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In vitro antioxidant activity and *in vivo* photoprotective effect of *Theobroma grandiflorum* butter emulgels on skin of mice exposed to UVB irradiation

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Introduction: Copoazú seed butter [*Theobroma grandiflorum* (Wild ex Spreng) K. Schum] is a rich source of fatty acids and polyphenols, active substances with high antioxidant and moisturizing activities with potential applications in the cosmetic industry. Some studies have demonstrated that sun-induced skin damage is partially mediated by oxidative pathways; in fact, there is evidence for the photoprotective roles of antioxidants.

Methods: We have developed a stable emulgel-type cosmetic formulation using Copoazú seed butter at different concentrations of 5, 10, and 20% and examined the antioxidant activity and the effect of Copoazú seed butter emulgels against UV-induced epidermal damage in mice to verify its use for topical photoprotective products.

Results and discussion: The antioxidant activity expressed as EC_{50} values varied from 8.47 ± 0.013 mg/ml to 4.53 ± 0.046 mg/ml for Copoazú seed butter emulgels. *In vitro* sun protection factor (SPF) assessment showed that Copoazú seed butter emulgel at 20% has the highest SPF of 11.67 ± 0.001, which is acceptable for the sunscreen products development, and these results were corroborated by the *in vivo* results since the mice were irradiated with UV light and treated with Copoazú seed emulgels and showed minor damage or significantly reduced the severity of the damage and were comparable with the standard photo-protector. The results showed that Copoazú seed butter is a promising compound for photoprotective formulations.

KEYWORDS

skin care ingredients, fatty acids, emollient, photoprotective effect, antioxidant, gelling agent

Introduction

Native and exotic fruit trees grow in the Amazon region, one of the most common is Copoazú [*Theobroma grandiflorum* (Wild ex Spreng) K. Schum], a typical tree that grows abundantly in the Peruvian rainforest, especially in Puerto Maldonado. The pulp is used for the preparation of food products such as juices, ice creams, liquors, and jellies, and the husk of the pod can be converted into handmade packaging. The seed receptacle is used in the production of fertilizers, whereas the seeds are employed to produce the cupulate, which is a product such as chocolate (Nazaré et al., 1990; Gondim et al., 2001; Ramos, 2015).

The Copoazú seeds are a rich source of fatty acids and polyphenols, both are active substances with high antioxidants (Yang et al., 2003) and nutritional value. Fat from seeds of this species is being investigated because of its increasing popularity as a new fruit crop (Faria et al., 2013), and the Peruvian Copoazú seed could be used as an alternative source of special fats. The percentage composition of fatty acids in the fat of Copoazú seed is 53.3, 40, and 3.4% for saturated, monounsaturated, and polyunsaturated fatty acids, respectively. Among the fatty acids predominating Copoazú seeds' fat are oleic (40%), stearic (32.7%), arachidic (15.1%), and palmitic acids (8.0%) (Itriago Yanesa et al., 2017). However, the by-product of its seeds usually is rejected by the cosmetic and food industries, but some studies showed that it has a high content of phenolic compounds with antioxidant activity (Silva da Costa et al., 2020).

Repeated exposure to solar ultraviolet (UV) radiation leads to several hazardous effects on human health by causing the generation of reactive oxygen species that exert harmful impacts through the biological oxidation of essential molecules and inducing oxidative injuries (Katiyar and Elmets, 2001). UVA and UVB rays can penetrate epidermal tissue and cause the generation of reactive oxygen species (ROS), activation of signal transduction pathways, DNA/protein/lipid damage, and disruption of skin defense systems (Fisher et al., 2002; Debacq-Chainiaux et al., 2012).

Excess free radicals cause many events, e.g., mitogen-activated protein kinase cascade and matrix metalloproteinase induction in the skin, leading to progressive cell structure and function deterioration. The skin has a very sophisticated antioxidant mechanism to cope with oxidative stress induced by UV light; however, chronic exposure to UV radiation may overcome the cutaneous antioxidant capacity and cause deleterious effects on the skin (Katiyar and Elmets, 2001). The topical application of sunscreen products has been the primary strategy employed to prevent sun damage since the 1930s (Ruszkiewicz et al., 2017).

Nowadays, people must use photo-protectors, and the formulation of these products has been changing and adapting to the needs of the population; although synthetic sunscreen chemicals have been used as a preventive/therapeutic strategy in the development of sunscreen products, with the observation of their adverse side effects (Napagoda et al., 2016), the concern about their use has increased. Numerous studies have shown the relationship between exposure to substances commonly found in sunscreens and developmental and endocrine disruptions (Ruszkiewicz et al., 2017). The growing tendency is the search for alternative human-friendly formulations with plant-based components (Napagoda et al., 2016), so there are some products developed using natural products such as extracts and butter that in addition to fulfilling the role of barrier can also act as antioxidants and moisturizer agents. Latin American countries have enormous plant biodiversity which is a great opportunity for the sustainable and innovative development of cosmetic ingredients and products (Bravo et al., 2020). The most well-known way to avoid the penetration of UV radiation through the skin is the use of topical sunscreen products containing active molecules that act as absorbers, reflectors, or scatterers of UV rays.

