Revista Virtual de Química

Article

Rev. Virtual Quim. **2026**, *18(6)*, 877-890 – http://dx.doi.org/10.21577/1984-6835.20260067

©2026 Sociedade Brasileira de Química

Investigation of the Photoprotective, Antioxidant, and Antiproliferative Properties of Pink Pepper Extracts (*Schinus terebinthifolius* Raddi)

Investigação das Propriedades Fotoprotetoras, Antioxidantes e Antiproliferativas dos Extratos de Pimenta Rosa (*Schinus terebinthifolius* Raddi)

Gabriela Cristina F. Mota,[a] Rafaela Fabiane de Sousa,[b] Andréa M. do Nascimento,[a,b] Lucas R. D. Sousa,[c] Thays Helena C. Duarte,[d] Paula M. de A. Vieira,[d] Laissa L. S. Feitoza,[e] Paulo Michel P. Ferreira,[e,f] Dalton Dittz,[g] Ângela L. Andrade,[a,h] Carolinne G. da Costa,[b] Viviane M. R. dos Santos[a,b,*]

[a] Universidade Federal de Ouro Preto, Department of Chemistry, Graduate Program in Chemistry, Zip Code 35402-136 Ouro Preto-MG, Brazil

[b] Universidade Federal de Ouro Preto-UFOP, Department of Chemistry, Laboratory of Natural Products and Organic Synthesis, Zip Code 35402-136, Ouro Preto-MG, Brazil

[c] Universidade Federal de Ouro Preto, Department of Pharmacy, Graduate Program in Pharmaceutical Sciences, Phytotechnology Laboratory, Zip Code 35400-000, Ouro Preto-MG, Brazil

[d] Universidade Federal de Ouro Preto, Center for Research in Biological Sciences, Morphopathology Laboratory, Zip Code 35402-136, Ouro Preto-MG, Brazil

[e] Universidade Federal do Piauí, Petrônio Portella Campus, Department of Biophysics and Physiology, Laboratory of Experimental Cancerology, Zip Code 64049-550, Teresina-PI, Brazil

[f] Universidade Federal do Ceará, Center for Research and Development of Medicines, Department of Physiology and Pharmacology, Laboratory of Experimental Oncology, Zip Code 60430-275, Fortaleza-CE, Brazil

[g] Universidade Federal do Piauí, Department of Biochemistry and Pharmacology, Laboratory of Antineoplastic Pharmacology, Zip Code 64049-550, Teresina-PI, Brazil

[h] Universidade Federal de Ouro Preto, Department of Chemistry, Laboratory of Biomaterials, Zip Code 35402-136 Ouro Preto-MG, Brazil

E-mail: vivianesantos@ufop.edu.br

Submitted: April 27th, 2026 – **Accepted:** October 16th, 2026 – **Published online:** October 29th, 2026

Associate Editor Handled: Bárbara Vasconcellos Silva

In Brazil, the use of natural products, mainly those derived from plants, for medicinal, cosmetic, and other applications represents a broad field for scientific investigation. *Schinus terebinthifolius* Raddi, popularly known as pink pepper, Brazilian pepper, red pepper, or aroeira, has attracted considerable interest from research groups due to its pharmacological properties. Phytochemical studies of this species have revealed the presence of phenols, flavonoids, steroids, triterpenes, anthraquinones, and saponins. In this study, the ethanolic extract of pink pepper and the *n*-hexane and dichloromethane fractions obtained by liquid-liquid extraction were investigated regarding their photoprotective, antioxidant, and antiproliferative potential. The photoprotective capacity of the ethanolic extract and its *n*-hexane and dichloromethane fractions was evaluated *in vitro* by determining the sun protection factor (SPF). The results showed that photoprotective activity was proportional to the concentration used, with the ethanolic extract presenting the highest activity. Antioxidant capacity was assessed using the DPPH and ABTS methods, and the ethanolic extract showed greater antioxidant activity in both assays, exhibiting lower IC_{50} values. The ethanolic extract also demonstrated *in vitro* antiproliferative potential against tumor cells and showed no signs of cytotoxicity.

Keywords: Sun Protection Factor (SPF); *Schinus terebinthifolius* Raddi; pink pepper; cytotoxic action; fibroblasts.

1. Introduction

The ultraviolet radiation (UV) consists in the region of the electromagnetic spectrum emitted by the sun between wavelengths of 200 to 400 nanometers (nm). UV radiation is divided into three groups based on wavelength: UVC (200–290 nm), UVB (290–320 nm), and UVA (320–400 nm). Each of these groups penetrates the skin at a different depth and has the capacity to harm live tissue.[1] The UVC radiation is absorbed by the ozone layer in the stratosphere and represents a low percentage of sunlight at the surface of the Earth. The ozone layer absorbs a significant amount of UV-B light, which is more energetic than UVA radiation and makes up around 5% of UV radiation that reaches the Earth's surface. UVB radiation not only causes sunburn but also directly breaks the DNA of skin cells, which contributes significantly to the development of skin cancer.[2]

Sunscreens are topical formulations incorporating filters that protect our skin against UV radiation emitted by the sun. UV filters are divided into physical (inorganic)

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

and chemical (organic) filters depending on their physicochemical properties. Physical filters usually reflect and scatter UV radiation, while chemical filters absorb light.[3] The most crucial information for determining the effectiveness of a sunscreen is its Sun Protection Factor (SPF), widely recognized and defined as the value obtained by the ratio between the minimum erythema dose (MED) of skin protected with sunscreen, applied in the amount of 2 mg cm^{-2}, and the MED on the same skin without protection.[4] SPF represents the ratio of the time it takes for skin to develop erythema (redness/sunburn) with and without sunscreen. Since UVB radiation is the primary cause of acute skin erythema, the SPF calculation focuses almost exclusively on the UVB spectrum.[5] Natural compounds have attracted considerable attention for skin photoprotection due to their UV-absorbing, antioxidant, and anti-inflammatory properties, making them promising alternatives or complementary agents to conventional UV filters. These photoprotective effects have been reported for a wide range of natural products, including individual compounds such as resveratrol, ferulic acid, and curcumin; phytochemical classes such as flavonoids, carotenoids, and the silymarin complex; as well as complex natural matrices, including propolis and botanical oils.[3]

In addition to being challenging and expensive to conduct, the typical *in vivo* assay necessitates irradiating participants' skin in small areas. This truth has some ethical ramifications that should be considered.[5] Because of this, it has become crucial to rely on easier techniques to measure sunscreens' photoprotective qualities. The methodology developed by Mansur *et al.*[6] has been widely used for the *in vitro* determination of SPF values. This simple UV spectrophotometric assay, originally applied to alcoholic extracts of commercial sunscreens, has been employed in several studies.[5] Mansur *et al.*[6] proposed an accessible and practical screening method that remains one of the most commonly used approaches for the preliminary assessment of *in vitro* SPF within the UVB region, particularly in the pharmaceutical and cosmetic industries.

