprotective emulsions containing organic UV filters and the most promising antioxidant selected by the previous step. The last step consisted of characterizing and assessing the photoprotective parameters including *in vitro* assessment of SPS and of the photostability of the developed formulations.

RESULTS AND DISCUSSION

Antioxidant Activity

Table II shows the results of the DPPH, ABTS, and ORAC tests for Vit. C, Vit. E, and Cq10.

TABLE II - Antioxidant activities of vitamin C, vitamin E, and coenzyme Q10 obtained in the DPPH, ABTS and ORAC tests

Sample	EC_{50} (µg/ml) - DPPH	µmol Trolox®/g – ABTS	µmol Trolox®/g – ORAC
Vitamin C	3.5 ± 0.1	9,000 ± 103	4,000 ± 54
Vitamin E	742.5 ± 1.7	910 ± 8	550 ± 3
Coenzyme Q10	N.D	N.D	720 ± 10

Legend: EC_{50}: 50% of the antioxidant effect; DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay; ABTS: 2,2'-azino-bis (ethylbenzthiazolin-6-sulfonic acid) cation assay; ORAC: Oxygen radical absorbing capacity assay; N.D: not determined. There was a statistically significant difference in antioxidant activity between vitamin E and vitamin C, vitamin E and Coenzyme Q10, vitamin C and Coenzyme Q10, according to the DPPH method ($p < 0.05$). Also, there was a significant difference in antioxidant activity between vitamin C and vitamin E, vitamin C and Coenzyme Q10, vitamin E and Coenzyme Q10, according to the ABTS method ($p < 0.05$) and between vitamin C and vitamin E, and vitamin C and Coenzyme Q10, according to ORAC method ($p < 0.05$).

The DPPH assay is an easy, fast, simple, and economical method for the assessment of the antioxidant capacity of a DP substance, where the free DP with an absorption band at 515 nm is reduced by an antioxidant agent and loses this absorption band (Ou, Hampsch, Prior, 2001). The DPPH assay showed that Vit. C had an EC_{50} of 3.5 ± 0.1 µg/mL and Vit. E had an EC_{50} of 742.5 ± 1.7. It was not possible to quantify the EC_{50} of Cq10.

Coutinho *et al.* (2015) also used ascorbic acid as a positive control, with an EC_{50} of 5.64 ± 0.09 µg/mL.

The ABTS assay measures the ability of antioxidants to scavenge the ABTS generated in the aqueous phase, as compared to a Trolox (water-soluble Vit. E analog) standard. ABTS is generated by the reaction of strong oxidizing agents (potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of blue green ABTS radical-colored solution by hydrogen-donating antioxidants is measured by the suppression of its characteristic long wave (734 nm) absorption spectra (Shalaby, Shanab, 2013). Vit. C and Vit. E showed 9,000 ± 103 µmol Trolox®/g and 910 ± 8 µmol Trolox®/g, respectively (Table II).

Cq10 did not show antioxidant activity in the DPPH and ABTS tests. This was expected since the formation of the reduced species ubiquinol-10, QH2, in a non-aqueous solution (methanol) requires the presence of a strong reducing agent such as sodium borohydride, and the reduction of Cq10 cannot be achieved by any component of the assay (Cervellati, Greco, 2016).

The ORAC assay measures the antioxidant activity of substances based on their peroxyl radical scavenging capacity since it evaluates the mechanism of hydrogen atom transfer (Rafiq *et al.*, 2012). Trolox was used as a standard, and the ORAC value was expressed as micromoles of Trolox per gram of sample (µmol TE/g). Vit. C, Vit. E, and Cq10 showed 4,000 ± 54 µmol Trolox®/g, 550 ± 3 µmol Trolox®/g, and 720 ± 10 µmol Trolox®/g, respectively (Table II).

Vit. C showed high antioxidant capacity as evaluated by the DPPH, ORAC, and ABTS methods; thus, it is possible to infer that Vit. C is more sensitive to reaction through hydrogen atom transfer (ORAC mechanism) and non-electron transfer (ABTS mechanism).