Currently, there is a growing demand for photo-protectors, due to the need to protect the skin from the harmful rays of the sun, and there are photo-protectors on the market that contain both synthetic and natural agents. However, many synthetic photo-protectors, such as oxybenzone, octinoxate, and homosalate, have serious adverse reactions such as contact allergies, reproductive toxicity, and endocrine disruption, and can cause skin irritation and even skin cancer (Shusterove and Romero, 2020). Sunscreen products that are vegetal component based are less toxic and irritant (Jangde and Daharwal, 2011), and therefore, the recent trend in the use of plant-based products is increasing (Rabinovich and Kazlouskaya, 2018). The herbal phytoconstituent absorbent of harmful rays has antioxidant properties (Korać and Khambholja, 2011). There are many secondary metabolites capable of acting as photo-protectors, including flavonoids (Nunes et al., 2018), but it is also known that some vegetable butter can have photoprotective effects, such as shea butter, which is widely employed in cosmetic formulation, is extracted from the shea nut, and is known to have an SPF value of 6–10 (Goswami et al., 2013). Copoazú seed butter, which originated in the pre-Columbian civilizations, contains a balanced composition of fatty acids with triglycerides and phytosterols (stigmasterol, β-sitosterol, and campesterol) and has an elevated water absorption capacity. It can be employed in the formulation of lip products, bath oils, creams, aftershave lotions, deodorants, and sunscreen products (Poljšak et al., 2020; Heusèle et al., 2022). This study determined the SPF and antioxidant values of an emulgel of Copoazú seed butter to increase the production values of this Amazonian butter, and it was possible to demonstrate its photoprotective effect on the skin of mice exposed to UVB irradiation.

Abbreviations: UV, Ultraviolet; EC_{50}, concentration which scavenges 50% radical; SPF, Sun Protection Factor; UVA, Ultraviolet A; UVB, Ultraviolet B; ROS, Reactive Oxygen Species; DNA, Deoxyribonucleic Acid; TEA, Triethanolamine; pH, Potential Hydrogen; rpm: revolutions per minute; nm, nanometer; DPPH: 2,2-diphenyl-1-picrylhydrazyl; H&E, Hematoxylin and Eosin; SD, Standard Deviation; SPSS, Statistical Package for the Social Sciences; FDA, Food and Drug Administration; o/w, oil-in-water; w/o, water-in-oil; PPARs, Peroxisome Proliferator-activated Receptors.

Materials and methods

Acquisition of Copoazú seed butter

The Copoazú seeds butter was obtained from the company Candela which obtains the Copoazú seeds from the region of Puerto Maldonado and then roasted and pressed them to obtain high-quality butter.

Preparation of emulgel formulations

The gel phase was prepared by dispersing Carbopol 940 in purified water with constant stirring, the pH was adjusted to 6.5–7.0 using triethanolamine (TEA), and a mechanical shaker was used. The oil phase of the emulsion was prepared by melting sodium cetearyl sulfate and *Theobroma grandiflorum* seed butter at different percentages (5, 10, and 20%), and the aqueous phase was prepared by dissolving polysorbate 80 and dehydroacetic acid benzyl alcohol in purified water. Both the aqueous and oil phases were heated at 70°C for about 1 min, and then, the aqueous phase was added to the oil phase with constant agitation until it cooled to room temperature. The gel was mixed with the emulsion in a 1:1 ratio with gentle agitation until the emulgel was obtained (Mohamed, 2004).

Physical examination

The physical characteristics such as color (visual observation), homogeneity, consistency, spreadability, and phase separation were evaluated in the final formulation.

pH measurements

The pH was measured at room temperature (18–20°C) using a pH meter (Advanced Jenway® pH meter 3510) by direct insertion of the electrode into the formulations placed in a beaker (Esprendor et al., 2019). All measurements were done for 12 weeks in duplicate.

Centrifugation

The emulgel samples were subjected to centrifugation using the centrifuge (EBA 200 Hettich) at 3,750 rpm for 5 h (Garg et al., 2002).

Evaluation of UV filtering potential

The UV absorption of each emulgel sample (5, 10, and 20%) diluted with ethanol (0.2 mg/ml) was measured using a UV–visible spectrophotometer (Evolution™ 300) in a range between 290 and 320 nm, and the sun protection factor (SPF) was determined using Mansur's equation (Mansur et al., 1986).

$$SPF\ spectrophotometric = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where EE(λ) is the erythemal effect spectrum, I(λ) solar intensity spectrum, Abs(λ) absorbance of sunscreen product, and CF correction factor (= 10).

A commercial sunscreen product (butyl methoxydibenzoylmethane, octocrylene, ethylhexyl methoxycinnamate, ethylhexyl triazone, and titanium dioxide as active ingredients and with SFP 50) was used as a reference substance. The analysis was developed in triplicate.