The species *S. terebinthifolius* Raddi, belonging to the Anacardiaceae family, is popularly known as pink pepper, red pepper, Brazilian pepper, mastic-red and mastic-pepper.[7-9] It is native to South America and mainly found in Brazil, Paraguay, and Argentina, and can be found in Europe, where it is cultivated as an ornamental species, Central and South America, mainly in Florida, where it has an invasive behavior.[8,10] It has evergreen leaves and its fruits are drupes that start out green and turn red as they ripen.[10]

The applications of pink pepper have been known for many years, and its properties were described in the first edition of the Brazilian Pharmacopeia, in 1926.[11] It is used in cooking, essentially to add flavor and refinement to the culinary dishes in the world, being especially suitable for making sauces to accompany white meats, poultry and fish, as it does not overpower their subtle taste.[12] In Brazil it is employed in popular medicine as antipyretic, analgesic, depurative and in the treatment of diseases of the urogenital system due to its pharmacological properties.[11] Pharmacological research using leaf extracts has revealed wound-healing, antioxidant, anti-allergic, antibacterial, anti-inflammatory, anti-ulcer, and anti-adherent qualities.[13] Antioxidant, antimicrobial, and anti-inflammatory properties have been attributed to *S. terebinthifolius* essential oil due to its rich and diverse chemical composition.[14]

Phenols, pentacyclic triterpenes, and anthraquinones were found in the ethanolic extract of *S. terebinthifolius* stem bark, according to Lima *et al*.[15] Phytochemical screening of the *n*-hexane extract obtained from the stem bark revealed the presence of flavones, xanthones, flavonoids, free steroids, anthraquinones, and pentacyclic triterpenes. Ethanolic leaf extract demonstrated favorable outcomes for phenols, flavones, flavonoids, xanthones, leucoanthocyanidins, flavanones, and free steroids.[15] The phytochemical study of the ethyl acetate fraction of the ethanolic leaf extract of *S. terebinthifolius* resulted in the isolation and identification of five active compounds: ethyl gallate, myricetrin, quercitrin, methyl gallate and myricetin, which contribute to the plant's antioxidant properties.[16] Additionally, the hydroalcoholic extract of *S. terebinthifolius* leaves has demonstrated significant anti-inflammatory effects in pleurisy and arthritis models, as evidenced by the marked reduction of zymosan-induced inflammatory mediators and the strong inhibition of neutrophil infiltration.[17]

The presences of secondary metabolites, mainly phenolic compounds, flavonoids, and terpenoids have been described in the literature, highlighting the antioxidant capacity of pink pepper. Phenolic phytochemicals are believed to have a positive effect on health, in part through their antioxidant and free radical scavenging effects, thus protecting cellular components from free radical-induced damage. Thus, for a substance to be defined as an antioxidant, it must be able to slow or inhibit the oxidation of oxidizable substrates and the activity of reactive oxygen species (ROS), mainly by reducing molecules. In this sense, regarding the antioxidant capacity, studies have demonstrated that the pink pepper extract presented antioxidant potential in DPPH, FRAP and ABTS assays.[18] Furthermore, *in vivo* analyses demonstrated the ability of *S. terebinthifolius* fruit to decrease the damage caused by oxidative stress promoted by hydrogen peroxide in *Saccharomyces cerevisiae* cells.[9] Therefore, pink pepper's potential application as a natural antioxidant for the cosmetics sector is indicated by the presence of phytochemicals with functional properties and its antioxidant capacity.

In this way, considering the need for the use of sunscreens against the harmful effects of solar radiation, as well as the tendency of natural products in the new cosmetic market, the realization of this work is justified by searching for a new alternative to natural-based sunscreens, as a way to promote the increase of the SPF without leaving the formulation with an irritating character. Brazil is the fourth largest consumer of cosmetic products in the world, but there has been little research on the pink pepper as a cosmetic in the country. Therefore, this work concentrates efforts on the study of photoprotection (SPF) of ethanolic extract from pink pepper (*S. terebinthifolius* Raddi) and on sunscreen formulations based on Pemulen TR-1 Gel UVA/UVB 5%, Pemulen TR-1 Gel and Polawax Cream. The selection of these three cosmetic bases was indeed carefully planned to analyze how different delivery systems affect the behavior of the active ingredients under study. In summary, the selection was based on: 1) Pemulen TR-1 Gel: a modern polymeric cosmetic emulsification system (gel-cream) characterized by low irritation potential and a dry feel; 2) Pemulen TR-1 Gel UVA/UVB 5%: enables the analysis of the behavior and stability of the cosmetic polymer matrix upon the addition of chemical sunscreens (highly lipophilic and polar substances); 3) Polawax Cream: serves as a traditional non-ionic cosmetic emulsion model, providing high emolliency and an occlusive barrier.

Therefore, this study assesses the photoprotective efficacy of *S. terebinthifolius* extract incorporated into three different cosmetic formulations. Ultimately, this research seeks to provide the green cosmetics sector a new, safer, and highly effective viewpoint.

2. Experimental

2.1. General conditions

The solvents used were ethanol 95%, dichloromethane and *n*-hexane acquired from New Química of the brands Synth and Vetec. The propylene glycol was purchased from NatureDerme Manipulation Pharmacy in Belo Horizonte, Minas Gerais, Brazil. The photoprotective formulations of Pemulen TR-1 Gel UVA /UVB 5% and Pemulen TR-1 Gel used in the analysis were obtained from BioFarma Manipulation Pharmacy in Itabirito, Minas Gerais, Brazil. The Polawax Cream was obtained from NatureDerme Pharmacy located in Belo Horizonte, Minas Gerais, Brazil. The Genesys 10S UV- VIS spectrophotometer and Thermo Scientific-Genesys 840 spectrophotometer coupled to a computer were used to determine the absorbance readings. For the chromatographic separation of the ethanolic extract, Polyamide CC6 (Macherey-Nagel, Germany, code 81561,) was used. Thin layer chromatography (TLC) (silica gel GF254; Merck) was used to analyze the 25 fractions obtained from column chromatography of the ethanolic extract.

Chemical composition of the base (Pemulen TR1 gel): purified water; polymeric agent (acrylate/C10-30 alkyl acrylate crosspolymer/Pemulen TR-1); neutralizing agent (pH adjustment to approximately 5.5–6.5) containing triethanolamine and sodium hydroxide; and a preservative system of phenoxyethanol and parabens. Chemical composition of the base (Pemulen TR1 gel 5% UVA/UVB): This base features the same polymeric structure as the previous gel but includes 5% solubilized chemical sunscreens—typically a combination of lipophilic and hydrophilic filters to cover both UVA and UVB rays. The sunscreens, constituting the 5% UVA/UVB fraction, include the UVB filter octyl methoxycinnamate (ethylhexyl methoxycinnamate), the UVA filters avobenzone (butyl methoxydibenzoylmethane) and octocrylene (a broad-spectrum filter). A light ester, such as dicaprylyl carbonate, is generally used to incorporate these filters into the Pemulen network. Chemical composition of the base (Polawax cream): the oil/emulsifier phase contains cetearyl alcohol and polysorbate 60 (Polawax wax); co-emollients include Paraffinum Liquidum and isopropyl myristate; and the formulation also contains purified water, glycerin, and a preservative system composed of phenoxyethanol, methylparaben, and propylparaben.

2.2. Plant material

Fruits of pink pepper (*S. terebinthifolius* Raddi) were collected in Ouro Preto, Minas Gerais, Brazil in April 2019. A voucher specimen was identified and deposited at the UFOP José Badini herbarium under the code OUPR31536.[19]

2.3. Preparation of ethanolic extract of pink pepper

The ethanolic extract was prepared by macerating 1.00 kg of fruits at room temperature with 95% ethanol (2 L, 2 consecutive extractions of 24 h each). The resulting mixture was filtered, and the solvent was evaporated under reduced pressure using a rotary evaporator to afford 123.64 g (12.4% yield) of crude ethanolic extract.[19]

2.4. Liquid-liquid extraction of the ethanolic extract using *n*-hexane and dichloromethane solvents

A part of the crude ethanolic extract (70.81 g) was dissolved in 100 mL of a 7:3 water/ethanol mixture and then a liquid-liquid extraction was performed in a separating funnel with the following solvents: first using *n*-hexane and then dichloromethane (4 consecutive extractions with

100 mL of each solvent). The fractions in *n*-hexane and in dichloromethane derived, were dried and weighed, the mass of the f *n*-hexane raction was 2.24 g (3.16% yield) and the mass of the dichloromethane fraction was 6.98 g (9.86% yield).