In vitro photoprotective potential of Vit. C, Vit. E and Cq10 for the CD138 (*ogg1*) strain against SSL irradiation-induced cytotoxicity

The photoprotection potential of Vit. C, Vit. E, and Cq10 against SSL irradiation-induced cytotoxicity was evaluated in the CD138 (*ogg1*) strain. The yeast model was used to assess the indirect photoprotection of antioxidant activity of the compounds in rescuing cells from UV-induced cytotoxicity. Figure 2 displays the cell survival of the CD138 (*ogg1*) strain in the absence (control) and in the presence of Vit. C, Vit. E, or Cq10 (100 μg/ml).

Vit. C was able to protect the CD138 (*ogg1*) strain cells against the highest SSL irradiation exposure (1 h 9' 25", 1 h 44' 9" and 2 h 18' 52") compared to control ($p < 0.05$). Vit. E and Cq10 did not significantly ($p > 0.05$) alter the survival of CD138 (*ogg1*) cells (Figure 2).

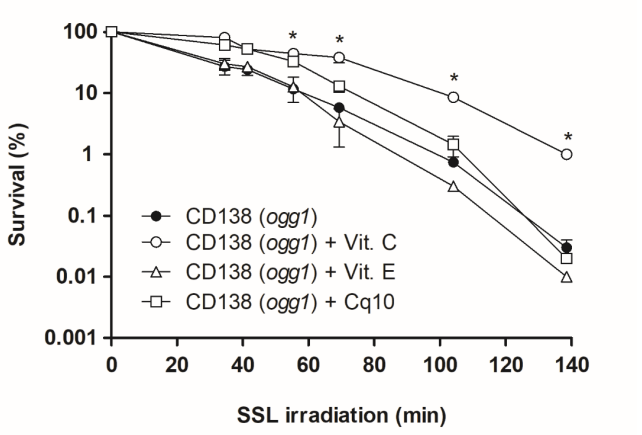

FIGURE 2 - Survival (%) of the CD138 mutant (*ogg1*) strain (●) to different SSL (Solar Simulated Light) irradiation times in minutes (min) in the presence of Vit. C (vitamin C) (○), Vit. E (vitamin E) (Δ) or Cq10 (coenzyme Q10) (□). Data are reported as means ± SD (standard deviation). * Significantly different ($p<0.05$) from control (CD138 strain plus SSL) as determined by the non-parametric Mann-Whitney test and the Kruskal-Wallis test followed by Dunn's multiple comparisons test).

Photoprotective assessment based on yeast assays is a promising tool in photo safety testing since *S. cerevisiae* is an interesting model for *in vitro* photobiological studies. Besides being a eukaryotic microorganism with repair genes orthologous and homologous to those of human cells, yeast strains are sufficiently resistant to support all UVA and UVB ranges, allowing irradiation protocols with UV doses and exposure times comparable to environmental sunlight (Paiva *et al.*, 2020). Previous studies have reported the use of the CD138 (*ogg1*) strain for the efficacy and safety assessment of UV filters. Notably, the inactivation of Ogg1 repair makes the CD138 (*ogg1*) strain a promising bioindicator model of oxidative events, allowing evaluation of the contribution of the antioxidant potential of compounds to photoprotection enhancement (Silva *et al.*, 2019; Diniz *et al.*, 2019).

In the present study, the CD138 (*ogg1*) strain model indicated that the high antioxidant activity of Vit. C evaluated by the DPPH, ORAC, and ABTS methods may be decisive to grant photoprotection against SSL irradiation-induced cytotoxicity by an indirect mechanism. In contrast, the level of antioxidant activity of Vit. E and Cq10 did not seem to have been sufficient to rescue cell survival from lethal oxidative cytotoxic lesions. In this screening phase, this result, taken together with those from the previously described techniques, highlighted that, Vit. C showed the best performance in terms of antioxidant activity.

Characterization of the emulsions

Vit. C was the best antioxidant, also showing a photoprotective potential, and therefore it was used to develop the emulsions. However, this substance is very unstable in an aqueous solution in the presence of oxygen and metal ions (Caritá *et al.*, 2020; Khalid *et al.*, 2013). Therefore, it was used in emulsions containing humectants such as glycerin and propylene glycol in order to enhance its photostability, as recommended by Ahmad *et al.* (2011).