Radical scavenging activity DPPH assay

The radical scavenging capacity of Copoazú seed butter emulgels was evaluated by measuring the reduction of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Mariotti and Frasson (2011). The samples were diluted to concentrations of 20, 10, 5, 2.5, 2.5, and 1.25 mg/ml in methanol. To 1 ml of 0.3 mM DPPH solution in methanol, 2.5 ml of each dilution was added. They were incubated for 30 min, and then, readings were taken at 517 nm using a spectrophotometer. A solution of 1 ml of 0.3 mM DPPH in 2.5 ml methanol was used as a negative control, and emulsions containing vitamin E at the same concentrations were used as standard (positive control). Methanol was used to zero the spectrophotometer, and solutions of each sample without the addition of DPPH were used as blank. The analysis was developed in triplicate, and the antioxidant activity was determined using the equation:

$$\%\ inhibition = \frac{100\ -\ [(Abs\ sample\ -\ Abs\ blank)\ \times\ 100]}{Abs\ control}$$

Where *Abs sample* is the absorbance of the sample formulations, *Abs blank* is the absorbance of the blank formulations without the addition of DPPH control, and *Abs control* is the absorbance of DPPH solution in methanol.

A graph of the percentage inhibition vs. extract concentration was plotted. The calculation of the effective concentration which scavenges 50% radical (EC_{50}) was performed using a linear equation.

Animals and UVB irradiation

Twenty-five Balb/c male mice (8 weeks old) from the National Center for Biological Products, Health National Institute, Lima, Perú, were used. The animals were acclimatized for 7 days under the following controlled conditions: humidity (50 ± 5%), temperature (20 ± 2 °C), and illumination (12-h light/dark cycles), and were given food and water *ad libitum*. The dorsal skin area (2.5 × 3 cm^2) of all mice was shaved using a razor (Zhan et al., 2016) 24 h prior to UVB radiation. The animals were anesthetized before the topical treatment using an i.p. injection of ketamine-xylazineacepromazine (75–15–2 mg/kg, respectively) (Romero-Fernandez et al., 2016). The following five groups containing five animals were used as follows: Group 1: mice exposed to UVB light without any treatment (negative control group), Group 2: mice exposed to UVB light and treated with a commercial sunscreen product (positive control), Group 3: mice exposed to UVB light and treated with Copoazú seed butter emulgel at 5%, Group 4: mice exposed to UVB light and treated with Copoazú seed butter emulgel at 10%, and Group 5: mice exposed to UVB light and treated with Copoazú seed butter emulgel at 20%.

Before UVB radiation, the animals received the topical treatment three times over 24 h. The mice in a chamber were subjected to UVB irradiation keeping a distance of 15 cm from the UVB lamp (5,000 J/m^2) for 15 consecutive sessions of 30 min. Twenty-four hours after the last UVB exposure, dorsal skin fragments from each animal were excised for histopathological study. This experimental procedure was developed following the protocol validated by Zhang et al. (2015), with some modifications.

All experiments were performed according to the approved guidelines for handling and care of laboratory animals: Mouse of the Health National Institute of Perú (Fuentes-Paredes et al., 2008), in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Council of Europe No. 123, Strasbourg 1985.

Histopathological study

Dorsal skin samples were taken from the middle area of the mice back and fixed in 10% buffered formalin, paraffin-embedded, sectioned at 5-μm sections, and stained with hematoxylin and eosin (H&E). These sections were used for the determination of general tissue morphology. The histological measurements were taken for the determination of both epidermal (including skin irritation, edema, erythema, hyperkeratosis, hypergranulosis, acanthosis, hyperplasia, dysplasia, ulceration, crust, and spongiosis) and dermal (including congestion, hemorrhage, angiogenesis, inflammatory infiltrate, and increased collagen) parameters using an Olympus CX31 optical microscope.

Statistical analysis

The data were indicated as the means ± SD and analyzed statistically using Tukey's test ($p < 0.05$) with the SPSS software.

Results

Emulgel formulations

The quantitative composition of Copoazú seed butter emulgel formulations at 5, 10, and 20% is presented in Table 1.

Physical examination

All prepared emulgel formulations (5, 10, and 20%) were pearl white, viscous, and creamy preparations with a smooth and homogeneous appearance, and they were easily spreadable. The evaluation was done for 12 weeks, and no changes were visible.

pH measurement

The pH values of emulgels at 5, 10, and 20% were 5.6–5.8, 6.2, and 6.1–6.2, respectively. The pH values of all the formulated emulgels ranged from 5.6 to 6.2 in a period of 12 weeks. The pH in the semisolid preparations often indicates stability concerning time and oxidation of the ingredients of the formulation. On the other hand, since these preparations are destined for skin treatment, the pH should be between 4 and 6.5 units to prevent the risk of irritation or alteration in the skin tissue (Clearly, 1984).

Centrifugation

After 5 h of centrifugation, all formulated emulgels maintained their consistency and homogeneity, and no phase separation was detected. It has been reported that the long-term stability of an emulsion could be assessed using a centrifugation process at 1,500 g (3,750 rpm) for 5 h (Garg et al., 2002). The centrifugation test helps to predict instability because it simulates the increase in gravitational force and consequently the mobility of the particles (Silva and Silva, 2007).