2.5. Fractionation of the ethanolic extract

A portion of the ethanolic extract (5.14 g) was purified by column chromatography using polyamide CC6 as the stationary phase (ratio between sample and adsorbent 1:3). Elution with ethanol 95% (100 mL), ethanol 95%-ethyl acetate 1:1 (100 mL) and ethyl acetate (50 mL) in decrescent gradient (because polyamide behaves as a reversed-phase stationary phase), afforded 25 fractions, which were monitored by TLC and pooled into 4 subfractions (PR-E1 to PR-E4). Subfraction PR-E1 (2.99 g, 58.2%), subfraction PR-E2 (0.20 g, 3.9%), subfraction PR-E3 (0.27 g, 5.3%) and subfraction 4 (0.06 g, 1.2%). The subfraction PR-E3 was chosen to identify its chemical composition due to its higher SPF value.

2.6. Phytochemical screening

Qualitative phytochemical screening was carried on the ethanolic extract of pink pepper fruits, as well as on the n-hexane and dichloromethane fractions obtained by liquid-liquid partitioning of the ethanolic extract, to identify the major classes of phytoconstituents, including alkaloids, flavonoids, phenolic compounds/tannins, saponins, terpenoids, carbohydrates, and anthraquinones.[20]

2.7. Evaluation of antioxidant activity

The antioxidant activity of ethanolic extract and dichloromethane partition from pink pepper was determined as the capacity of the antioxidants present in the sample to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2'-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS$^{\bullet+}$) (Sigma), applying a previously described methodology.[21,22] In both methods, the ethanolic crude extract was dissolved in ethanol to obtain stock solutions of approximately 200 μg mL^{-1}. For the DPPH method, different aliquots were taken out of the stock solution to obtain final solutions ranging from 0.3 to 10.0 μg mL^{-1}. This solution was homogenized with 1250 μL of DPPH solution (0.2 mmol L^{-1}). The negative control only used ethanol and DPPH. Quercetin was analyzed under the same experimental conditions as a positive antioxidant control. After 30 min at room temperature, protected from light, the spectrophotometric reading was done at 517 nm. Ethanol 70% (v/v) was used as control and subject to the same procedure. Experiments were done in triplicate. The percentage of free-radical inhibition (%I) was calculated using Equation 1.

$$\%\,I = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100 \quad (1)$$

where $Abs_{control}$ is the absorbance of the control and $Ab_{ssample}$ is the absorbance of the sample. The concentration required to obtain a 50% antioxidant effect (IC_{50}) was calculated by linear regression for samples.

To prepare the ABTS radical cation, an ABTS solution was oxidized in water by treating it with potassium persulfate (molar ratio = 1 : 0.35) for 12–16 h in the dark, and then diluted with ethanol, prior to the assays, to give an absorbance of 0.7 + 0.02 at 734 nm. A suitable amount of the sample (3.78 to 40.0 μg mL^{-1}) was added to the reagent and the mixture was incubated at 25 °C. Quercetin was used as a positive control antioxidant and evaluated under the same experimental conditions. Absorbance was measured after 6 min, and an ethanol blank was used for baseline correction. The percentage inhibition of absorbance at 734 nm was then calculated. The IC_{50} values, defined as the concentration required to scavenge 50% of the ABTS$^{\bullet+}$ radical, were determined by plotting percentage inhibition (I, %) against sample concentration. IC_{50} values (μg mL^{-1}) were calculated by linear regression using the linear portion of the concentration-response curve.

2.8. *In vitro* determination of the SPF

Ethanolic extract, the *n*-hexane fraction, the dichloromethane fraction, isolated natural compounds (quercetin and gallic acid) and subfractions PR-E1 to PR-E4, obtained from the fractionation of the ethanolic extract, were dissolved in ethanol (1 mg mL^{-1}) and diluted until obtaining concentrations of 0.020, 0.030, 0.050, 0.070 and 0.1 mg mL^{-1}. After the solution preparations, the absorbances sweepings were scanned in the UV-Vis spectrophotometer and the SPF values were obtained. The SPF model used in this study was according to the methodology described by of Mansur *et al.*[6] The absorbance of the samples was measured across the UVB wavelength range (290-320 nm) at 5 nm intervals, and each measurement was performed in triplicate. The SPF values were calculated using Mansur's equation (Equation 2).

$$SPF = FC \sum_{290}^{320} EE(\lambda) Abs(\lambda) \quad (2)$$

where FC (factor of correction) = 10; EE (λ) is the erythemal efficiency spectrum; I (λ) is the solar intensity spectrum; Abs (λ) is the absorbance of the solution.

2.9. Preparation of formulations and determination of the SPF *in vitro*

The ethanolic extract, the dichloromethane fraction, and the pure compounds (quercetin and gallic acid) were weighed and dissolved in a 1:1 mixture of 70% ethanol and propylene glycol. After solubilization, incorporations were made into Pemulen TR-1 UVA/UVB Gel 5%, Pemulen TR-1 Gel and Polawax Cream. The mixtures were placed on a stir plate in the presence of a magnetic stirrer to aid in the incorporation process. The incorporations remained under the stir plate for a period of 15 to 20 min. Subsequently, 1 g of each formulation of the ethanolic extract and the dichloromethane fraction, previously prepared, was weighed and transferred to a 10 mL volumetric flask. In the same way, 0.5 g of each formulation of the pure compound's quercetin and gallic acid was weighed and transferred to a 10 mL volumetric flask. Then, the formulations were diluted in a mixture of ethanol/propylene glycol at a ratio of 1:1. A solution containing only Pemulen TR-1 UVA-UVB 5% Gel, Pemulen TR-1 Gel and Polawax Cream was also made for the positive control of the study. After preparation of the solutions, the absorbances were swept in the UV-Vis spectrophotometer, obtaining the SPF values. All readings were performed in triplicate.

2.10. Silylation and Gas Chromatography–Mass Spectrometry (GC-MS) analysis of subfraction PR-E3

The subfraction PR-E3, obtained from the fractionation of the ethanolic extract, was chosen to identify its chemical composition due to its higher SPF value. In a glass vial with a screw cap, 5 mg of subfraction PR-E3 was weighed. To this glass were added 350 µL of the BSTFA reagent + 1% TMCS (N,O-bis(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane) and 65 µL of pyridine. The reaction occurred at 70 °C for 40 min with occasional stirring. After that, ethyl acetate was added to complete the volume of 1 mL and immediately followed by GC-MS analysis.