Moreover, higher viscosity formulations offer greater protection against the oxidation of Vit. C (10). This study used carboxy polymethylene as a rheology modifier, aluminum starch octenyl succinate as a thickening agent, and caprylic/caprylic acid triglyceride, which is an emollient and can also function as a thickener in emulsions (Rowe, Sheskey, Quinn, 2009).

All the emulsions were homogenous and white systems with the same characteristics, which were maintained after 120 days, without instability.

The droplet size and PDI of the emulsions were also characterized. The droplet size of the emulsion blank, emulsion with 0.5% w/w of Vit. C, and emulsion with 1 % w/w of Vit. C had an average size of 840 ± 17 nm, 788 ± 21 nm, and 769 ± 16 nm, respectively. According to the mean diameter of the droplets, all the emulsions can be classified as macroemulsions since the size of the droplets was more than 400 nm (Callender *et al*., 2017).

There was a significant difference in droplet size between the emulsion blank and the emulsion with 0.5% w/w of Vit. C and between the emulsion blank and the emulsion with 1 % w/w of Vit. C ($p < 0.05$), whereas there was no significant difference between the emulsion with 0.5% w/w of Vit. C and the emulsion with 1% w/w of Vit. C. Thus, emulsions with Vit. C had a lower droplet size that was not influenced by an increase in this vitamin.

The PDI values of the emulsion blank, the emulsion with 0.5% w/w of Vit. C and the emulsion with 1 % w/w of Vit. C were 0.4 ± 0.05, 0.42 ± 0.08, and 0.40 ± 0.09, respectively, with no significant difference among them ($p > 0.05$). PDI is a measure that evaluates the droplet size distribution, ranging from 0 to 1. All emulsions showed values around 0.4, indicating a polydispersion distribution over time (Ardani *et al*., 2017) which could be attributed to the production method that employed mechanical homogenization.

Viscosity analysis

The apparent viscosity values obtained for all emulsions are shown in Figure 3. All formulations exhibited a non-newtonian, pseudo-plastic behavior since viscosity decreases as velocity or shear rate increases (Saez *et al*., 2019).

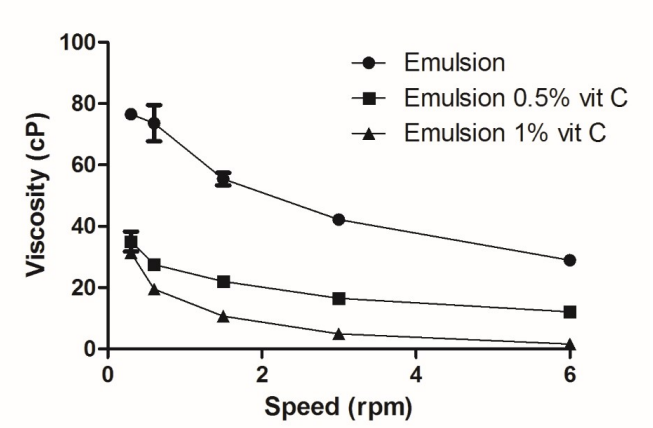

FIGURE 3 - Rheograms of the emulsion blank, emulsion with 0.5% w/w of Vit. C (vitamin C), and emulsion with 1% w/w of Vit. C (vitamin C). Data are reported as means ± SD (standard deviation). One-way Analysis of Variance (ANOVA) was used to demonstrate statistical differences ($p < 0.05$).

Lower viscosity values were observed in all ranges for the emulsions with Vit. C compared to the emulsion blank ($p < 0.05$). This can be explained by the fact that carboxy polymethylene, which is the gelling agent of the aqueous phase, needs to have the charges of its surface neutralized in order to acquire greater viscosity. With the incorporation of Vit. C, which is an acid, there was a reversal of the neutralization process and a reduction in the system's viscosity (Rowe, Sheskey, Quinn, 2009).

The lower viscosity of emulsions with Vit. C may have facilitated the dispersal of the oil phase droplets which caused these systems to have smaller droplet sizes. Viscosity is one of the key fluid properties used to investigate the droplet size of any emulsion since the gradual increase of viscosity causes a slow increase in the average droplet diameter of the emulsion (Khalid *et al*., 2013).