Evaluation of UV filtering potential

The determination of SPF values for emulgels and commercial sunscreen product samples was made using the UV spectrophotometric method and the Mansur equation. The results are shown in Table 2. The sunscreen formulations should absorb UV radiation in the range of 290 nm to 400 nm to be effective in the prevention of skin cancer, wrinkle formation, photoaging, sunburn, and other types of skin disorders, in this case, the results showed that Copoazú seeds butter emulgel at 20% have the highest SPF (11.67 ± 0.001), which is acceptable for the development of sunscreen products

TABLE 1 Formulation of emulgels containing Copoazú seed butter at 5, 10, and 20%.

No.	Constituent	Quantity (%)	Function
1	Sodium Cetearyl Sulfate	4.5	Anionic surfactant
2	*Theobroma grandiflorum* seed butter	5, 10, 20	Emollience/antioxidant
3	Polysorbate 80	4.5	Emulsifier
4	Dehydroacetic acid/benzyl alcohol	0.1	Preservative
5	Carbomer	1	Gelling agent
6	Triethanolamine	c.s.p. pH = 7	pH corrector
7	Water (Aqua)	c.s.p 100 ml	Vehicle

TABLE 2 SPF found in the emulgels formulated.

Formulation	SPF*
Copoazú seed butter emulgel at 5%	$9.44^a \pm 0.003$
Copoazú seed butter emulgel at 10%	$9.63^a \pm 0.001$
Copoazú seed butter emulgel at 20%	$11.67^b \pm 0.001$
Commercially sunscreen product	$44.74^c \pm 0.003$

n = 3. Different superscripts indicate a significant difference ($p < 0.05$) among formulations.
*Experimental data obtained in this research.

(Prasanna Kumar et al., 2015). In addition, the labeled SPF of the commercial product was 50, and in the analysis, an SPF of 44.74 ± 0.003 was obtained. According to Pissavini et al. (2003), it is more difficult to measure high SPF values, due to biological variations in volunteers, a high SPF usually leads to higher uncertainty in the test *in vivo*. For these considerations, a formulator must know the physicochemical principles, not just the UV absorbance of the active substances to formulate safe sunscreen products with a high SPF. *In vitro*, SPF determination is a complement to *in vivo* measurements for screening tests during product development (Abreu et al., 2004). The UV spectrophotometric method is fast, simple, and low cost.

Radical scavenging activity DPPH assay

The radical scavenging activity of Copoazú seed butter against the DPPH radical can be seen in Figure 1. The antioxidant activity expressed as EC_{50} values varied from 8.47 ± 0.013 mg/ml to 4.53 ± 0.046 mg/ml for Copoazú seed butter emulgels.

Vitamin E emulgel at 5, 10, and 20% had EC_{50} values of 7.91± 0.034, 4.71± 0.004, and 3.89 ± 0.005, respectively. Copoazú seed butter emulgel at 20% had an EC_{50} value of 4.53 ± 0.046 mg/ml, showing a comparable scavenging activity to the standard at the same concentration.

Effects of emulgel formulations on UVB-induced inflammation in mice skin

For UV-induced skin inflammation and skin cancer assessment, histopathological analysis of skin lesions by H&E staining was performed. Table 3 showed that the mice skin in the control group (without any treatment) displayed histological changes in both epidermal and dermal structures. At the epidermal level, crust, spongiosis, hyperkeratosis, acanthosis, and hypergranulosis were observed in the first week and ulceration occurred in the second week, while at the dermis level increased collagen was observed in the first week and focal atrophy of skin adnexa and low inflammatory infiltrate was observed in the second week. The commercial sunscreen product caused hyperkeratosis and hypergranulosis at the epidermal level during the first and second weeks. While at the dermis level, it caused focal atrophy of the skin adnexa and increased collagen. The application of the Copoazú seed butter emulgel at 20% produced crust, acanthosis, mild dysplasia, and ulcer at the epidermal level in the first week, while in the second week the ulceration disappeared and hypergranulosis, hyperplasia, and dysplasia could be seen. At the dermis level, there were only changes in the first week showing increased inflammatory collagen infiltrate and focal atrophy of the skin adnexa. On the other hand, Copoazú seed butter emulgels at 10 and 5% concentrations showed milder changes at both the epidermis and dermis levels as can be seen in Table 3. These results demonstrate that there is the protection of the skin of mice when using 5 and 10% Copoazú seed butter emulgels against UVB radiation, which coincides with the result of the antioxidant activity. It should be noted that it appears that the Copoazú seed butter emulgel at 20% did not achieve adequate penetration of the fatty acids and polyphenols through the skin of the mice due to its high concentration.

