cosmetics

MDPI

Article

Development of an Antioxidant, Anti-Aging, and Photoprotective Phytocosmetic from Discarded *Agave sisalana* Perrine Roots

Guilherme dos Santos Mazo [1], Julia Amanda Rodrigues Fracasso [2,*], Luísa Taynara Silvério da Costa [2], Valdecir Farias Ximenes [3], Natália Alves Zoppe [1], Amanda Martins Viel [4,5], Lucas Pires Guarnier [6], Beatriz de Castro Silva [1,2], Luan Victor Coelho de Almeida [1,2] and Lucinéia dos Santos [1,2]

[1] Department of Biotechnology, School of Sciences and Languages, São Paulo State University (UNESP), 2100, Dom Antonio Avenue, Assis 19806-900, SP, Brazil; na.zoppe@unesp.br (N.A.Z.); beatriz.c.silva@unesp.br (B.d.C.S.); luan.coelho@unesp.br (L.V.C.d.A.); lucineia.santos@unesp.br (L.d.S.)
[2] School of Dentistry, São Paulo State University (UNESP), 1193, José Bonifacio Street, Araçatuba 16015-050, Brazil; luisa.silverio@unesp.br
[3] Department of Chemistry, Faculty of Sciences, São Paulo State University (UNESP), Bauru 17033-360, SP, Brazil; valdecir.ximenes@unesp.br
[4] São Camilo University Center, Sao Paolo 04263-200, SP, Brazil; amanda.mviel@hotmail.com
[5] Nossa Senhora do Patrocínio University Center (CEUNSP), Itu 13300-200, SP, Brazil
[6] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto 14048-900, SP, Brazil; lucasguarnier@usp.br
* Correspondence: j.fracasso@unesp.br

Abstract: The primary source of hard fiber globally is *Agave sisalana* Perrine, also known as sisal. In areas where sisal is grown, the roots of the plant are usually left in the field after it has stopped producing, which leads to soil degradation and decreased sisal productivity. It is, therefore, critical to find alternatives to reuse this waste. This study explores the potential use of sisal waste in the cosmetic industry by incorporating a hydroethanolic extract (HER) into a cream–gel formulation, taking advantage of the plant's recognized ethnopharmacological value. The study involves analyzing the extract's phytochemical composition (flavonoids) and evaluating its cytotoxicity. Subsequently, the antioxidant and antiglycation activities of the extract and cream–gel are evaluated, as well as ex vivo ocular toxicity, photoprotective activity, and preliminary stability analyses. The HER extract showed a flavonoid composition (catechin, kaempferol, isorhamnetin, and chrysin) and maintained cell viability above 70% throughout all time points analyzed in the MTT assay. Furthermore, the extract and the formulation demonstrated proven antioxidant and antiglycation activities. The cream–gel's UVB and UVA protection effectiveness with the HER was comparable to that of synthetic UVB/UVA sunscreens, with the samples proving nonirritating and stable. In conclusion, the extract has a significant presence of flavonoids, and the cream–gel developed with it did not present cytotoxicity and met the stability requirements, indicating phytocosmetic potential with antioxidant, antiglycation, and photoprotective properties.

Keywords: agave; flavonoids; phytocosmetic

Citation: Mazo, G.d.S.; Fracasso, J.A.R.; da Costa, L.T.S.; Farias Ximenes, V.; Zoppe, N.A.; Viel, A.M.; Guarnier, L.P.; Silva, B.d.C.; de Almeida, L.V.C.; dos Santos, L. Development of an Antioxidant, Anti-Aging, and Photoprotective Phytocosmetic from Discarded *Agave sisalana* Perrine Roots. *Cosmetics* **2024**, *11*, 104. https://doi.org/10.3390/cosmetics11030104

Academic Editor: Antonio Vassallo

Received: 11 April 2024
Revised: 2 May 2024
Accepted: 9 May 2024
Published: 20 June 2024

1. Introduction

Sisal, the primary hard fiber produced in the world, is extracted from the leaves of *Agave sisalana* Perrine plant species. Originally from the Yucatan Peninsula in Mexico, sisal was exported to Brazilian territory during the 19th century [1]. Nowadays, sisal is crucial for the socioeconomic development of the semi-arid region of the northeast region, particularly in the state of Bahia, which accounts for 90% of its production [2]. Brazil is currently the world's largest producer of sisal, with an average annual production of approximately 140 thousand tons of its hard fiber [3–5]. In areas where sisal is cultivated,

the roots of the plant are often left in the field after they have served their useful purpose or to prevent an excessive number of new seedlings. This accumulation of organic material at the production site can reduce soil oxygenation and disrupt the balance of microorganisms present, ultimately damaging sisal production [5–7].

Given this scenario, alternative methods for reusing and reducing waste from sisal roots are necessary [8]. *Agave sisalana* is recognized worldwide for its ethnopharmacological value. According to the literature, it possesses anti-inflammatory, antioxidant, and larvicidal properties. These properties are attributed to the significant secondary metabolites in their phytochemical composition, such as phenolic compounds [9–12].

Phenolic compounds have antioxidant properties that can reduce the formation of reactive oxygen species (ROS) in the deeper layers of the skin. Chronic exposure to the sun can cause photoaging and increase the risk of developing cancer. Therefore, using xqq1sisal residue, which has antioxidant and photoprotective properties, as a phytocosmetic can be a sustainable alternative to its improper disposal. Incorporating antioxidant compounds, either alone or in combination with synthetic photoprotectors, can broaden the spectrum of action of formulations and enhance their effectiveness [13,14]. Furthermore, with the growing development of the sustainable market, consumers are increasingly interested in cosmetics of natural origin, which are less harmful to health and the environment, replacing synthetic compounds.

This study aimed to explore the potential use of sisal residue in the cosmetic industry due to its high tolerability, biodegradability, and diverse biological and therapeutic activities. To achieve this, phytochemical, toxicological, pharmacological, and stability tests were conducted. The first step was to prepare a hydroethanolic extract from sisal roots and analyze its phytochemical composition to confirm the presence of flavonoids, a class of phenolic compounds. The extract's cytotoxicity was then analyzed to ensure its safety for use. The extract was incorporated into a cream-gel formulation for topical use, and the antioxidant and antiglycating activities of the extract and cream-gel were evaluated. Finally, the cream-gel underwent an ex vivo ocular toxicity evaluation, a photoprotective activity assessment, and a preliminary stability analysis.

2. Materials and Methods

2.1. Plant Material

The root was obtained from rural producers in the municipality of Valente, Bahia, as designated by the Secretariat of Science, Technology, and Innovation of Bahia. The mucilage resulted from the defibration process of sisal leaves, while the root, typically disregarded, was collected directly from the soil. The mucilage and root were frozen and transported to the Faculty of Sciences and Letters of Assis, São Paulo State University "Júlio de Mesquita Filho"—UNESP. The species under study was identified as Agave sisalana in the Herbarium Assisense (HASSI) of the State University of São Paulo (Assis, state of São Paulo). A voucher specimen was deposited under the number 2597.