Formulation efficacy: photoprotective parameters

The *in vitro* photoprotection tests have been developed because they are rapid, less expensive, and do not require the involvement of human volunteers. These methods are based on the measurement of the spectral transmittance of the sunscreen substance incorporated into a formulation and applied to a synthetic support

simulating the human skin (COLIPA, 2007) or on the spectrophotometric analysis of dilute solutions of sunscreen products (Dutra *et al*., 2004).

The following photoprotective parameters were evaluated: SPF, which is the indicator of the photoprotective performance of sunscreens against UVB radiation and consequently indicates the ability of a sunscreen to reduce UV-induced erythema; the UVA/UVB ratio which measures the level of protection offered by the sunscreen in the UVA range and can be classified by *Boot`s Star Rating System,* and λc which is the wavelength at which the area under the integrated optical density curve starting at 290 nm is equal to 90% of the integrated area between 290 and 400 nm (FDA, 2012).

The *in vitro* SPF values, UVA/UVB ratio, and λc were obtained for the emulsion blank, the emulsion with 0.5% Vit. C, and the emulsion with 1% of Vit. C, at times 0, 30, 60, 90, and 120 days, and are shown in Table III.

TABLE III - Sun protection factor (SPF), UVA/UVB ratio, and critical wavelength (λc) of the emulsion blank, the emulsion with 0.5 % vitamin C, and the emulsion with 1% vitamin C

	Emulsion Blank	**Emulsion with 0.5% vitamin C**	**Emulsion with 1% vitamin C**
		Time zero	
SPF	31 ± 1.5	30 ± 1.6	31 ± 3.6
UVA/UVB ratio	0.57 ± 0.03	0.84 ± 0.02	0.7 ±0.17
Critical wavelength (λ)	370 ± 0.57	376 ± 0.47	373 ± 3.78
		30 days	
SPF	32 ± 2.08	32 ± 1.24	33 ± 1.5
UVA/UVB ratio	0.57 ± 0.03	0.53 ± 0.03	0.53 ± 0.03
Critical wavelength (λ)	370 ± 0.57	370 ± 1.24	370 ± 1
		60 days	
SPF	32 ± 1.52	31 ± 1.4	32 ± 2.9
UVA/UVB ratio	0.58 ± 0.05	0.55 ± 0.02	0.55 ± 0.01
Critical wavelength (λ)	371 ± 0.01	371 ± 1.24	370 ± 0.6
		90 days	
SPF	33 ± 2.51	31 ± 1.24	32 ± 1.3
UVA/UVB ratio	0.560 ± 0.04	0.59 ± 0.04	0.58 ± 0.03
Critical wavelength (λ)	370 ± 1.15	372 ± 2.1	370 ± 1.2
		120 days	
SPF	32 ± 3,4	31 ± 4.1	32 ± 2.1
UVA/UVB ratio	0,596 ± 0.02	0.569 ± 0.03	0.571± 0.02
Critical wavelength (λ)	376 ± 0.6	374 ±1.7	371 ± 0.8

Legend: SPF: sun protection fator. The SPF values of all formulations at time zero and after 30, 60, 90, and 120 days did not show statistically significant differences ($p < 0.05$). Also, the UVA/UVB ratio and critical wavelength (λc) did not show statistically significant differences ($p < 0.05$) for any formulation at times 0, 30, 60, 90, and 120 days. Statistical analyses were performed using One-way Analysis of Variance (ANOVA), with the level of significance set at $p < 0.05$.

The SPF values of all formulations did not show a statistically significant difference ($p < 0.05$) at time zero and after 30, 60, 90, and 120 days. Also, the UVA/UVB ratio and λc did not show a statistically significant difference ($p < 0.05$) for any formulation at times 0, 30, 60, 90, and 120 days. The SPF of all formulations was around 30 and, according to the FDA (2012), the formulations offered high protection against UVB radiation.