Perivascular dermatitis exists when inflammatory cell infiltrates are predominately distributed in circumvascular patterns. The following four variants are recognized: (1) perivascular dermatitis involving the superficial or

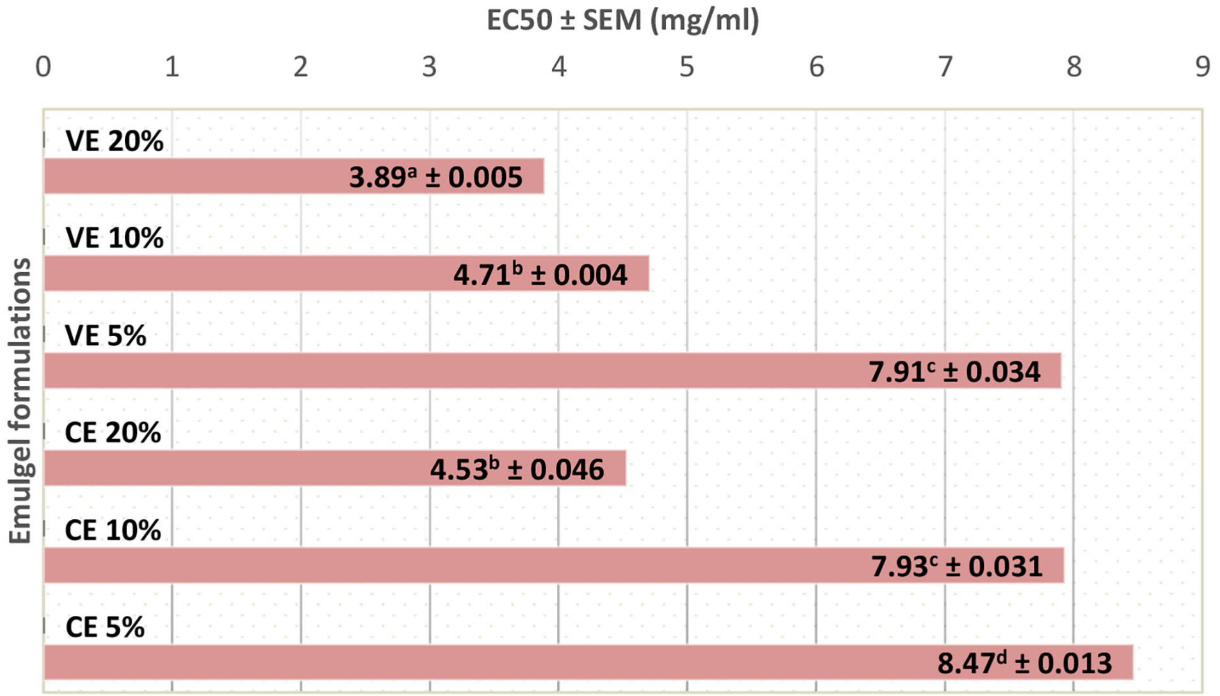

FIGURE 1
Antioxidant and radical scavenging activity of emulsion formulations against the DPPH radical. VE 20%: vitamin E emulgel at 20%, VE 10%: vitamin E emulgel at 10%, VE 5%: vitamin E emulgel at 5%, CE 20%: Copoazú seed butter emulgel at 20%, CE 10%: Copoazú seed butter emulgel at 10%, and CE 5%: Copoazú seed butter emulgel at 5%. n = 3. Different superscripts indicate a significant difference ($p < 0.05$) among formulations.

deep dermal blood vessels; (2) interface dermatitis where inflammatory cell infiltrates closely follow the dermal–epidermal junction as well as perivascular distributions; (3) spongiotic dermatitis which is characterized by intercellular edema in the epidermal layer which may progress to form intraepidermal vesicles and bullae; and (4) hyperplastic dermatitis which is a variant associated with chronic irritation and varying degrees of epidermal hyperplasia and hyperkeratosis. Any of these four variants of perivascular dermatitis might be indicative of contact with noxious agents, food or drug allergies or several additional infections, or toxic or physical agents. Interface dermatitis may be associated with toxic epidermal necrolysis, drug reactions, and autoimmune skin diseases. Spongiotic dermatitis is perhaps the most common initial change following contact with a dermal irritant (Hobson, 1991). To observe the skin damage caused by UV irradiation, pictures of the macroscopic skin lesions of mice were taken with a digital camera. The typical images of macroscopic skin lesions induced by UV irradiation in the second week were shown in Figure 2, and skin damages such as erythema, coarse, edema, thickening, and leathery appearance were observed. In addition, many ulcerative red maculopapules were examined in the group of any treatment after UVB irradiation.

Discussion

The present study shows the potential use of Copoazú seed butter in the formulation of sunscreen products. There is a growing need for formulations against UV radiation to prevent its side effects such as sunburn, premature aging, wrinkles, and decreased immunity against infections and cancer (Korać and Khambholja, 2011). Copoazú seed butter restores elasticity to the skin because it is an excellent emollient providing antioxidant and hydration (Yang et al., 2003). It is considered a "super-moisturizer" because transporting water into the skin makes the skin more supple, soft, and elastic (Winkler, 1977). Copoazú seed butter has a high content of phytosterols that fight against free radical damage and fatty acids that protect and moisturize the skin. This natural fat has been used by the Amazonian people since ancient times to heal the skin from sun damage by restoring its natural beauty and acting as other FDA-approved active ingredients, which gives true restructuring and healing properties to the skin, increases the moisture barrier of the skin, and provides hydration by making it more elastic (Fleck and Newman, 2012).