2.2. Hydroethanolic Extract Roots of Sisal

The dried roots of the plant material were powdered and macerated in an EtOH/H_2O (70:30 v/v) solution (dry plant: EtOH/H_2O—1:5 w/v) at room temperature (25–30 °C) protected from light for seven days, filtered, and evaporated under reduced pressure at 60 °C to obtain the crude extract of Hydroethanolic Extract Roots (HERs). The extract was stored at 6–10 °C and protected from light until use.

2.3. Analysis of Flavonoids Present in the Extract

The phytochemical characterization of the HER was performed through high-performance liquid chromatography (HPLC). The HPLC analyses were carried out on a Luna® C18 reversed-phase column (Phenomenex, 250 × 4.6 mm, 5 µm) at 35 °C. The mobile phase consisted of solvent (A), 0.1% aqueous formic acid, and solvent (B), 0.1% formic acid in acetonitrile. Solvent gradient program: 0–3 min (80%A), 3–6 min (80–65%A), 6–11 min

(65%A), 11–14 (65–30%A), 14–19 min (30%A), 19-21 min (30–80%A), and 21–24 min (80%A). The flow rate was 1 mL min^{-1}. The detector was set at 364 nm. The experiments used a JASCO system in line with a diode array detector (JASCO, Easton, MD, USA). The peaks were determined by comparison with authentic flavonoid standards (Sigma-Aldrich, Sao Paolo, Brazil). The flavonoids (10 mM) were dissolved in DMSO and diluted in water to 0.01 mM. The injection volume was 20 µL.

2.4. Determination of Cytotoxicity of the Extract with the MTT Assay [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide]

The MTT cytotoxicity assay was carried out as described previously by Kumar et al. [15]. For this assay, mouse fibroblasts of dermal origin (NIH/3T3, ATCC® CRL-1658™, Washington, DC, USA) were inoculated in 96-well microtiter plates and incubated in culture medium for a period of 24 h at 37 °C and 5% CO_2. After a confluence of approximately 75% (24 h), these cells were exposed to five different concentrations of HER isolated (100 µg/mL, 200 µg/mL, 400 µg/mL, 800 µg/mL, and 1.600 µg/mL) to the negative control—NC (physiological solution)—and the positive control—PC (2% (*v*/*v*) Tween 80). The treatment time was 24, 48, and 72 h.

2.5. Preparation of the Cream-Gel Formulation Containing the HER

To prepare the phytocosmetic formulation for topical administration, the components of the aqueous phase were initially weighed, and, with the help of a planetary mixer, the components were slowly dispersed. Then, the pH was adjusted to 5.5 with 20% aqueous NaOH solution (*w*/*v*). The aqueous and oil phases were heated to 70 °C, and then the aqueous phase was slowly poured into the oil phase under stirring until cooling (20–25 °C). The composition of the formulation is again described in Table 1. It is important to point out that all the components present in the formulations are widely used in the cosmetic and pharmaceutical sectors and, therefore, pharmaceutically and cosmetically accepted.

Table 1. Formulation of cream-gel containing extract.

Phase	Function	Composition	Concentration (% *w*/*w*)
Oil	Emollient	Mineral oil	2
	Emulsifier	Tween 80	2.5
	Emulsifying wax	Polawax 400	10
	Preservative	Methylparaben	0.5
Aqueous	Humectant	Glycerin	4
	Active compound	HER	5
	Agent of viscosity	Carbopol	0.5
	Vehicle	Distilled water	qsp 100

2.6. Determination of In Vitro Antioxidant Activity of the Extract and Formulations

2.6.1. Treatments

In all antioxidant tests, the analyzed samples were as follows: the HER (50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, and 600 µg/mL) and formulations with the HER (200 µg/mL—C1, 400 µg/mL—C2, and 600 µg/mL—C3). In the positive control, the extract doses were replaced by quercetin (300 µg/mL), and in the negative control, they were replaced by saline solution.

2.6.2. DPPH Assay

The antiradical effectiveness of the HER and formulations was assessed using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. This assay is preferred because it is used to provide a stable free-radical scavenging assay [16]. The test was conducted in triplicate, and the absorbance was measured with an ELISA reader at 517 nm. The scavenging of the

DPPH radical was calculated using Equation (1), and the results were expressed as IC50 values, representing the concentration required to inhibit 50% of the DPPH radicals.

$$\%\ Antioxidant\ Activity = [(\text{Abs Control} - \text{Abs Sample})/\text{Abs Control}] \times 100 \quad (1)$$

where "Abs Control" is the absorbance of the DPPH control and "Abs Sample" is the absorbance of the sample.

2.6.3. Lipid Peroxidation Inhibition

The capacity of the HER and formulations as lipid peroxidation inhibitors was evaluated using the egg yolk extract method with minor modifications [17]. To summarize, 0.4 mL of treatments were individually added to 4 mL of egg yolk prepared in phosphate-buffered saline (PBS), followed by 0.4 mL of 17 mM ferrous sulfate. The mixture was shaken vigorously and incubated at 37 °C for 30 min. Next, 2 mL of 20% (*w*/*v*) trichloroacetic acid and 2 mL of treatments were centrifuged at 4000× *g* for 10 min. Then, 2 mL of the supernatant was collected, and 2 mL of 0.8% (*w*/*v*) thiobarbituric acid was added to the mixture. After boiling at 95 °C and cooling for 30 min, the absorbance was measured at 532 nm (Femto 600 Plus, Sao Paolo, Brazil). The test was performed in triplicate. The results were expressed as the sample concentration providing 50% inhibition (IC50) values.

2.7. Determination of In Vitro Anti-Aging Activity of the Extract and Formulations

2.7.1. Treatments

The treatments included the HER (50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, and 600 µg/mL) and formulations with the HER (200 ug/mL—C1, 400 ug/mL—C2, and, 600 ug/mL—C3). In the positive control, the extract doses were replaced by quercetin (300 µg/mL), and in the negative control, they were replaced by saline solution.