The Boots Star Rating System classifies a product according to the UVA/UVB ratio. The higher this ratio, the better the product protects against UVA radiation. All emulsions showed a UVA/UVB ratio higher than 0.5 according to the Boots Star Rating System, indicating good UVA photoprotection (COLIPA, 2007).

According to COLIPA (2007) and FDA (2012), a photoprotective product with a λc of 370 nm or higher is considered a broad-spectrum product that protects against longer wavelengths of radiation, above 370 nm. All formulations showed a λc of 371 nm and thus can be classified as broad-spectrum products protecting against longer radiation, above 370 nm.

Indeed, OMC and OCT filters absorb mostly in the UVB and UVA II wavelength range, while the DHHB and BEMT filters are broad spectrum and UVA I filters according to the absorption profile obtained with a BASF sunscreen simulator (Továr-Sanchez *et al.,* 2020). Therefore a combination of these filters provides ample protection against UVA and UVB radiation.

None of the formulations developed here showed changes in photoprotective parameters (SPF values, UVA/UVB ratio, and λc) at any time point evaluated ($p < 0.05$). Consequently, the formulations maintained their photoprotective action and remained photostable in the absence of radiation.

This result agrees with data reported by Coutinho *et al.* (2015), who developed an oil-in-water photoprotective and antioxidant nanoemulsion (NE) and observed that the mixture of sunscreens remained photostable in the absence of radiation.

Vit. C did not influence the photoprotective parameters of the formulations during a period of 120 days without radiation, with higher absorption values in the UVC region and lower absorption values in the UVB and UVA regions (Burke, 2011; Galanakis, Tsatalas, Galanakis, 2018). This explains why Vit. C was found to have no effect on the photoprotective parameters when a UV transmittance analyzer was used, since this method correlates the sample absorption in the UVA and UVB region with its photoprotection efficiency.

Photostability assay

The photostability of sunscreen formulations measures the stability of UV filters against solar radiation and is an important factor that should be considered during the development of sunscreen formulations. The present formulations were irradiated for 2 hours, which is the period recommended for the reapplication of a sunscreen (Teixeira *et al.*, 2019).

After irradiation, the emulsion blank, the emulsion with 0.5 % Vit. C, and the emulsion with 1% Vit. C showed SPF, UVA/UVB ratio, and λc of 22 ± 1.2; 24 ± 1.8; 26.7 ± 2.0, 0.6 ± 0.004, 0.58 ± 0.03, 0.58 ± 0.02, 370 ± 0.58, 369 ± 0, and 370 ± 0.51, respectively.

There was a statistically significant difference in SPF values between the emulsion blank and the emulsion with 1% Vit. C ($p < 0.05$). Based on these results, it can be suggested that Vit. C protects OMC, which is the only photolabile UV absorber in the composition. On the other hand, as expected, there were no changes in UVA parameters, since the sunscreen formulations contained only photostable UVA filters.

This result agrees with data reported by Damiani *et al.* (2006), who found that the addition of antioxidants increased sunscreen stability. Moreover, sunscreen photodegradation can generate reactive oxygen species (ROS) that can damage skin structures, and an antioxidant such as Vit. C added to the formulation could act by capturing these reactive species (Caritá *et al.*, 2020).

Vit. C is used in the pharmaceutical and cosmetic industries due to its bioactive properties such as acting on collagen biosynthesis, and to its antioxidant action, scavenging free radicals (Amina *et al.*, 2016). Therefore, it can be used before sun exposure, minimizing the damage induced by radiation and neutralizing the ROS generated by sun exposure and environmental

factors such as smoke and pollution (Caritá *et al*., 2020). Furthermore, Afonso *et al.* (2014) showed that Vit. C increased the photostability of avobenzone and reduced UV-induced skin damage.

Skin damage and skin aging are biological processes which are significantly amplified by exposome factors including chronic UV exposure, pollution, and their association (photo pollution), mediated by ROS generation. In this scenario, the incorporation of antioxidant agents into cosmetic formulations such as sunscreens seems particularly interesting. In fact, several studies have pointed out the benefits of associating antioxidants with photoprotective formulations, protecting the skin against the formation of MMP-1, with a reduction of lipid peroxidation by-products, a reduction of the pro-inflammatory effect of nuclear factor kappa ß, and the preservation of collagen, among other parameters related to the preservation of skin health (Grether-Beck *et al*., 2014; Valacchi *et al*., 2016; Ferrara *et al*., 2020; Neves *et al*., 2022). In this context, the present study contributed to the development of new effective photoprotective formulations containing antioxidants, capable of reducing skin damage caused by exposomes.