Ultraviolet B radiation provokes the alteration in the skin connective tissue mainly because there is an elevation of lipid peroxides, reactive oxygen species (ROS), and degradation of collagen and elastin leading to the formation of wrinkles and loss

TABLE 3 Microscopic analysis of the epidermis and dermis of the mice skin exposed to UVB radiation.

Groups	Epidermal changes		Dermal changes	
	First week	Second week	First week	Second week
without any treatment (G1)	Crust Spongiosis Hyperkeratosis Acanthosis Hypergranulosis Hyperplasia	Crust Hyperkeratosis Ulcer	Increased collagen Focal atrophy of skin adnexa	Low inflammatory infiltrate
Commercial sunscreen product (G2)	Hyperkeratosis	Minor hyperkeratosis Hypergranulosis	Focal atrophy of skin adnexa	Increased collagen Decreased cutaneous adnexa
Copoazú seed butter emulgel at 5% (G3)	Low spongiosis Hyperkeratosis Mild acanthosis	Spongiosis Hyperkeratosis Acanthosis Hyperplasia	Mild congestion	Focal inflammatory infiltrate
Copoazú seed butter emulgel at 10% (G4)	Focal Hyperkeratosis Mild Acanthosis Mild dysplasia	Hyperplasia	Mild angiogenesis Low inflammatory infiltrate Focal atrophy of skin adnexa	
Copoazú seed butter emulgel at 20% (G5)	Crust Acanthosis Mild dysplasia Ulcer	Hypergranulosis Hyperplasia Dysplasia	Inflammatory infiltrate Increased collagen Focal atrophy of skin adnexa	

of skin elasticity (Leccia et al., 2019). In addition, free radicals induced by UV radiation produce harmful effects on human skin by activating DNA damage and the formation of inflammatory cytokines to damage the extracellular matrix (ECM) (Kwon et al., 2019). Fatty acids are important metabolites with crucial cellular functions, including cell proliferation, differentiation, migration, and development (Jia et al., 2018). Some previous studies have demonstrated a relevant therapeutic application of fatty acids for skin wound healing (Pereira et al., 2008; Cardoso et al., 2011), because they can modulate the release of proinflammatory cytokines, affecting neutrophil migration, increasing the wound healing tissue mass while decreasing the thickness of the necrotic cell layer edge around the wound, and ultimately modulating the closure of skin wounds (Pereira et al., 2008). Oleic acid, for instance, is an important constituent of Copoazú seeds and has been demonstrated to induce a faster wound closure (Cardoso et al., 2004). According to Scapagnini et al. (2014), the lipids present in different kinds of butter such as *Theobroma cacao* benefit skin health through skin elasticity improvement and dry skin relief also plays an outstanding role in the proinflammatory cytokines downregulation, resulting in the anti-inflammatory effect. For this consideration, a topical application of *Theobroma grandiflorum* butter (a close relative of *T. cacao*) based on its triglycerides and fatty acids and the unsaponifiable matter would increase dermal antioxidant capacity and protects the epidermis from oxidative stress and exogenous ROS.

Sustainability, which is a word used lately by most industries worldwide, has been responsible for leading raw material and product development in new directions due to changing consumer and companies' behavior (Adams and Jeanrenaud, 2008). The selection of products, packaging, and production processes have a great impact on the environment, so there is a growing interest in sustainable products, and because of this, many cosmetic products are formulated with natural ingredients. Thus, in the last decades, there was a growing interest in natural products, especially in European countries where there is a consuming market called "the green consumer" (Fonseca-Santos et al., 2015).

Molecules with a sun protection factor (SPF) value of 15 or greater are highly appreciated in the development of sunscreen products and can be incorporated into various cosmetic products such as gels, lotions, creams, and oils (Korać and Khambholja, 2011). Many synthetic sunscreen products have been limited in their use due to their adverse side effects such as hypersensitivity, allergies, irritant dermatitis, and even melanoma (Chanchal and Swarnlata, 2009). In recent years, the use of natural sunscreen products has obtained great attention