The chemical composition analysis of the subfraction PR-E3 was performed using a gas chromatography mass spectrometer (QP 2010 Ultra, Shimadzu, Kyoto, Japan) equipped with a fused silica capillary column Zebron (ZB-5MS 30 m × 0.25 mm × 0.25 µm, Phenomenex, Torrance, CA, USA). The relative percentages (% content) of all components were calculated by integrating their respective peak areas in the total ion chromatogram (TIC, *m/z* 35–600). Sample preparation involved combining the sample with ethyl acetate, as described above, followed by injection under the following conditions: helium (99.999%) as the carrier gas at a constant flow of 1.4 mL min^{-1}; an injection volume of 1 µL; an injector split ratio of 1:5; injector temperature of 250 °C; electron impact mode at 70 eV; ion-source temperature of 250 °C. The oven temperature was programmed from 60 °C (isothermal for 3 min), to increase from 60 °C to 320 °C in 20 min. The identification of the constituents present in the subfraction PR-E3 was accomplished by comparing the spectrum fragmentation pattern with those stored in Wiley 7 and NIST 11 databases.

2.11. Phenolic quantification

Total phenolic content was determined by the Folin-Ciocalteu method with slight modifications.[23] For quantification of total phenolics, 9.8 mg of the extract ethanol from pink pepper and the dichloromethane fraction were dissolved in 50 mL of 95% ethyl alcohol and 1.6 mL of this solution was mixed with 1.2 mL of deionized water and 0.2 mL of Folin–Ciocalteu reagent. The mixture was stirred for 1 min, followed by the addition of 0.8 mL of sodium carbonate solution (7.5% w/v). After stirring for 30 s, 0.2 mL of water was added and the mixture was incubated for 2 h. The absorption of the reaction mixture was measured at 725 nm against a 95% ethanol blank using a Genesys 10S UV-Vis spectrophotometer. The Gallic Acid (GA) was used as standard. A standard calibration plot was generated at 725 nm using known concentrations of GA ($y = 0.0374x - 0.0394$). Phenol concentrations in test samples are calculated from calibration chart and expressed in milligrams of GA phenol equivalent g^{-1} of sample.

2.12. Cell viability

Murine fibroblasts L-929 cells cultivated in RPMI 1640 medium (Sigma®) were distributed in 96-well microtiter plate (density of 5 x 10^4 cells). They were incubated at 37 °C with 5% of CO_2 for 24 h. Cells were treated with samples dissolved in 2% DMSO, at concentrations ranging from 62.5 to 250 µg mL^{-1}. Cell viability was evaluated using the sulforhodamine B assay (SRB).[24] After 24 and 48 h incubation the media was removed and cells were fixed with cold 20% trichloroacetic acid for 1 h at 4 °C. The microtiter plate was washed with distilled water and dried. Thereafter, fixed cells were stained for 30 min with 0.1% SRB dissolved in 1% acetic acid. The plate washed again with 1% acetic acid, again allowed to dry and 200 µL of 10 mmol L^{-1} Tris buffer (pH 10.5) were added to stain solubilization at room temperature for approximately

30 min. Samples absorbance was read in the spectrophotometer (490 nm) and the values were expressed as percentage of cell viability using GraphPad Prism 8.0.1 software.

2.13. Antiproliferative assay on tumor cells

The antiproliferative potential of the crude extracts was evaluated by Alamar Blue assay[25] against two murine tumor cells lineages (4-T1 breast carcinoma and B-16/F10 melanoma lines), all obtained from the cell bank of the Federal University of Rio de Janeiro (BCRJ, Rio de Janeiro, Brazil). Both lineages were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U mL^{-1} penicillin and 100 µg mL^{-1} streptomycin (Cultilab™, Brazil) at 37 °C and 5% CO_2. The cells were plated in 96-well plates, incubated to allow cell adhesion at 37 °C, 5% CO_2 and 95% humidity (Incubator, Shel Lab CO_2, USA), and crude extract was added to each well (0.1-500 µg mL^{-1}). Twenty-four hours before the end of the incubation (72 h), 20 µL of stock solution (0.156 mg mL^{-1}) of Alamar Blue were added to each well. Subsequently, the absorbance was measured at 570 nm and 595 nm using a multiplate reader and the drug effect was quantified as the percentage of the control (GloMax, Progema, EUA).[25] Negative and positive controls cells and those cells treated with crude extract were exposed to the same DMSO percentage (0.1%). Doxorubicin (0.3 µg mL^{-1} Sigma Aldrich, EUA) was used as positive control. Negative and positive controls were exposed to the same DMSO percentage (0.1%). The values of IC_{50} [mean inhibitory concentration capable of causing 50% of the maximum effect ± standard deviation (S.D.)] were calculated using the GraphPad Prism software, version 9.0.

3. Results and Discussion

3.1. Phytochemical screening

The tests were based on the color changes after the reaction of the extract with standard reagents to detect secondary metabolites. The appearance of a red-orange spot in the Dragendorff test was considered a positive result for the presence of alkaloids. An intense yellow color indicated the presence of flavonoids. Brownish green or a blue-black color indicated the presence of tannins and phenols. A stable persistent froth indicated the presence of saponins. A reddish-brown coloration of the interface was formed to indicate positive results for the presence of terpenoids. A dark blue/purple coloration indicates the presence of carbohydrates and the presence of a pink, red or violet color in the ammoniacal (lower) phase indicates the presence of free anthraquinones.

The results of the phytochemical screening are summarized in Table 1. The ethanolic extract of pink pepper fruits showed a positive result for flavonoids, phenols/tannins and terpenoids. Previous UPLC-QTOF-MS/MS studies reported in the literature identified flavonoids and phenolic compounds in this ethanolic extract, aligning with the results of our qualitative screening.[19] The dichloromethane fraction, obtained by liquid-liquid extraction of the ethanolic extract, tested positive for flavonoids, phenols/tannins, and terpenoids, whereas the *n*-hexane fraction, also obtained from liquid-liquid extraction of the ethanolic extract, showed a positive result only for terpenoids.

3.2. *In vitro* determination of the SPF

Based on the data presented in Table 2, it can be seen that the ethanolic extract of pink pepper presented higher SPF values consistent with the phenolic compounds and flavonoids identified in the phytochemical screening of the extract. According to literature data, chemical analysis of pink pepper ethanolic extract by UPLC-QTOF-MS/MS allowed the identification of its components, categorizing the compounds into three main groups: gallic acid derivatives, flavonoids, and catechins.[19] The analysis of the SPF of gallic acid and quercetin, compounds present in the ethanolic extract of pink pepper, demonstrated high values of photoprotection, being possibly responsible for the higher SPF value of the ethanolic extract of pink pepper when compared with the dichloromethane fractions and *n*-hexane. In relation to liquid-liquid extraction, it is

Table 1. Phytochemical screening of the ethanolic extract and the dichloromethane and *n*-hexane fractions from pink pepper

Phytochemicals	Ethanolic Extract[a]	Dichloromethane Fraction[a]	Hexane Fraction[a]
Alkaloids (Dragendorff's test)	(–)	(–)	(–)
Flavonoids (alkaline reagent test)	(+)	(+)	(–)
Phenols/Tannins (ferric chloride test)	(+)	(+)	(–)
Saponins (froth test)	(–)	(–)	(–)
Terpenoids (Salkoski test)	(+)	(+)	(+)
Carbohydrate (iodine test)	(–)	(–)	(–)
Anthraquinones (Borntrager's test)	(–)	(–)	(–)

[a]Key: + = present ; – = not detected.