The proposed screening approach highlighted that Vit. C had a photoprotective potential against SSL irradiation during exposure times of more than 1 h and a high antioxidant potential, also increasing sunscreen photostability. It can be used to protect the skin from harmful UV radiation, preventing the damage caused by the free radicals generated by solar irradiation and photo pollution.

CONCLUSION

Vit. C showed high antioxidant capacity as evaluated by the DPPH, ORAC, and ABTS methods; thus, it is possible to infer that it is more sensitive to reaction through hydrogen atom transfer (ORAC mechanism), and non-electron transfer (ABTS mechanism). Its photoprotective potential associated with antioxidant activity was also investigated by an *in vitro* approach using a *S. cerevisiae* strain model. The yeast test was useful as a screening tool for selecting the best compound to be part of a sunscreen formulation in the next step since the model indicated that the high antioxidant potential of Vit. C assessed by DPPH, ORAC, and ABTS seemed to contribute to the increase in photoprotection of the strain tested. Consequently, Vit. C was chosen to be incorporated into a photoprotective formulation and evaluated as an *in vitro* sun protection factor. The SPF values, UVA/UVB ratio, and λc of the emulsion blank, with 0.5 % w/w and 1% w/w of Vit. C at time zero and after 30, 60, 90 and 120 days did not show a statistically significant difference ($p < 0.05$), with the preparations maintaining their photoprotective action. However, Vit. C increased sunscreen photostability compared to the emulsion blank after irradiation. Consequently, Vit. C can be considered a good antioxidant for sunscreen formulations to be used before and during sun exposure and the proposed screening approach was suitable for the development of new and improved sunscreen formulations.

REFERENCES

Addor FAS. Antioxidants in dermatology. An Bras Dermatol. 2017;92(3):356-362.

Afonso S, Horita K, Sousa e Silva JP, Almeida IF, Amaral MH, Lobão PA, et al. Photodegradation of avobenzone: Stabilization effect of antioxidants. J Photochem Photobiol B. 2014;140:36-40.

Ahmad I, Sheraz MA, Ahmed S, Shaikh RH, Vaid FH, Ansari SA. Photostability and interaction of ascorbic acid in cream formulations. AAPS Pharm Sci Tech. 2011;12(3):917-23.

Al-Niaimi F, Chiang N, Yi Z. Topical Vitamin C and the Skin: Mechanisms of Action and Clinical Applications. J Clin Aesthet Dermatol. 2017;10(7):14–17.

Alves PE, Gomes ACC, Gomes AKC, Nigro F, Kuster RM, Freitas ZMF, et al. Development and Characterization of Phytocosmetic Formulations with Saccharum officinarum. Revista Brasileira de Farmacognosia. 2020;30:406–415.

Amina RM, Elfeky SA, Verwanger T, Krammer B. A new biocompatible nanocomposite as a promising constituent of sunscreens. Mater Sci Eng C Mater Biol Appl. 2016;63:46-51.

Ardani HK, Imawan C, Handayani W, Djuhana D, Harmoko A, Fauzia V. Enhancement of the stability of silver nanoparticles synthesized using aqueous extract of Diospyros discolor Willd. Leaves using polyvinyl alcohol. IOP Conference Series: Materials Science and Enginnering. 2017;188:1-5.

Arias G. Estudio de la composición química y la actividad antioxidante de aceites esenciales y extractos aislados de *Salvia officinalis*, *Rosmarinus officinalis*, *Thymus vulgaris* y *Lippia citriodora*, cultivadas en el municipio Sucre (Santander). Tesis de Maestría. Escuela de Química, Universidad Industrial de Santander. 2012.

Barcelos IP, Haas RH. CoQ10 and Aging. Biology. 2019;8(2):28-50.