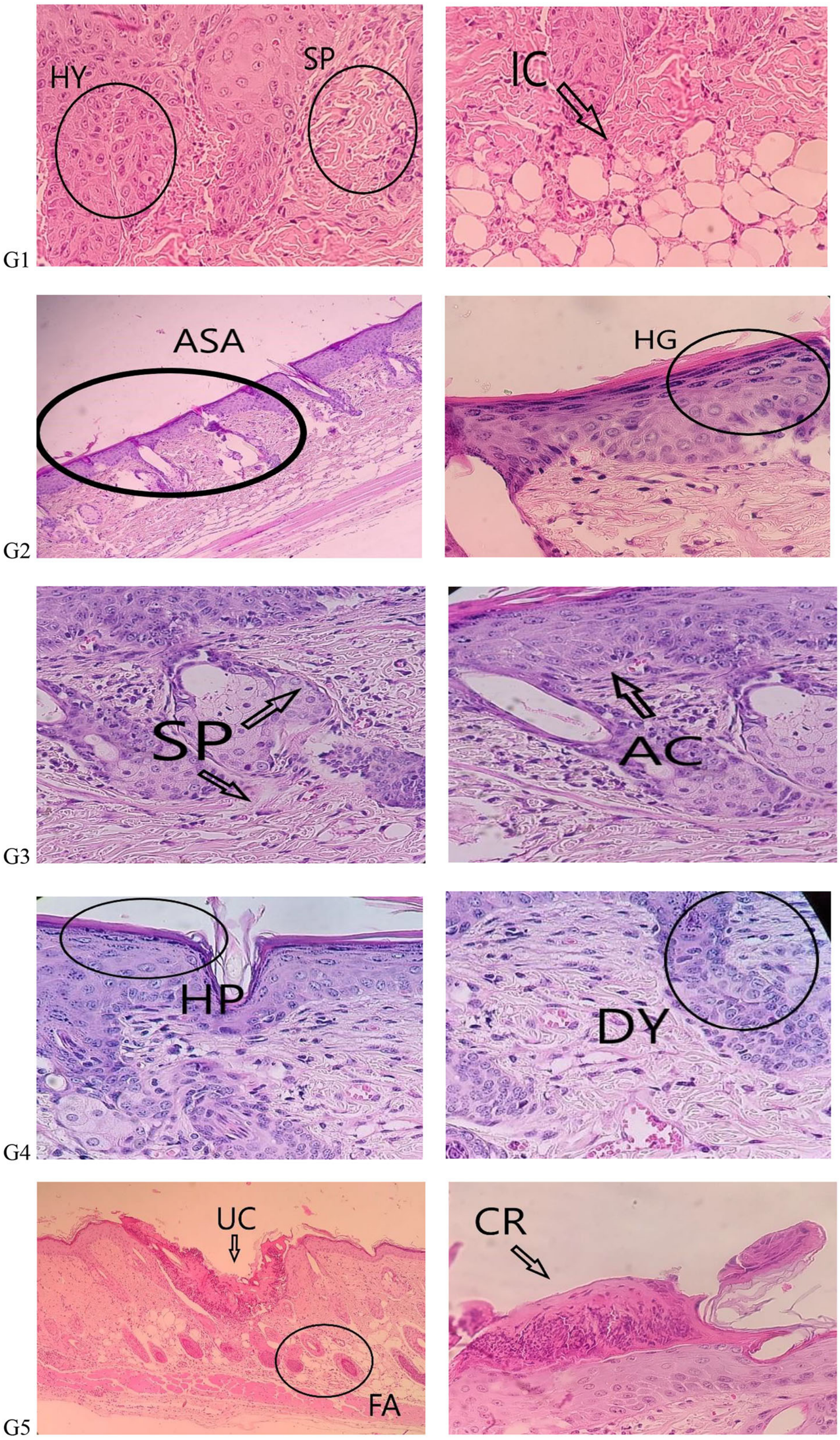

FIGURE 2
Histopathological evaluation of the skin of mice at the end of the experiment period. G1, mice exposed to UVB light without any treatment (negative control group): **(Left)** HY, hypergranulosis; SP, spongiosis; **(Right)** IC, increased collagen. G2, mice exposed to UVB light treated with commercial sunscreen product (positive control): **(Left)** ASA, absence of skin attachments; **(Right)** HG, hypergranulosis. G3, mice exposed to UVB light treated with Copoazú seed butter emulgel at 5%: **(Left)** SP, spongiosis; **(Right)** AC, acanthosis. G4, mice exposed to UVB light treated with Copoazú seed butter emulgel at 10%: **(Left)** HP, hyperplasia; **(Right)** DY, dysplasia. G5, mice exposed to UVB light treated with Copoazú seed butter emulgel at 20%: **(Left)** UC, ulcer; FA, focal atrophy of hair follicles; **(Right)** CR, crust.

and many natural components with UV-absorbing properties have been used to replace or reduce the number of synthetic sunscreen products (Nichols and Katiyar, 2010).

Topical drug delivery provides direct access to the skin for the treatment and prevention of various skin diseases. Emulgel is one of the new cosmetic technologies employed for the treatment of fungal infections, acne, psoriasis, and other skin disorders (Arora et al., 2017). It is an emulsion (w/o or o/w) that is gelled by mixing with a gelling agent such as Carbopol and has several desirable properties, such as being thixotropic, non-greasy, easy to spread, easy to remove, water soluble, non-staining, emollient, longer shelf life, biodegradable, transparent, and pleasant appearance (Stanos, 2007).