Table 2. SPF values of the pink pepper ethanolic extract, *n*-hexane and dichloromethane fractions, and isolated compounds

Concentration (mg mL^{-1})	SPF Ethanolic Extract	SPF Gallic Acid	SPF Quercetin	SPF *n*-Hexane Fraction	SPF Dichloromethane Fraction
0.020	2.5264 ± 0.0075	2.6300 ± 0.0021	5.0641 ± 0.0022	0.3338 ± 0.0100	1.7950 ± 0.0065
0.030	3.2303 ± 0.0299	4.3755 ± 0.0036	7.9957 ± 0.0678	0.2505 ± 0.0043	1.9789 ± 0.1613
0.050	3.7646 ± 0.0428	6.9608 ± 0.0041	13.7637 ± 0.0041	0.2677 ± 0.0634	2.5622 ± 0.0022
0.070	5.7510 ± 0.0045	10.1446 ± 0.0159	18.5198 ± 0.0888	0.3061 ± 0.0032	2.8679 ± 0.0065
0.1	7.7599 ± 0.0139	14.3754 ± 0.0670	27.4117 ± 0.2285	0.5414 ± 0.0043	4.8033 ± 0.0157

Data are presented as SPF values means ± standard deviation (S.D.)

noted that the dichloromethane fraction presents a higher SPF value, compared to the *n*-hexane fraction, supposedly due to the presence of flavonoids and phenols/tannins that were not identified in the *n*-hexane fraction. Moreover, it was observed that both the dichloromethane fraction and the ethanolic extract of pink pepper, at a concentration of 0.1 mg mL^{-1}, showed a higher SPF value compared to the other concentrations. This fact is justified since it presents a higher concentration of extract and consequently provides greater photoprotective efficacy.

The SPF values of the subfractions obtained from the fractionation of the pink pepper ethanolic extract are described in Table 3. The PR-E3 subfraction showed the highest SPF value at a concentration of 0.1 mg mL^{-1}. The increase in the photoprotective activity of the isolated subfractions in relation to the pink pepper ethanolic extract demonstrates that the chromatographic separation was efficient, increasing the concentration of photoprotective compounds in the subfractions, which increased the SPF value due to greater purity when compared to the raw extract.

3.3. Preparation of formulations and determination of the SPF *in vitro*

Based on the data presented in Table 4, it can be seen that the ethanolic extract of pink pepper incorporated to Pemulen TR-1 Gel formulation 5% UVA/UVB showed a higher SPF value, followed by the incorporation with Pemulen TR-1 Gel and Polawax Cream. The results found with the incorporation of the ethanolic extract of pink pepper and the dichloromethane fraction to the formulations of Pemulen TR-1 UVA/UVB 5% Gel, Pemulen TR-1 Gel and Polawax Cream demonstrated that the pink pepper promoted

Table 3. SPF values of the isolated subfractions of the fractionation of the pink pepper ethanolic extract

Concentration (mg mL^{-1})	SPF PR–E1	SPF PR–E2	SPF PR–E3	SPF PR–E4
0.020	0.3625 ± 0.0027	1.1570 ± 0.0034	2.5815 ± 0.0022	2.0343 ± 0.0011
0.030	0.5263 ±0.0092	1.7279 ± 0.0028	3.8609 ± 0.0011	3.1876 ± 0.0050
0.050	0.8258 ± 0.0025	2.8312 ± 0.0014	5.9896 ± 0.0056	5.2863 ± 0.0187
0.070	1.1481 ± 0.0047	4.0832 ± 0.0049	8.5179 ± 0.0763	7.0709 ± 0.0081
0.1	1.6012 ± 0.0005	5.6188 ± 0.0079	12.1959 ± 0.0327	10.2399 ± 0.0105

Data are presented as SPF values means ± standard deviation (S.D.)

Table 4. SPF values of the ethanolic extract and dichloromethane fraction in the formulations of the Gel Pemulen TR-UVA/UVB 5%; Gel Pemulen TR-1 and Creme Polawax at concentration 0.1 g mL^{-1}

Formulations	SPF
Pemulen TR-1 Gel	9.8289 ± 6.8027
Pemulen TR-1 Gel formulation 5% UVA/UVB	28.9694 ± 1.3159
Polawax Cream	10.7705 ± 5.3119
Ethanolic extract in Pemulen TR-1 Gel 5% UVA/UVB	33.1018 ± 0.4116
Ethanolic extract in Pemulen TR-1 Gel	24.1730 ± 1.2872
Ethanolic extract in Polawax Cream	19.9046 ± 7.7978
Dichloromethane fraction Pemulen TR-1 Gel 5% UVA/UVB	32.8190 ± 0.6513
Dichloromethane fraction Pemulen TR-1 Gel	17.5588 ± 1.6880
Dichloromethane fraction Polawax Cream	28.5290 ± 2.6843

Data are presented as SPF values means ± standard deviation (S.D.)

the intensification of the SPF values of the formulations, ensuring greater protection against solar radiation.

The values are consistent with the solubility of each of the bases used in the formulation, as Pemulen TR-1 Gel better incorporates polar substances while Polawax Cream better incorporates non-polar substances. The difference between the affinity of the gel and the cream explains why the dichloromethane fraction incorporated into Polawax Creme presents a significant SPF value due to greater solubility.

The incorporations of the natural compounds quercetin and gallic acid obtained excellent SPF values even at lower concentrations, shown in Table 5, which justifies the ethanolic extract of pink pepper having excellent SPF compared to the others because of presence of these substances in its composition. The lower concentration of formulations with pure compounds is due to their high photoprotective activity, which prevents their reading on a UV-Vis spectrophotometer caused by the high absorption in the UV region. Its SPF values follow the consistency of solubility in Pemulen TR-1 Gel and Polawax Cream, with higher results in the gel due to greater affinity, corroborating the values initially found.

Interestingly, the individual incorporation of gallic acid and quercetin into the formulations resulted in remarkably high SPF values, even at concentrations lower than those employed for the crude extract. This observation reinforces the contribution of these phenolic constituents to the photoprotective activity of *S. terebinthifolius*. Nevertheless, the superior performance of the ethanolic extract is likely associated with the combined action of different phenolic compounds naturally present in the extract, suggesting a synergistic effect among these constituents. Such synergism has been widely reported for plant extracts and may explain why the crude extract simultaneously exhibited high antioxidant activity and excellent photoprotective performance.[19]

Both the U.S. Food and Drug Administration (FDA) and the European Union recommend that photoprotective formulations contain ingredients capable of providing SPF values greater than 15 to ensure adequate protection against harmful UV radiation.[18] Therefore, the two extracts investigated in this study can be incorporated into Polawax cream at a concentration of 0.1 g mL^{-1}, contributing to an increase in the formulation's sun protection capacity.

3.4. Evaluation of antioxidant activity

Antioxidant activity is correlated with photoprotective activity, since compounds capable of absorbing UV radiation can also neutralize reactive oxygen species and free radicals generated as a result of UV exposure. Thus, the ethanolic extract of pink pepper and the dichloromethane fraction were tested for their antioxidant activity as they presented the best photoprotective activities. The free radical-scavenging activity of the samples was determined by the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods and the results are shown in Table 6.

The results shown in Table 6 demonstrate the antioxidant potential and phenolic quantification of the extracts used in this study. Based on these findings, we can conclude that the ethanol extract from pink pepper displayed lower IC_{50} values in both assays, indicating higher antioxidant potential, compared to the dichloromethane fraction.