Burke KE. Photoprotection of the Skin with Vitamins C and E: Antioxidants and Synergies. Nutrition and Skin. 2011;43-58.

Callender SP, Mathews JA, Kobernyka K, Wettig SD. Microemulsion utility in pharmaceuticals: Implications for multi-drug delivery. Int J Pharm. 2017;526(1-2):425-442.

Caritá AC, Santos BF, Shultz JD, Michniak-Kohn B, Chorilli M, Leonardi GR. Vitamin C: One compound, several uses. Advances for delivery, efficiency, and stability. Nanomedicine. 2020;24(102117):1-15.

Cervellati R, Greco E. *In vitro* Antioxidant activity of Ubiquinone, compared to vitamin E. Helvetica Chimica Acta. 2016;99:41-45.

Chen AC, Damian DL, Halliday GM. Oral and systemic photoprotection. Photodermatol Photoimmunol Photomed. 2014;30(2-3):102-11.

Comité de Liaison des Industries de La Parfumerie. COLIPA. Method for the *in vitro* determination of UVA protection provided by sunscreen products. 2007.

Coutinho CC, Santos-Oliveira R, Santos EP, Mansur CR. Development of a photoprotective and antioxidant nanoemulsion containing chitosan as an agent for improving skin retention. Eng Life Sci. 2015;15:593-604.

Damiani E, Astolfi P, Cionna L, Ippoliti F, Greci L. Synthesis, and application of a novel sunscreen-antioxidant. Free Radic Res. 2006;40(5):485-94.

Diniz, RR, Paiva JP, Aquino RM, Gonçalves TCW, Leitão AC, Santos BAMC, et al. Saccharomyces cerevisiae strains as bioindicators for titanium dioxide sunscreen photoprotective and photomutagenic assessment. J Photochem Photobiol B. 2019;198:11158-11179.

Dutra EA, Oliveira DAGC, Kedorhackmann ERM, Santoro MIRM. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Braz J Pharm Sci. 2004;40(3):381-38.

Ferrara F, Woodby B, Pecorelli A, Schiavone ML, Pambianchi E, Messano N, et al. Additive effect of combined pollutants to UV induced skin OxInflammation damage. Evaluating the protective topical application of a cosmeceutical mixture formulation. Redox Biol. 2020;34:101481.

Food and drug administration. FDA. Guidance for industry Labeling and effectiveness Testing: Sunscreen drug products for over-the-counter human use-small Entity Compliance Guide. 2012.

Galanakis CM, Tsatalas P, Galanakis JM. Phenols from olive mill wastewater and other natural antioxidants as UV filters in sunscreens. Environ Technol Innov. 2018;9:160-168.

Garcia EJ, Oldoni TLC, Alencar SM, Reis A, Loguercio AD, Grande RHM. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Braz Den J. 2012;23(1):22-27.

Grether-Beck S, Marini A, Jaenicke T, Krutmann J. Effective Photoprotection of Human Skin against Infrared A Radiation by Topically Applied Antioxidants: Results from a Vehicle Controlled, Double-Blind, Randomized Study. Photochem and Photobiol. 2014;91(1):248-50.

He H, Li A, Li S, Tang J, Li L, Xiong L. Natural components in sunscreens: Topical formulations with sun protection factor (SPF). Biomed Pharmacother. 2021;134:111161.

Huang D, Ou B, Prior R. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005;53:1841-1856.

Jesus A, Mota S, Torres A, Cruz M T, Sousa E, Almeida I F, Cidade H. Antioxidants in Sunscreens: Which and What For? Antioxidants. 2023;12(138):4-22.

Keen MA, Hassan I. Vitamin E in dermatology. Indian Dermatology Online J. 2016;7(4):311-315

Khalid N, Kobayashi I, Neves MA, Uemura K, Nakajima M. Preparation, and characterization of water-in-oil emulsions loaded with high concentration of L-ascorbic acid. Food Sci Tech. 2013;51(2):448-454.

Knott A, Achterberg V, Smuda C, Mielke H, Sperling G, Dunckelmann K, et al. Topical treatment with coenzyme Q10-containing formulas improves skin's Q10 level and provides antioxidative effects. Biofactors. 2015;41(6):383–390.