2.7.2. Bovine Serum Albumin (BSA-GLU) Assay

The anti-aging assay was carried out according to the methodology previously reported by Mridula et al. [17]. Briefly, 3 mL of reaction mixture containing 1 mL of BSA (10 mg/mL) and 1 mL of treatments was prepared in 0.2 M sodium phosphate buffer (pH 7.4). The reaction mixture was incubated for 5 min. Then, 0.5 mL of sodium azide (0.5 mM) was added and incubated for 7 days at 37 °C in the dark. The fluorescence intensity (excitation of 370 nm and emission of 440 nm) was measured using an Omega microplate reader. The experiment was carried out in triplicate. The percent inhibition was calculated by using Equation (2):

$$\%\ \text{Inh} = \text{FLU Negative control group} - \text{FLU Test group FLU Negative control group } 1 \times 100 \quad (2)$$

2.7.3. BSA–Methylglyoxal (BSA-MGO) Assay

A bovine serum albumin (BSA)–methylglyoxal assay was performed as described by Mridula et al. [17]. BSA (20 mg/mL) and methylglyoxal (60 mM) were dissolved in 0.1 M sodium phosphate buffer (pH 7.4). Three milliliters (3 mL) of reaction mixture containing 1 mL of BSA, 1 mL of methylglyoxal, and 1 mL of the treatments were prepared. The reaction mixture was mixed thoroughly and incubated for 5 min at room temperature. After incubation, 0.5 mL of sodium azide (0.2 g/L) was added to each tube. The tubes were tightly capped and incubated at 37 °C for 7 days in the dark. After 7 days of incubation, the fluorescence intensity of the samples was measured at an excitation wavelength of 370 ηm and an emission wavelength of 440 ηm using an Omega microplate reader. The experiment was carried out in triplicate. The percentage of inhibition was calculated by using Equation (2).

2.8. Determination of the Sun Protection Factor (SPF) of the Formulation Containing 5% Extract

The device meets the regulatory requirements of Colipa (The European Cosmetics Association) and the FDA (Food and Drug Administration) of the United States. The cream–

gel with a synthetic UVB/UVA filter—Merck, 15% (PC)—and the cream–gel enriched with 5% HER (CGHER) were analyzed. Merck sunscreen contains 2–phenylbenzimidazole–5-sulfonic acid and 2–hydroxy–4–methoxybenzophenone–5-sulfonic acid. The determination of the SPF is a technique that proves the effectiveness of sunscreen filters for the ultraviolet B (UVB) radiation portion of the electromagnetic spectrum. With wavelengths of higher energy (290–320 ηm), UVB is responsible for causing erythema in the skin and cellular changes that predispose to skin cancer. As a positive control, the HER was replaced by a water-soluble UVB/UVA solar filter from Merck, which has 2–phenylbenzimidazole–5-sulfonic acid and 2–hydroxy–4–methoxybenzophenone–5-sulfonic acid in its composition, incorporated into the emulsion at a concentration of 15% [17].

For the determination of the SPF, spectrophotometric readings of the diluted samples were performed using a UV-1000s spectrophotometer, and, subsequently, the obtained absorbance values were applied to Equation (3).

$$SPF = CF \times \sum EE(\lambda) \times I(\lambda) \times \mid (\lambda) \mid \ 320\ \eta m\ 290\ \eta m \quad (3)$$

where SPF is the sun protection factor, CF is the correction factor, equal to 1030, EE(λ) is the erythematogenic effect of solar radiation at each λ, I(λ) is the intensity of the solar radiation at each λ, and Abs(λ) is the absorbance at each λ. The EE(λ) and I(λ) values are previously known, as they are obtained from the literature.

2.9. Ex Vivo Ocular Irritability Test of the Formulation Containing 5% Extract in the Chorioallantoic Membrane of Chicken Eggs (MCA)

This test was carried out according to the methodology described by Fracasso et al. [18] and Lebrun et al. [19]. Four fertilized eggs from White Leghorn chicken were used per treatment group: negative control (saline 0.9%, *w*/*v*) and cream–gel without and with 5 mg/g of HER.

On the tenth day of incubation, the treatments were applied to the MCA, and the presence or absence of irritating effects was observed. After visual analysis, thiopental solution was injected into the fertilized eggs. The graduation of each phenomenon was determined in 5 min, with numerical values (1, 3, 5, 7, and 9) depending on time (Table 2). Visual analysis of the MCA was performed with a magnifying glass.

Table 2. Numerical graduation (1, 3, 5, 7, and 9) of the phenomena as a function of the elapsed time (seconds) before their occurrence.

Phenomenon	30 s	30 and 60 s	60 and 300 s
Hyperemia	5	3	1
Bleeding	7	5	3
Coagulation	9	7	5

The classification of the analyzed samples was obtained with the mean value of the sum of the scores of three independent tests (n = 3), and the degree of irritation was divided into four categories: between 0.0 and 0.99—nonirritating (NI); 1.0 and 4.99—mild irritant (MI); 5.0 and 8.99—moderate irritant (MI); and 9.0 and 21—severe irritant (SI) [18].

2.10. Determination of the Stability of the Formulation Containing 5% Extract

2.10.1. Stability Properties

The stability properties of the cream–gel with the HER, such as its color, physical appearance, and homogeneity, were evaluated through visual perception and the odor directly through smell. The samples were stored at an ambient temperature of 25 °C, at 40 °C in an oven, and at 4 °C in a refrigerator. The analyses occurred weekly for a period of 30 days [11].

2.10.2. Spreadability

The spreadability of the cream–gel with the HER was determined by measuring the spreading diameter of 1 g of sample between two horizontal glass plates (10 cm × 20 cm) after one minute. The standard weight applied to the upper plate was 25 g. Each formulation was tested three times.

2.10.3. pH Determination

The pH was determined potentiometrically, according to Romanian Pharmacopoeia (FR X), using a portable digital pH meter (Sension™ 1, Hach Company, Loveland, CO, USA). A five-gram ointment/cream–gel was added to 20 mL of distilled water previously heated to 37 ± 2 °C and stirred vigorously for 1 min. After cooling, the dispersion was filtered, and the pH of the filtrate was determined. Each determination was made in triplicate.

2.11. Statistical Analysis

The data are expressed in terms of the mean ± standard deviation. Statistical analysis was performed in Prism 8. To verify the statistical differences between the groups, a one-way analysis of variance (ANOVA) was performed according to the experimental protocol, followed by Tukey's multiple-comparison test. For all analyses, a p-value of <0.05 was considered significant.

3. Results

3.1. Analysis of Flavonoids Present in the Extract

The results obtained in this study by high-performance liquid chromatography revealed in an unprecedented way in the HER the presence of the flavonoids catechin, kaempferol, and isohamnetin (Figure 1).