Table 5. SPF values of the gallic acid and quercetin in the formulations of the Gel Pemulen TR-UVA/UVB 5%, Gel Pemulen TR-1 and Creme Polawax at concentration 0.05 g mL^{-1}

Formulations	SPF
Quercetin in Pemulen TR-1 Gel 5% UVA/UVB	42.6838 ± 1.4128
Quercetin in Pemulen TR-1 Gel	20.5744 ± 0.7040
Quercetin in Polawax Cream	7.4123 ± 2.0067
Gallic acid in Pemulen TR-1 Gel 5% UVA/UVB	37.4141 ± 1.9642
Gallic acid in Pemulen TR-1 Gel	18.5135 ± 0.5915
Gallic acid in Polawax Cream	8.6846 ± 1.3015

Data are presented as SPF values means ± standard deviation (S.D.)

Table 6. Antioxidant activity and phenolic quantification of the ethanolic extract from pink pepper and dichloromethane fraction

Samples From Pink Pepper	IC_{50} (μg mL^{-1})		Phenolic Quantification (mg GA g^{-1} of Sample)
	DPPH	ABTS	
Ethanolic extract	2.7 ± 0.1	2.9 ± 0.3	8.82 ± 0.01
Dichloromethane fraction	67.5 ± 0.1	74.4 ± 0.1	7.04 ± 0.01
Quercetin (control)	0.3 ± 0.01	0.24 ± 0.02	

IC_{50}: concentration of a sample required to inhibit 50% of a radical Data are presented as mg GA g^{-1} of sample values means ± standard deviation (S.D.).

This was expected since the more polar extracts generally exhibit higher antioxidant activities, thus providing justification for the observed values. The reduction of DPPH molecules appears to be correlated with the presence of available hydroxyl groups.[21] It is strongly suggested that the DPPH free radical abstracts the phenolic hydrogen from the molecule that donates electrons, which could serve as a general mechanism for the scavenging action of antiperoxidative flavonols, among others.[21] Based on the mechanism of reduction of the DPPH molecule extensively described in the literature[26-28] that is correlated with the presence of hydroxyl groups on the antioxidant molecule, we can infer that the very good activity of the polar extracts is probably due to the presence of substances with an available hydroxyl group (phenolic or not). This structural requirement could be linked to the presence of flavonols or condensed tannins, which are known to occur in plant species belonging to the Anacardiaceae family, for example.[29] Furthermore, the antioxidant activity of the extract may be related to its photoprotective activity.

3.5. Phenolic quantification

Phenolic quantification corroborates the photoprotective and antioxidant activities, demonstrated in the Table 6, in which the ethanolic extract of pink pepper presented the highest concentration of phenols, obtaining a value of 8.82 mg GA g^{-1} of sample, which justifies its greater photoprotective activity since phenolic compounds have high absorption in the region UV due to its unsaturations and hydroxyl groups.[30]

These findings are further supported by the phenolic profile previously reported by our research group for the same ethanolic extract of *S. terebinthifolius* fruits, in which HPLC analysis identified gallic acid, quercetin, rutin, catechin, chlorogenic acid, caffeic acid, and kaempferol as the major phenolic constituents.[19] These compounds are well recognized for their antioxidant properties due to the presence of hydroxyl groups capable of donating hydrogen atoms or electrons, while their conjugated aromatic structures also exhibit strong absorption in the UV region. Therefore, the higher total phenolic content observed for the ethanolic extract is consistent with both its superior antioxidant activity and its higher SPF values.

Taken together, the results obtained in this study demonstrate a close relationship between phenolic composition, antioxidant activity, and photoprotective performance. The ethanolic extract, which exhibited the highest total phenolic content, also showed the lowest IC_{50} values in the DPPH and ABTS assays and the highest SPF values among the evaluated extracts. Moreover, gallic acid and quercetin, previously identified in the same ethanolic extract by HPLC analysis, displayed remarkably high SPF values when individually incorporated into the formulations. These findings provide complementary evidence that phenolic constituents play a major role in both the antioxidant and photoprotective properties of *S. terebinthifolius*, although synergistic interactions among the different phenolic compounds naturally present in the extract are also likely to contribute to its overall biological activity.

3.6. Cell viability

Assessing the cell viability of extracts, partitions and fractions is important in order to comprehend potential toxic effects and determine safe dosages for the intended application.[31] In this research, ISO 10993-5 of 2009 was used as a reference, which recommends that samples are considered cytotoxic when viability is less than 70%.[32] Thus, L-929 fibroblasts are a murine fibroblast cell line widely used *in vitro* investigations. Such cells have been employed in a variety of studies related to cell biology, wound healing and evaluation of cytotoxicity of drugs, materials or extracts.[33,34]

The ethanolic extract did not exhibit cytotoxicity towards L-929 cells after 24 h and 48 h of treatment. In contrast, the dichloromethane fraction was cytotoxic at 250 μg mL^{-1} after 24 h of treatment and at 125 and 250 μg mL^{-1} after 48 h of treatment (Figure 1).

A comparison with existing literature reveals similarities. The study of authors who developed sodium alginate hydrogels to obtain a nano-based semi-solid formulation for application on the skin using the hydroalcoholic extract of *S. terebinthifolius* Raddi leaves demonstrated the absence of toxicity against L-929 fibroblasts in the concentration range of 0.025 mg mL^{-1} and 10 mg mL^{-1}. This finding corroborates the study's results and suggests the safety of this type of extract for topical use.[35] Furthermore, the dichloromethane fraction in the study showed toxicity only at concentrations higher than those necessary to achieve photoprotective and antioxidant activity, which suggests that it could be safe for its intended purpose.

3.7. Antiproliferative assay on tumor cells

From the cell viability assay, it was determined that the ethanolic extract of pink pepper has the lowest toxicity offering the greatest potential for selective antiproliferative activity. The IC_{50} values for pink pepper ethanolic extract determined by the Alamar Blue assay are summarized in Table 7. The results demonstrated moderate *in vitro* antiproliferative potential. According to the U.S. National Cancer Institute (NCI), an $IC_{50} \leq 30$ μg mL^{-1} is a desirable result for considering a crude extract a

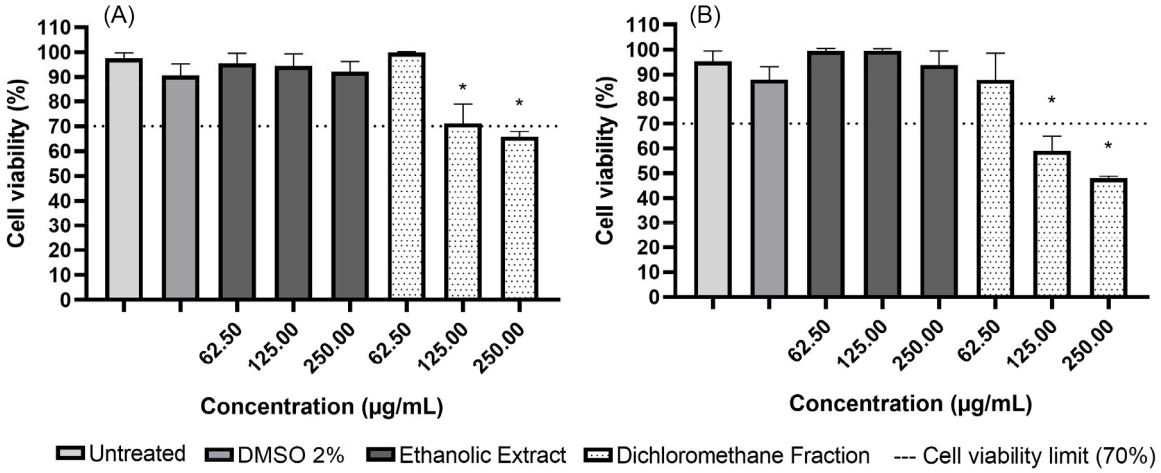


Figure 1. Cell viability of L-929 fibroblasts after 24 h (A) and 48 h (B) of treatment with ethanolic extract and dichloromethane fraction; *: significant difference in relation to untreated group ($p < 0.05$), according to a one-way ANOVA test with Dunnett's multiple comparisons post test; Results expressed as the mean of triplicates ± standard deviation