Percutaneous drug absorption has been increased by a wide variety of long-chain fatty acids, the most popular of which is oleic acid (Trommer and Neubert, 2006), and in this field, Akram et al. (2013) developed an insulin emulgel formulation for transdermal drug delivery using oil with high oleic acid contents and also contains other fatty acids, such as palmitic acid, stearic acid, linoleic acid, linolenic acid, palmitoleic acid, and myristic acid with very interesting results in penetration enhancing activities achieving good blood insulin levels. Copoazú seed butter has a good content of oleic acid, which makes it an invaluable product for the development of skin products. Jiang and Zhou (2003) studied the effect of oleic acid on stratum corneum lipids in rats using electron microscopy, and it was found that oleic acid enhances permeability through its ability to perturb the stratum corneum and lacunae formation. It was also studied by Larrucea et al. (2001) that the combination of oleic acid with propylene glycol in carbomer gel enhanced the absorption of tenoxicam. Many oils and butter show the ability to resist UV radiation; for example, sesame oil resists 30% of UV rays, while cottonseed, coconut, peanut, and olive oils block out about 20%. The traditional use of plants in medication or beautification is the basis for research and development of new trends in cosmetics (Korać and Khambholja, 2011). Fat from *Theobroma grandiflorum* (Copoazú) seeds has been investigated because there is an increasing demand as a new fruit crop. There is a growing market for natural products, and the Peruvian Theobroma species could be employed as an alternative source of special fats. Considering the assays performed in this study, *Theobroma grandiflorum* seed butter had a good photoprotective effect with a high potential for use in the development of sunscreen products.

These days, sustainability is a key factor in the beauty sector in general. Environmental protection and the competitiveness of the globalized market strongly demand innovations. Over the past decades, researchers have been studying the sources of environmentally friendly ingredients that ensure sustainability, traceability, integrity of supply, and transparency; in fact, the cosmetics world is doing efforts to search for new vegetable oils and fats to replace synthetic chemicals in formulation (Archambault and Bonté, 2021). Recent investigation data on the content and organization of the different fats responsible for the skin barrier support the utilization of vegetable lipids to improve or care for the skin (Jia et al., 2018, 2019; Berkers et al., 2019; Zhou et al., 2020). The cosmetics industry has been developing an eco-design approach in all its dimensions, especially with the use of more sustainable ingredients, as the progressive closing of refining centers in Europe and the demand for natural ingredients is challenging formulators to research sources other than paraffin derivatives. Thus, some vegetable raw materials are being employed in the synthesis of surfactants, solubilizers, and other substances used in cosmetic formulation. Now, a high number of fatty acids present in oils from vegetable sources and the increasing need from users for more natural, even vegan, products have led to their use in the development of new products on the market (Archambault and Bonté, 2021). This is the case of a sunflower oil extract rich in unsaponifiable (>5%) developed by some cosmetic industry suppliers as an active ingredient to stimulate the synthesis of key lipids in the skin barrier where the peroxisome proliferator-activated receptors (PPARs) are activated. These intracellular receptors are expressed in the epidermis and play an essential function in the maintenance of the skin barrier and their activation drives the stimulation of the synthesis of skin lipids and anti-inflammatory activity (De Belilovsky et al., 2011).

Butters and waxes are commonly employed in cosmetic and grooming products, for example, eye shadows or blushes, make use of fat blends as binders to make the powder particles more dispersible, more moisturizing, and more cohesive during compaction and make them adhere better to the skin. Among the most used kinds of butter in cosmetics are shea (*Butyrospermum parkii*), shorea (*Shorea robusta*), palm (*Elaeis guineensis*), mango (*Mangifera indica*), coconut (*Cocos nucifera*), Copoazú (*Theobroma grandiflorum*), cocoa (*Theobroma cacao*), and wild mango (*Irvingia gabonensis*) from tropical regions of the world. However, the intensive cultivation and production of coconut, palm, and cocoa butter have had a significant impact on the loss of biodiversity, and this is the main problem pending a solution (Archambault and Bonté, 2021).

Conclusion

A stable emulgel-type cosmetic formulation was developed using Copoazú seed butter at different concentrations of 5, 10, and 20%. The antioxidant activity and the effect of Copoazú seed butter emulgel against UV-induced epidermal damage in mice were evaluated to verify its use for topical photoprotective products. The antioxidant activity expressed as EC_{50} values was 8.47 ± 0.013 mg/ml, 7.93 ± 0.031 mg/ml, and 4.53 ± 0.046 mg/ml for Copoazú seed butter emulgels at 5, 10, and 20%, respectively. The *in vitro* determination of the sun protection factor (SPF) showed that Copoazú seed butter emulgels at 5, 10, and 20% have SPF values of 9.44 ± 0.003, 9.63 ± 0.001, and 11.67 ± 0.001, respectively. The *in vivo* results in mice irradiated with UV light and treated with Copoazú emulgels showed minor damage and a significant reduction in the severity of the damage. Copoazú seed butter could be useful in the development of photoprotective formulations. Also, the use of this butter is advantageous since this is considered waste and is discarded, providing savings, as well as adding value to the product. Today, there is a necessity to have cosmetics more "eco-friendly" by using new raw materials, plants, and herbs which tend to make the market for cosmetic products more sustainable, so the companies can get more market share.

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for this animal study, in accordance with the local legislation and institutional requirements.

Author contributions

CA-Á and CE-S were involved in the investigation, formal analysis, writing—original draft preparation, and writing—review and editing. CD was involved in conceptualization, writing—review and editing, supervision, project administration, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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