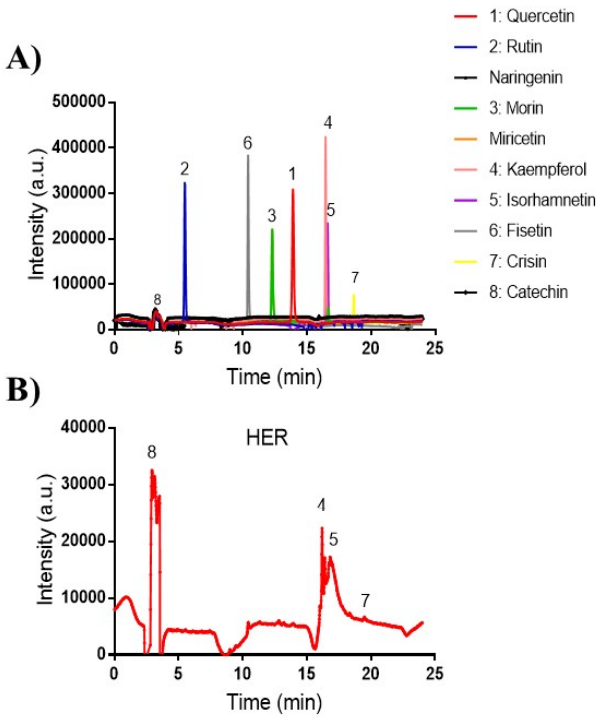

Figure 1. Chromatograms of (**A**) reference flavonoids and (**B**) HER fingerprint obtained by high-performance liquid chromatography. (1) rutin; (2) morin; (3) kaempferol; (4) isorhamnetin; (5) fisetin; (7) chrysin; and (8) catechin.

3.2. Determination of the Cytotoxicity of the Extract with the MTT Assay

The viability of NIH 3T3 cells was confirmed by the formation of formazan crystals. During all the analyzed times, the HER and PC (Tween 80%) showed significant differences from the NC (physiological solution), $p < 0.05$, but in opposite directions, as shown in Figure 2. This is because the HER, at various concentrations and times, promoted a cell viability greater than 70% ($p < 0.05$), and at 48 and 72 h, it induced an over 100% increase in fibroblast cell proliferation. In contrast, the PC led to intense cytotoxicity at all the analyzed times. Furthermore, the CC50% (50% cytotoxic concentration) was calculated as 3912 µg/mL for 24 h, 3349 µg/mL for 48 h, and 6324 µg/mL for 72 h.

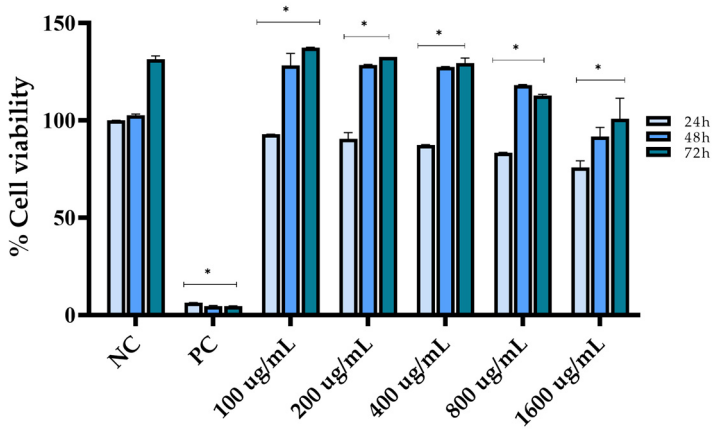

Figure 2. Mean ± SD of cell viability of the NC—negative control (physiologic solution 0.9%), PC—positive control (2% Tween 80%), and the HER (100 µg/mL, 200 µg/mL, 400 µg/mL, 800 µg/mL, and 1600 µg/mL) by the MTT method. A one-way ANOVA followed by Tukey's post hoc test was performed. An asterisk (*) indicates if there is a significant difference ($p < 0.05$) from the negative control.

3.3. Determination of In Vitro Antioxidant Activity of the Extract and Formulations

The antioxidant activity obtained in the DPPH test for the groups analyzed was NC—0.00 + 0.00%; PC—95.60 ± 0.58%; HER 50 µg/mL—15.45 ± 0.96%; HER 100 µg/mL—29.16 ± 0.3%; HER 200 µg/mL—45.90 ± 0.66; HER 400 µg/mL—100.00 ± 1.75%; HER 600 µg/mL—100.00 ± 0.43%; C1—71.88 ± 1.08%; C2—98.12 ± 0.99%; and C3—100.03 ± 0.00. All the treatments showed a significant difference from the NC ($p < 0.05$). The IC50% for the HER was 90 µg/mL, and for the cream–gel formulation, it was 275 µg/mL (Figure 3A).

Subsequently, the antioxidant activity was measured through the dosage of MDA formed in the lipid lipoperoxidation test induced by ferrous sulfate. The NC showed antioxidant activity equal to 0.00 ± 0.00%; PC—95.06 ± 1.55%; HER 50 ug/mL—43.67 ± 1.0% 100 µg/mL—76.87 ± 0.45%; 200 µg/mL—84.62 ± 2.00%; 400 µg/mL—86.98 ± 3.10%; and 600 µg/mL—91.94 ± 2.76%. For the formulations, the antioxidant activities were as follows: C1—83.26 ± 1.36%, C2—89.66 ± 4.53%, and C3—98.77 ± 5.84%. All treatments showed a significant difference in NC ($p < 0.05$). The IC50% of the HER was 169 µg/mL, and that of the formulation was 479 µg/mL (Figure 3B).

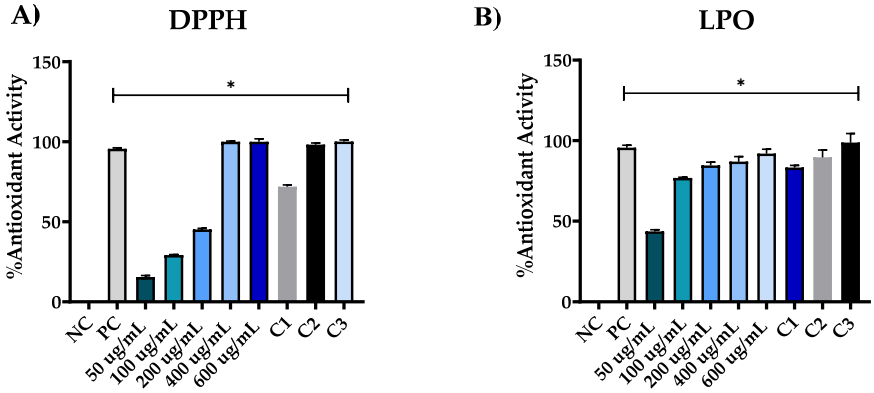

Figure 3. Mean ± SD of values in % of antioxidant activity in the (**A**) DPPH and (**B**) Lipoperoxidation tests after the following treatments: NC—negative control (physiologic solution 0.9%), PC—positive control (quercetin solution 300 µg/mL), HER (50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, and 600 µg/mL), and the cream–gel with HER (C1—200 µg/mL, C2—400 µg/mL, and C3—600 µg/mL). A one-way ANOVA followed by Tukey's post hoc test was performed. An asterisk (*) indicates if there is a significant difference ($p < 0.05$) from the negative control.