Table 7. *In vitro* antiproliferative activity of the sample after 72 h of incubation

Samples	IC_{50} (µg mL^{-1})	
	4-T1	B-16/F10
Ethanolic extract from pink pepper	46.54 ± 1.05	44.40 ± 1.07
Doxorubicin	0.40 ± 0.10	0.02 ± 0.01

Data are presented as IC_{50} values ± standard deviation (S.D.) for murine lines (4-T1, breast and B-16/F10, melanoma). All experiments were performed in duplicate. Doxorubicin was used as positive control.

promising substance for further purification and biological analysis.[36-38] Conversely, a previous study reported that the methanolic extract of *S. terebinthifolius* leaves and its isolated compounds showed selectivity toward ovarian cancer cell lines (NCI-ADR/RES; OVCAR-3), with growth inhibitory activity values ranging from 1.9 to 6.5 mg mL^{-1}.[13] In a previous study conducted by our research group, quercetin was identified as one of the components present in the ethanolic extract of pink pepper, by UPLC-QTOF-MS/MS.[19] Quercetin is a natural flavonoid with well-established antiproliferative activities against a variety of cancers.[36] In the study of Zhaorigetu *et al*.[37], quercetin was examined against lung human cancer cells using A549 cell lines and showed good anti-proliferative action with IC_{50} values on A549 being 8.65, 7.96 and 5.14 µg mL^{-1} at 24, 48 and 72 h respectively. Quercetin showed a high antiproliferative activity against human tumor cell lines HL-60 (promyelocytic leukemia) presenting 90.86% inhibition of cell growth.[38] So, quercetin is partly responsible for *in vitro* antiproliferative findings of the pink pepper ethanolic extract.

3.8. Silylation and Gas Chromatography–Mass Spectrometry (GC-MS) analysis of subfraction PR-E3

Silylation of organic compounds is the classical and most widely used derivatization procedure to facilitate analysis by GC-MS.[39] In silylation, the active hydrogen in organic molecules is easily substituted by an alkylsilyl group, generally trimethylsilyl (TMS). Compared to their parent compounds, silyl derivatives are often more volatile and thermally stable and less polar.[40] The identification of the compounds present in the subfraction PR-E3, obtained from the fractionation of the ethanolic extract, was possible by comparing the retention times of the silylated compounds with those of the standards and the spectral data obtained from the Wiley 7 and Nist 11 libraries. From the GC-MS profiling, 67 phytochemicals were detected in the subfraction PR-E3, which led to the identification of 2 phenolic compounds; the other compounds are non-phenolic (Figure S1, Supporting Information). The results obtained are recorded in Table 8. Phenolic compounds are well known in the literature for their high photoprotective activity, caused by the high absorption in the UV region of conjugated unsaturations and the hydroxyl groups attached to these unsaturations.[41] It is possible that important photoprotective constituents were not detected by GC-MS due to low volatility or thermal instability. In a previous study by our research group, some phenolic compounds were identified in the ethanolic extract from pink pepper by UPLC QTOF–MS/MS.[19]

The phenolic compounds identified in the subfraction PR-E3, by GC-MS, were 3-hydroxybenzoic

Table 8. Compounds detected in the subfraction PR-E3

Peak	Retention Time (min)	Name of the Compound	Base *m/z*	Formula
28	17.634	3-Hydroxybenzoic acid	266.95	$C_7H_6O_3$
66	33.642	1,8-Dihydroxyanthraquinone	368.90	$C_{14}H_8O_4$

acid and 1,8-dihydroxyanthraquinone (Table 8). The 1,8-dihydroxyanthraquinone, also known danthron, is a compound found in natural sources like rhubarb and is known for its role in inhibiting inflammatory processes, specifically by targeting the NLRP3 inflammasome.[42] It is a derivative of anthraquinone, a class of compounds known to possess UV-absorbing properties, however, danthron is better viewed as a photosensitive anticancer agent, not as a compound that shields skin or tissues from UV damage.[43] The compound 3-hydroxybenzoic acid has no photoprotective activity described in the literature. These results suggest that the photoprotective action of the PR-E3 subfraction was caused by some phenolic compound that was not identified by GC-MS.

Phytochemical investigations of *S. terebinthifolius* Raddi extracts have identified phenolic compounds as their primary constituents. Due to their ability to scavenge reactive oxygen and nitrogen species and absorb UV radiation, these compounds exhibit potent antioxidant and photoprotective activities. Exposure to UVA (320–400 nm) and UVB (290–320 nm) radiation causes irreversible cutaneous damage, including photoaging and skin cancer. Additionally, UVB radiation triggers oxidative stress and DNA damage, leading to erythema, sunburn, and immune dysfunction. Consequently, utilizing sunscreens is a crucial preventive strategy, and there is a growing interest in incorporating natural, antioxidant-rich extracts into these formulations.[44]

4. Conclusions

The present study has demonstrated the importance of using plant extracts in photoprotection preparations incorporated to photoprotective formulations, since an increase in the SPF value was observed when the incorporation of the ethanolic extract of pink pepper was performed, resulting in a greater protection against solar radiation. Given the data presented through the spectrophotometry of the Mansur method, it was possible to verify that the ethanolic extract of pink pepper has significant photoprotective activity. Moreover, in this work it was shown that the ethanolic extract of pink pepper demonstrated a potentiating effect on the photoprotective activity of the formulations of Pemulen TR-1 UVA/UVB 5% Gel, Pemulen TR-1 Gel and Polawax Cream, with the photoprotective effect being more effective in the formulation of Pemulen TR-1 UVA/UVB 5% Gel. Therefore, the use of natural substances such as ethanolic extract of pink pepper in sunscreen formulations becomes of paramount importance, since it was observed an increase in SPF values associated with the formulations used in this research. The crude ethanolic extract of pink pepper did not show cytotoxicity toward L-929 fibroblasts under the tested conditions. Thus, even though the SPF values obtained via the Mansur method provide an important indication of the filters' intrinsic photoprotective potential and their synergy within the formulation, they should not be considered equivalent to clinical SPF. They serve as an initial guide for formula optimization, where as *in vivo* testing is absolutely essential to substantiate clinical efficacy and for regulatory product labeling. Although the incorporation of plant extracts into the formulations led to a promising increase in the *in vitro* SPF suggesting the potential of these natural matrices as photoprotective agents or synergistic adjuvants—these findings remain preliminary. Due to experimental limitations, such as the absence of long-term physicochemical stability assays, organoleptic analyses, compatibility tests, or *in vivo* clinical efficacy evaluations, the current results do not yet support immediate practical application in commercial cosmetic products or advanced pharmaceutical systems.

Supporting Information

Supporting Information (chromatogram and mass spectra) is available free of charge at https://rvq.sbq.org.br/pdf/AR2026-5186-MS.

Acknowledgments

We are grateful to the Federal University of Ouro Preto (UFOP) and the Dean of Research, Graduate Studies and Innovation at the Federal University of Ouro Preto (PROPPI/UFOP). Dr. Paulo Michel Pinheiro Ferreira thanks the Brazilian agency "Conselho Nacional de Desenvolvimento Científico e Tecnológico" for his personal scholarship (CNPq #304803/2022-7).