Krutmann J, Bouloc A, Sore G, Bernard BA, Passeron T. The skin aging exposome. J Dermatol Sci. 2017;85(3):152-161.

Marrot L. Pollution and Sun Exposure: A Deleterious Synergy. Mechanisms and Opportunities for Skin Protection. Curr Med Chem. 2019;25(40):5469-5486.

Neves JR, Grether-Beck S, Krutmann J, Correia P, Gonçalves Jr JE, Sant'Anna B, et al. Efficacy of a topical serum containing L-ascorbic acid, neohesperidin, pycnogenol, tocopherol, and hyaluronic acid in relation to skin aging signs. J Cosmet Dermatol. 2022;21(10):4462-4469.

Ou B, Hampsch M, Prior R. Development, and validation of an improved Oxygen Radical Absorbance Capacity assay

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

using fluorescein as the fluorescent probe. J Agric Food Chem. 2001;49:4619-4626.

Paiva JP, Diniz RR, Leitão AC, Cabral LM, Fortunato RS, Santos BAMC, et al. Insights and controversies on sunscreen safety. Crit Rev Toxicol. 2020;50(8):707-723.

Rafiq M, Azeemuddin M, Anturlikar SD, Viswanatha GL, Patkiet PS. Application of oxygen radical absorbance capacity (ORAC) assay in the estimation of antioxidant value of botanicals. Oxid Antioxid Med Sci. 2012;1(2):87-90.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice C. Antioxidant activity applying as improved ABTS radical cation decolorization assay, Free Radic Biol Med. 1999;26(9-10):1231-7.

Rowe R, Sheskey PJ, Quinn ME. Handbook of pharmaceutical excipients. 6th Edition. Pharmaceutical Press. 889 p, 2009.

Ruvolo E, Boothby-Shoemaker W, Kumar N, Hamzavi IH, Lim HW, Kohli I. Evaluation of efficacy of antioxidant-enriched sunscreen prodcuts against long wavelength ultraviolet A1 and visible light. Int J Cosmet Sci. 2022;44(3):394-402.

Saez V, Menezes FD, Santos CC, Alencar LMR, Ricci-Júnior E, Mansur CRE, et al. Graphene quantum dots nanoparticles changed the rheological properties of hydrophilic gels (carbopol). J Mol Liq. 2019;287:110949-110967.

Silva ACP, Paiva JP, Diniz RR, Dos Anjos VM, Silva ABSM, Pinto AV, et al. Photoprotection assessment of olive (Olea europaea L.) leaves extract standardized to oleuropein: *in vitro* and *in silico* approach for improved sunscreens. J Photochem Photobiol B. 2019;193:162-171.

Teixeira J, Freitas ZMF, Monteiro MSSB, Vermelho AB, Ricci Júnior E, Santos EP. Development and characterization of photoprotective formulations containing keratin particles. Braz J Pharm Sci. 2019;55:1-14.

Thiele JJ, Hsieh SN, Ekanayake-Mudiyanselage S. Vitamin E: critical review of its current use in cosmetic and clinical dermatology. Dermatol Surg. 2005;31:805-813.

Továr-Sanchez A, Sparaventi E, Gaudron A, Rodríguez-Romero A. A new approach for the determination of sunscreen levels in seawater by ultraviolet absorption spectrophotometry. Plos One. 2020;16: 1-10.

Valacchi G, Muresan XM, Sticozzi C, Belmonte G, Pecorelli A, Cervellati F, Demaude J, Krol Y, Oresajo C. Ozone-induced damage in 3D-Skin Model is prevented by topical vitamin C and vitamin E compound mixtures application. J Dermatol Sci. 2016;82(3):209-212.

Valverde TM, Soares BNGS, Nascimento AM, Andrade AL, Sousa LRD, Vieira PMA, et al. Anti-Inflammatory, Antimicrobial, Antioxidant and Photoprotective Investigation of Red Propolis Extract as Sunscreen Formulation in Polawax Cream. Int J Mol Sci. 2023;24(6):5112.

Received for publication on 05th April 2023
Accepted for publication on 20th July 2023