3.4. Determination of In Vitro Anti-Aging Activity of the Extract and Formulations

The results obtained confirmed the anti-aging activity of the HER in the BSA/GLU assay: NC—0.00 ± 0.00%; PC—95.60 ± 0.69%; HER 50 µg/mL—33.60 ± 3.24%; 100 µg/mL—74.33 ± 2.29; 200 µg/mL—100.00 ± 0.10%; 400 µg/mL—100.00 ± 0.23%; and 600 µg/mL—100.00 ± 2.24%. The formulations' values obtained were as follows: C1—100.61 ± 0.16%; C2—100.91 ± 0.11%; and C3—100.99 ± 0.02%. All the treatments showed a significant difference from NC ($p < 0.05$), as shown in Figure 4A.

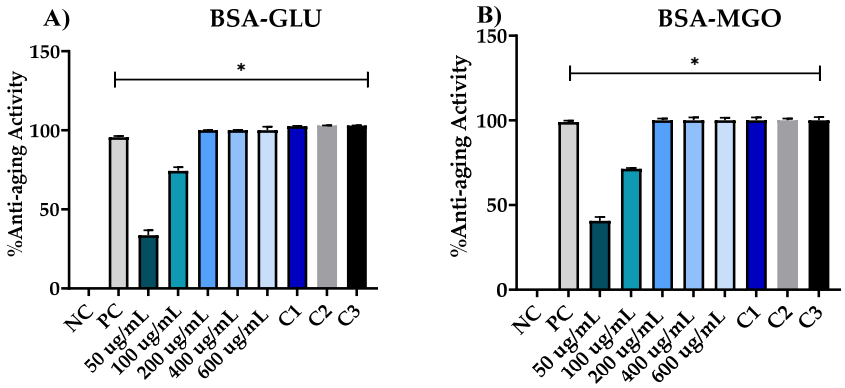

Figure 4. Mean ± SD of the values in % of anti-aging activity through (**A**) BSA/GLU and (**B**) BSA/MGO after the following treatments: NC—negative control (physiologic solution 0.9%), PC—positive control (quercetin 300 ug/mL), HER (50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, and 600 µg/mL), and the cream–gel with HER (C1—200 µg/mL, C2—400 µg/mL, and C3—600 µg/mL). A one-way ANOVA followed by Tukey's post hoc test was performed. An asterisk (*) indicates if there is a significant difference ($p < 0.05$) from the negative control.

Using the BSA/MGO methodology, the following results were obtained: NC—0.00 ± 0.00%; PC—99.01 ± 0.78; HER 50 µg/mL—40.67 ± 2.19%; 100 µg/mL—71.36 ± 0.48; 200 µg/mL—99.99 ± 1.07; 400 µg/mL—100.00 ± 1.75; and 600 µg/mL—100.00 ± 1.54. For the formulations, the results were as follows: C1—100.00 ± 1.75; C2—100.00 ± 1.08; and C3—100.00 ± 1.91 (Figure 4B).

3.5. Photoprotection against UVA/UVB

Table 3 presents the results obtained in evaluating the UVB and UVA protection of the cream–gel with the HER and the cream–gel containing the synthetic UVB/UVA sunscreen.

Table 3. Photoprotective Activity with cream-gel with synthetic 15% UVB filter (PC) and cream-gel containing 5% HER (CGHER). The in vitro FPS values correspond to anti-UVB protection, and those of the UVA/UVB ratio and critical point (λc) refer to anti-UVA protection.

	PC	CGHER
UVA/UVB	0.1	0.17
Λc	324.66	335.5
FPS	27.71 ± 0.06	28.59 ± 0.09

3.6. Ex Vivo Ocular Irritability Test of the Formulation Containing 5% Extract

When analyzing the degree of irritation of the chorioallantoic membranes of fertilized eggs, all the groups analyzed differed significantly from the negative control group. However, the differences were not enough to change the final classification of the analysis in relation to the negative control. Thus, all the analyzed samples did not demonstrate any harmful action on the membrane (Table 4).

Table 4. Mean ± SEM of the degree of irritation of the chorioallantoic membranes of fertilized chicken eggs after interaction with the formulations and their respective classifications.

Sample	Mean ± EPM *	Classification
NC	0.75	NI
PC	0.25 **	NI
C1	0.25 **	NI

NC—negative control, with the addition of distilled water only; PC—cream-gel without the addition of extract; and C1—cream-gel with 5% HER. NI—nonirritating. * All Mean Standard Error values were less than 0.005. ** indicates whether there was a significant difference between the samples and the negative control group ($p < 0.05$). A one-way ANOVA followed by Tukey's post hoc test was performed.

The results presented in Table 4 show that PC and C1 differed significantly in relation to the negative control group in terms of the average degree of irritation of the chorioallantoic membranes of eggs. However, according to the classification presented previously in Table 2, in the materials and methods, the differences were not sufficient to change the final classification of the analysis in relation to the negative control group. Thus, the analyzed samples were shown to be nonirritating.

3.7. Determination of the Stability of the Formulation Containing 5% Extract

Finally, considering the possibility of developing a phytocosmetic according to the demands of the international consumer market, the stability of the formulations was evaluated. In the centrifugation test and in the spreadability test, the base gel (the gel without the addiction of extract) and the HER cream-gel did not present any changes in the three storage conditions. Furthermore, the base gel and the HER cream-gel did not present any statistically significant differences.

In the pH analysis, the measured values of the base gel (the gel without the addition of extract) and the HER cream-gel did not present any statistically significant differences. In the pH analysis, the cream-gel showed a mean of 5.91 ± 0.05, and the base gel showed a mean of 5.74 ± 0.06. These values are within the threshold range of compatibility with the physiological pH of the skin, ranging from 5.5 to 7.3.

4. Discussion

Animal testing is being replaced by in vitro tests in cosmetic product development. The cream-gel analyzed in this study followed this trend [20,21].

Flavonoids are natural phenolic compounds found in plant roots [22]. Due to their proven potential antioxidant activity, these compounds contribute greatly to photoprotection and anti-aging for the skin [14,20,23]. In the extract derived from *Agave sisalana* roots, a significant concentration of flavonoids, particularly catechin and quercetin, was observed. These compounds are responsible for several crucial pharmacological activities, including antioxidant properties, which have been extensively reported [21].

The MTT method was used to evaluate the cytotoxicity of a cream-gel containing sisal root extract on cells of the NIH 3T3 lineage. The results showed that the extract was safe for use in a phytocosmetic product as it did not exhibit any harmful effects on the cells. The MTT test is a commonly used method of assessing the cytotoxicity of plant extracts as well as cosmetic formulations [11,24]. In a similar study conducted by Pegorin-Brasil et al. [11], a phytocosmetic containing Pequi residue extract was found to be noncytotoxic during evaluations conducted at 24, 48, and 72 h, which is consistent with our findings. Saewan [21], while studying extracts of caffeine and chlorogenic acid at concentrations of 0–5 mg/mL for 24 h in human dermal fibroblast (HDF) cells, found that concentrations lower than 2 mg/mL are not cytotoxic, a finding compatible with our study. Lee et al. [23] analyzed Bunge root extract from *Anemarrhena asphodeloides* at concentrations of 4–12 μg/mL, and they exhibited more than 95% cell viability in the HDF strain.

During testing, it was found that the cream-gel containing the HER did not generate any harmful signals on the chorioanonoid membrane compared to the negative control. The positive control was the only substance that caused any harmful signals to the membrane. This means that the cream-gel with 5% HER is safe and does not damage the membrane, which is consistent with the results of the MTT test, where only the plant extract was analyzed [25–27].

The study evaluated the antioxidant activity of an extract and formulation obtained from sisal root. The results showed that the extract and formulation have significant antioxidant potential. This is the first report demonstrating the antioxidant activity of sisal root extract. Behera [27] analyzed the antioxidant activities of the methanolic extract of *A. racemosus,* which were approximately 94.92% at concentrations ranging from 10 to 500 μg/mL, results similar to those obtained in our analysis. Gabriele et al. [28] found that the highest antioxidant activity (98.39 ± 0.56%) was observed at a concentration of 3.33 mg/mL. Li et al. [29] analyzed extracts from Aralia taibaiensis roots at a concentration of 1 mg/mL, achieving an elimination rate of 90.5 ± 1.4%. All the cited studies administered additional doses of phenolic compounds, suggesting that the observed pharmacological activity may be attributed to secondary metabolites, especially flavonoids, which are more abundant in the crude extract and enriched formulation [19,23,30–36].

As we age, our bodies undergo significant physiological changes that can lead to the development of secondary pathologies. To mimic protein glycation, we used the BSA-GLU and BSA-MGO methodologies. We found that both the crude extract and the formulation at all concentrations performed well when compared to the positive control used. Similar results have been reported in the literature for studies of formulations enriched with plant extract using the same protocol as our study [28–32]. Starowicz and Ziellinski [33] evaluated different spices and found that the most potent glycation inhibitors, according to the BSA-MGO assay, were Star Anise (88%), Cinnamon (85%), Allspice (81%), and Clove (79%), being analyzed at an initial concentration of 300 mg/mL.

Formulations that contain plant extracts rich in flavonoids are commonly used in photoprotective products. This is because flavonoids are known to have the ability to absorb solar radiation and act as antioxidants, which can enhance the overall protection of the product and neutralize free radicals that are produced in the skin after exposure to the sun. The 5% HER gel was found to have superior UVAB solar photoprotection (SPF-UVAB) compared to commercial sunscreen products. This is also compatible with other studies on animal bioactive and plant extracts. Batista et al. [34] evaluated formulations with red propolis and found the presence of isoflavones, and Kraokaew et al. [35] analyzed ethyl acetate extract from seaweed containing flavonoid compounds. Koch et al. [35],

in their characterization of formulations enriched with *Camellia sinensis* tea extract, state that there are flavonoids such as thearubigins (TRs), theaflavins (TFs), and theobrownins (TBs), among others, assessed in the same methodology [25,36–38]. Andrade et al. [39] valuated the hydroalcoholic extract of Bordeaux grape pomace, observing that this extract achieved an SPF of 12. A study conducted by Albuquerque et al. [40] analyzed a nontoxic hydroalcoholic extract of *Clariasia racemosa* at a concentration of 100 µg/mL, resulting in an SPF of 8.93 ± 0.5.

The formulation's stability was confirmed by methods demonstrating effective stability, pH compatibility with the skin, and an absence of changes after centrifugation [18,21,29,41]. It was possible to demonstrate that sustainability and developing the circular economy by producing a phytocosmetic enriched with discarded sisal roots are promising, and this study also proves to be pioneering in validating the pharmacological properties of sisal roots.

5. Conclusions

The extract has a high level of flavonoids, which provide significant antioxidant, antiglycating, and photoprotective activity. The cream-gel containing the extract was nontoxic and stable, indicating its potential for use in phytocosmetics with antioxidant, antiglycating, and photoprotective properties.

6. Patents

The patent for the HER gel was granted by Instituto Nacional da Propriedade Industrial (INPI, Brasília, Brazil) on 11 April 2023 with the process number BR 10 2021 019122 8 A2.

Author Contributions: All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript; G.d.S.M., J.A.R.F., L.T.S.d.C., L.P.G., A.M.V., N.A.Z. and L.d.S. conducted the experiments; G.d.S.M., V.F.X., B.d.C.S., L.V.C.d.A. and J.A.R.F. were involved with the chemical analysis of the extract and formulations and in vitro experiments. A.M.V., J.A.R.F. and L.d.S. were responsible for data discussion and manuscript correction. L.d.S., G.d.S.M. and V.F.X. were the senior researchers responsible for this work. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the FAPESP (São Paulo Research Foundation—Process nº 15/22314-2 and 13/09903-3).

Informed Consent Statement: The manuscript has been read and approved by all the authors; the requirements for authorship have been met; and all authors believe that the manuscript represents honest work.

Data Availability Statement: The data that support the findings of this research are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Trejo-Torres, J.C.; Gann, G.D.; Christenhusz, M.J.M. The Yucatan Peninsula Is the Place of Origin of Sisal (*Agave sisalana*, Asparagaceae): Historical Accounts, Phytogeography and Current Populations. *Bot. Sci.* **2018**, *96*, 366–379. [CrossRef]
2. Queiroz, S.R.d.O.D.; Ortolani, F.A.; Mataqueiro, M.F.; Osuna, J.T.A.; Moro, J.R. Chromosomal Analysis of Immature Bulbs of Sisal (*Agave* Spp.) Cultivated in Different Districts in Bahia, Brazil. *Acta Bot. Bras.* **2012**, *26*, 842–848. [CrossRef]
3. Cantalino, A.; Torres, E.; Silva, M. Sustainability of Sisal Cultivation in Brazil Using Co-Products and Wastes. *J. Agric. Sci.* **2015**, *7*, 64. [CrossRef]
4. Santos, E.M.C.; Silva, O.A.D. Sisal in Bahia—Brazil. *RM* **2017**, *16*, 1–13. [CrossRef]
5. Raya, F.T.; Quintanilha-Peixoto, G.; de Oliveira, A.B.; Marone, M.P.; dos Reis, W.J.V.; Marques, J.G.O.; Almeida, R.L.; Falcão Filho, C.A.T.; Costa, O.D.V.; Ribeiro, R.V.; et al. Molecular Epidemiology of Sisal Bole Rot Disease Suggests a Potential Phytosanitary Crisis in Brazilian Production Areas. *Front. Chem. Eng.* **2023**, *5*, 1174689. [CrossRef]
6. Hübner, A.A.; Demarque, D.P.; Lourenço, F.R.; Rosado, C.; Baby, A.R.; Kikuchi, I.S.; Bacchi, E.M. Phytocompounds Recovered from the Waste of Cabernet Sauvignon (*Vitis vinifera* L.) Vinification: Cytotoxicity (in Normal and Stressful Conditions) and In Vitro Photoprotection Efficacy in a Sunscreen System. *Cosmetics* **2023**, *10*, 2. [CrossRef]

7. Abbasi-Parizad, P.; De Nisi, P.; Scaglia, B.; Scarafoni, A.; Pilu, S.; Adani, F. Recovery of Phenolic Compounds from Agro-Industrial by-Products: Evaluating Antiradical Activities and Immunomodulatory Properties. *Food Bioprod. Process.* **2021**, *127*, 338–348. [CrossRef]
8. Gasparrini, M.; Forbes-Hernandez, T.Y.; Afrin, S.; Reboredo-Rodriguez, P.; Cianciosi, D.; Mezzetti, B.; Quiles, J.L.; Bompadre, S.; Battino, M.; Giampieri, F. Strawberry-Based Cosmetic Formulations Protect Human Dermal Fibroblasts against UVA-Induced Damage. *Nutrients* **2017**, *9*, 605. [CrossRef] [PubMed]
9. Araldi, R.P.; dos Santos, M.O.; Barbon, F.F.; Manjerona, B.A.; Meirelles, B.R.; de Oliva Neto, P.; da Silva, P.I.; dos Santos, L.; Camargo, I.C.C.; de Souza, E.B. Analysis of Antioxidant, Cytotoxic and Mutagenic Potential of *Agave sisalana* Perrine Extracts Using Vero Cells, Human Lymphocytes and Mice Polychromatic Erythrocytes. *Biomed. Pharmacother.* **2018**, *98*, 873–885. [CrossRef]
10. Dunder, R.J.; Luiz-Ferreira, A.; de Almeida, A.C.A.; de-Faria, F.M.; Takayama, C.; Socca, E.A.R.; Salvador, M.J.; Mello, G.C.; dos Santos, C.; de Oliva-Neto, P.; et al. Applications of the Hexanic Fraction of *Agave sisalana* Perrine Ex Engelm (Asparagaceae): Control of Inflammation and Pain Screening. *Mem. Inst. Oswaldo Cruz* **2013**, *108*, 263–271. [CrossRef]
11. Pegorin Brasil, G.S.; Borges, F.A.; Machado, A.d.A.; Mayer, C.R.M.; Udulutsch, R.G.; Herculano, R.D.; Funari, C.S.; dos Santos, A.G.; Santos, L. A Sustainable Raw Material for Phytocosmetics: The Pulp Residue from the *Caryocar brasiliense* Oil Extraction. *Rev. Bras. Farmacogn.* **2022**, *32*, 827–833. [CrossRef]
12. Subramanian, S.; Duraipandian, C.; Alsayari, A.; Ramachawolran, G.; Wong, L.S.; Sekar, M.; Gan, S.H.; Subramaniyan, V.; Seethalakshmi, S.; Jeyabalan, S.; et al. Wound Healing Properties of a New Formulated Flavonoid-Rich Fraction from *Dodonaea viscosa* Jacq. Leaves Extract. *Front. Pharmacol.* **2023**, *14*, 1096905. [CrossRef] [PubMed]
13. Lan, A.; Lui, Y.; Zuo, J.; Lohan, S.B.; Schanzer, S.; Wiemann, S.; Keck, C.M.; Lademann, J.; Meinke, M.C. Methodology to Reach Full Spectral Photo-Protection by Selecting the Best Combination of Physical Filters and Antioxidants. *Cosmetics* **2023**, *10*, 1. [CrossRef]
14. Guan, L.L.; Lim, H.W.; Mohammad, T.F. Sunscreens and Photoaging: A Review of Current Literature. *Am. J. Clin. Dermatol.* **2021**, *22*, 819–828. [CrossRef]
15. Kumar, P.; Nagarajan, A.; Uchil, P.D. Analysis of Cell Viability by the MTT Assay. *Cold Spring Harb. Protoc.* **2018**, *2018*. [CrossRef]
16. Sirivibulkovit, K.; Nouanthavong, S.; Sameenoi, Y. Paper-Based DPPH Assay for Antioxidant Activity Analysis. *Anal. Sci.* **2018**, *34*, 795–800. [CrossRef]
17. Mridula, S.; Masroor, W.S.; Xavier, M.; Hui, T.W.; Chan, H.K.; Chirara, K.; Nwabueze, O.P. Antioxidant and Anti-Advanced Glycation End Products Formation Properties of Palmatine. *J. Pharm. Pharmacogn. Res.* **2021**, *9*, 366–378. [CrossRef]
18. Fracasso, J.A.R.; Ibe, M.B.; da Costa, L.T.S.; Guarnier, L.P.; Viel, A.M.; de Brito, G.R.; Parron, M.C.; do Pereira, A.E.S.; Pegorin Brasil, G.S.; Farias Ximenes, V.; et al. Anti-Inflammatory Effect and Toxicological Profile of Pulp Residue from the *Caryocar brasiliense*, a Sustainable Raw Material. *Gels* **2023**, *9*, 234. [CrossRef] [PubMed]
19. Lebrun, S.; Nguyen, L.; Chavez, S.; Chan, R.; Le, D.; Nguyen, M.; Jester, J.V. Same-Chemical Comparison of Nonanimal Eye Irritation Test Methods: Bovine Corneal Opacity and Permeability, EpiOcular™, Isolated Chicken Eye, Ocular Irritection®, OptiSafe™, and Short Time Exposure. *Toxicol. In Vitro* **2021**, *72*, 105070. [CrossRef]
20. Popova, T.P.; Ignatov, I.; Petrova, T.E.; Kaleva, M.D.; Huether, F.; Karadzhov, S.D. Antimicrobial Activity In Vitro of Cream from Plant Extracts and Nanosilver, and Clinical Research In Vivo on Veterinary Clinical Cases. *Cosmetics* **2022**, *9*, 122. [CrossRef]
21. Saraiva, S.M.; Crespo, A.M.; Vaz, F.; Filipe, M.; Santos, D.; Jacinto, T.A.; Paiva-Santos, A.C.; Rodrigues, M.; Ribeiro, M.P.; Coutinho, P.; et al. Development and Characterization of Thermal Water Gel Comprising *Helichrysum italicum* Essential Oil-Loaded Chitosan Nanoparticles for Skin Care. *Cosmetics* **2023**, *10*, 8. [CrossRef]
22. Krzemińska, M.; Owczarek, A.; Gonciarz, W.; Chmiela, M.; Olszewska, M.A.; Grzegorczyk-Karolak, I. The Antioxidant, Cytotoxic and Antimicrobial Potential of Phenolic Acids-Enriched Extract of Elicited Hairy Roots of *Salvia bulleyana*. *Molecules* **2022**, *27*, 992. [CrossRef]
23. Saewan, N. Effect of Coffee Berry Extract on Anti-Aging for Skin and Hair—In Vitro Approach. *Cosmetics* **2022**, *9*, 66. [CrossRef]
24. Orsine, J.V.C.; Marques Brito, L.; Silva, R.C.; Santos Almeida, M.d.F.M.; Novaes, M.R.C.G. Cytotoxicity of *Agaricus sylvaticus* in Non-Tumor Cells (NIH/3T3) and Tumor (OSCC-3) Using Tetrazolium (MTT) Assay. *Nutr. Hosp.* **2013**, *28*, 1244–1254. [CrossRef]
25. Bouilloux, J.; Kiening, M.; Yapi, S.; Lange, N. Double-PEGylated Cyclopeptidic Photosensitizer Prodrug Improves Drug Uptake from In Vitro to Hen's Egg Chorioallantoic Membrane Model. *Molecules* **2021**, *26*, 6241. [CrossRef] [PubMed]
26. Tavakkoli, H.; Derakhshanfar, A.; Moayedi, J.; Poostforoosh Fard, A. Utilization of a Chicken Embryo Membrane Model for Evaluation of Embryonic Vascular Toxicity of Dorema Ammoniacum. *Avicenna J. Phytomed.* **2020**, *10*, 152–160. [PubMed]
27. Marquardt, C.; Matuschek, E.; Bölke, E.; Gerber, P.A.; Peiper, M.; V Seydlitz-Kurzbach, J.; Buhren, B.A.; van Griensven, M.; Budach, W.; Hassan, M.; et al. Evaluation of the Tissue Toxicity of Antiseptics by the Hen's Egg Test on the Chorioallantoic Membrane (HETCAM). *Eur. J. Med. Res.* **2010**, *15*, 204–209. [CrossRef] [PubMed]
28. Sugiura, S.; Minami, Y.; Taniguchi, R.; Tanaka, R.; Miyake, H.; Mori, T.; Ueda, M.; Shibata, T. Evaluation of Anti-Glycation Activities of Phlorotannins in Human and Bovine Serum Albumin-Methylglyoxal Models. *Nat. Prod. Commun.* **2017**, *12*, 1934578X1701201137. [CrossRef]
29. Mejía-Giraldo, J.C.; Scaiano, J.C.; Gallardo-Cabrera, C.; Puertas-Mejía, M.A. Photoprotection and Photostability of a New Lignin-Gelatin-Baccharis Antioquensis-Based Hybrid Biomaterial. *Antioxidants* **2021**, *10*, 1904. [CrossRef]

30. Arachchige, S.P.G.; Abeysekera, W.P.K.M.; Ratnasooriya, W.D. Antiamylase, Anticholinesterases, Antiglycation, and Glycation Reversing Potential of Bark and Leaf of Ceylon Cinnamon (Cinnamomum Zeylanicum Blume) In Vitro. *Evid.-Based Complement. Alternat. Med.* **2017**, *2017*, 5076029. [CrossRef]
31. Dedvisitsakul, P.; Watla-Iad, K. Antioxidant Activity and Antidiabetic Activities of Northern Thai Indigenous Edible Plant Extracts and Their Phytochemical Constituents. *Heliyon* **2022**, *8*, e10740. [CrossRef] [PubMed]
32. Hanif, M.; Khan, M.-A.; Rafey, M.R.A.; Zaman, A.; Aziz, I.; Amanullah; Amin, A. Inhibitive Efficacy of Nymphoides Indica Rhizome Extract on α-Glucosidase, and Cross-Link Formation of Advanced Glycation End Product. *J. Tradit. Chin. Med.* **2021**, *41*, 376–380. [CrossRef] [PubMed]
33. Starowicz, M.; Zieliński, H. Inhibition of Advanced Glycation End-Product Formation by High Antioxidant-Leveled Spices Commonly Used in European Cuisine. *Antioxidants* **2019**, *8*, 100. [CrossRef] [PubMed]
34. Batista, C.M.; de Queiroz, L.A.; Alves, Â.V.F.; Reis, E.C.A.; Santos, F.A.; Castro, T.N.; Lima, B.S.; Araújo, A.N.S.; Godoy, C.A.P.; Severino, P.; et al. Photoprotection and Skin Irritation Effect of Hydrogels Containing Hydroalcoholic Extract of Red Propolis: A Natural Pathway against Skin Cancer. *Heliyon* **2022**, *8*, e08893. [CrossRef] [PubMed]
35. Kraokaew, P.; Manohong, P.; Prasertsuksri, P.; Jattujan, P.; Niamnont, N.; Tamtin, M.; Sobhon, P.; Meemon, K. Ethyl Acetate Extract of Marine Algae, Halymenia Durvillei, Provides Photoprotection against UV-Exposure in L929 and HaCaT Cells. *Marine Drugs* **2022**, *20*, 707. [CrossRef] [PubMed]
36. Koch, W.; Zagórska, J.; Marzec, Z.; Kukula-Koch, W. Applications of Tea (*Camellia sinensis*) and Its Active Constituents in Cosmetics. *Molecules* **2019**, *24*, 4277. [CrossRef] [PubMed]
37. Grether-Beck, S.; Marini, A.; Jaenicke, T.; Krutmann, J. French Maritime Pine Bark Extract (Pycnogenol®) Effects on Human Skin: Clinical and Molecular Evidence. *Skin Pharmacol. Physiol.* **2016**, *29*, 13–17. [CrossRef] [PubMed]
38. Abiola, T.T.; Auckloo, N.; Woolley, J.M.; Corre, C.; Poigny, S.; Stavros, V.G. Unravelling the Photoprotection Properties of Garden Cress Sprout Extract. *Molecules* **2021**, *26*, 7631. [CrossRef] [PubMed]
39. Andrade, L.B.; Hollas, F.F.; Locatelli, C. Antioxidant and Photoprotector Effect from Grape Marc Hydroalcoholic Extract: In Vitro Assays/Efeito Antioxidante e Fotoprotetor Do Extrato Hidroalcoólico de Bagaço de Uva: Ensaios in vitro. *Braz. J. Dev.* **2021**, *7*, 10433–10443. [CrossRef]
40. de Albuquerque Nerys, L.L.; Jacob, I.T.T.; Silva, P.A.; da Silva, A.R.; de Oliveira, A.M.; da Rocha, W.R.V.; Pereira, D.T.M.; da Silva Abreu, A.; da Silva, R.M.F.; da Cruz Filho, I.J.; et al. Atividades Fotoprotetoras, Biológicas e Composição Química Do Extrato Hidroalcoólico Atóxico de *Clarisia racemosa* Com Aplicações Cosméticas e Farmacêuticas. *Ind. Crops Prod.* **2022**, *180*, 114762. [CrossRef]
41. Pegorin, G.S.; Marques, M.O.M.; Mayer, C.R.M.; Santos, L. Development of a Phytocosmetic Enriched with Pequi (*Caryocar brasiliense* Cambess) Oil. *Braz. Arch. Biol. Technol.* **2020**, *63*, e20190478. [CrossRef]