Author Contributions

Gabriela Cristina Ferreira Mota - Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, and Validation. Rafaela

Fabiane de Sousa - Data curation, Investigation. Andréa Mendes do Nascimento- Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, Validation, Formal analysis, Funding acquisition and Project administration. Lucas Resende Dutra Sousa - Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, and Validation. Thays Helena Chaves Duarte - Data curation, Investigation. Paula Melo de Abreu Vieira - Data curation, Investigation. Laissa Lindes Sousa Feitoza - Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, and Validation. Paulo Michel Pinheiro Ferreira - Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, and Validation. Dalton Dittz - Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, and Validation. Ângela Leão de Andrade - Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, Validation, Supervision and Resources. Carolinne Gomes da Costa: Conceptualization, Data curation, Investigation, Visualization, Writing – original draft. Viviane Martins Rebello dos Santos - Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, Validation, Formal analysis, Funding acquisition, Project administration and Supervision.

Bibliographic References

1. Sullivan, M.; Obezo, C. G.; Lipsky, Z.; Panchal, A.; Jensen, J.; Frontiers in Topical Photoprotection. *Cosmetics* **2025**, *12*, 96. [Crossref]
2. Mmbando, G. S.; Ngongolo, K.; Environmental & health impacts of ultraviolet radiation: current trends and mitigation strategies. *Discover Sustainability* **2024**, *5*, 436. [Crossref]
3. Milutinov, J.; Pavlovic, N.; Cirin, D.; Krstonosic, M. A.; Krstonosic, V.; The potential of natural compounds in UV protection products. *Molecules* **2024**, *29*, 5409. [Crossref] [PubMed]
4. Schalka, S.; dos Reis, V. M. S.; Sun protection factor: meaning and controversies. *Anais Brasileiros de Dermatolologia* **2011**, *86,* 507. [Link] [PubMed]
5. Mesías, L. G. G.; Qwisgaard, A. M. R.; Untiveros, G. P. C.; Kobayashi, L. A. P.; Shimabukuro, L. E. M.; Sugahara, A. A. K.; Comparison of the photoprotective effects of sunscreens using spectrophotometric measurements or the survivability of yeast cells exposed to UV radiation. *Revista de la Sociedad Química del Perú* **2017***, 83*, 294. [Crossref]
6. Mansur, J. S.; Breder, M. N. R.; Mansur, M. C. A.; Azulay, R. D.; Determinação do fator de proteção solar por espectrofotometria. *Anais Brasieleiros de Dermatologia* **1986**, *61*, 121. [Link]
7. Oliveria, K. C.; Franciscato, L. S. S. M.; Mendes, S. S.; Barizon, F. M. A.; Gonçalves, D. D.; Barbosa, L. N.; Faria, M. G. I.; Valle, J. S.; Casalvara, R. F. A.; Gonçalves, J. E.; Gazim, Z. C.; Ruiz, S. P.; Essential oil from the leaves, fruits and twigs of *Schinus terebinthifolius*: chemical composition, antioxidant and antibacterial potential. *Molecules* **2024**, *29*, 469. [Crossref] [PubMed]
8. Martins, C. F.; Santos Junior, W. A. C.; Silva, L. S.; de Paula, J. R.; *Schinus terebinthifolius* Raddi: scientometric analysis. *Research, Society and Development* **2021**, *10*, e11110817016. [Crossref]
9. De Oliveira, V. S.; Augusta, I. M.; Braz, M. V. C.; Riger, C. J.; Prudêncio, E. R.; Sawaya, A. C. H. F.; Sampaio, G. R.; Torres, E. A. F. S.; Saldanha, T.; Aroeira fruit (*Schinus terebinthifolius* Raddi) as a natural antioxidant: chemical constituents, bioactive compounds and *in vitro* and *in vivo* antioxidant capacity. *Food Chemistry* **2020**, *315*, 126274. [Crossref] [PubMed]
10. Barraqui, N. S. G.; Ventura, J. A.; Ruas, F. G.; Gomes, R. B. A.; Lovatti, B. P. O.; da Silva, S. R. C.; Filgueira, P. R.; Kuster, R. M.; Physical and chemical characteristics of *Schinus terebinthifolia* Raddi (Brazilian peppertree) fruits at different stages of maturation. *Biochemical Systematics and Ecology* **2023**, *110*, 104704. [Crossref]
11. Carvalho, M. G.; Melo, A. G. N.; Aragão, C. F. S.; Raffin, F. N.; Moura, T. F. A. L.; *Schinus terebinthifolius* Raddi: chemical composition, biological properties and toxicity. *Revista Brasileira de Plantas Medicinais* **2013**, *15*, 158. [Crossref]
12. Degáspari, C. H.; Waszczynskyj, N.; Prado, M. R. M.; Antimicrobial activity of *Schinus terebenthifolius* Raddi. *Ciência e Agrotecnologia* **2005**, *29*, 617. [Crossref]
13. Silva, M. M.; Iriguchi, E. K. K.; Kassuya, C. A. L.; Vieira, M. C.; Foglio, M. A.; Carvalho, J. E.; Ruiz, A. L. T. G.; Souza, K. P.; Formagio, A. S. N.; *Schinus terebinthifolius*: phenolic constituents and *in vitro* antioxidant, antiproliferative and *in vivo* anti-inflammatory activities. *Revista Brasileira de Farmacognosia* **2017**, *27*, 445. [Crossref]
14. Bernardi, J. L.; Ferreira, J. A.; Puton, B. M. S.; Camargo, S. D.; Junges, A.; Cansian, R. L.; Paroul, N.; Exploring the essential oil of aroeira (*Schinus terebinthifolius* Raddi): chemical composition, biological potentials, and future directions. *Food Bioscience* **2025**, *68*, 106670. [Crossref]
15. Lima, M. R. F.; Luna, J. S.; Santos, A. F.; Andrade, M. C. C.; Sant'ana, A. E. G.; Genet, J.-P.; Marquez, B.; Neuville, L.; Moreau, N.; Anti-bacterial activity of some Brazilian medicinal plants. *Journal of Ethnopharmacology* **2006**, *105*, 137. [Crossref] [PubMed]
16. Ceruks, M.; Romoff, P.; Fávero, O. A.; Lago, J. H. G.; Polar phenolic constituents from *Schinus terebinthifolius* Raddi (Anacardiaceae). *Química Nova* **2007**, *30*, 597. [Link]
17. Rosas, E. C.; Correa, L. B.; Padua, T. A.; Costa, T. E. M. M.; Mazzei, J. L.; Heringer, A. P.; Bizarro, C. A.; Kaplan, M. A. C.; Figueiredo, M. R.; Henriques, M. G.; Anti-inflammatory

effect of *Schinus terebinthifolius* Raddi hydroalcoholic extract on neutrophil migration in zymosan-induced arthritis. *Journal of Ethnopharmacology* **2015**, *175*, 490. [Crossref] [PubMed]

18. Silva, T. L.; de Oliveira, V. S.; Augusta, I. M.; Keller, L. M.; Gamallo, O. D.; Saldanha, T.; Aroeira (*Schinus terebinthifolius* Raddi) fruit: chemical composition and antioxidant capacity. *Revista Virtual de Químic*a **2019**, *11*, 1614. [Crossref]
19. Pinto, I. C.; Seibert, J. B.; Pinto, L. S.; Santos, V. R.; de Sousa, R. F.; Sousa, L. R. D.; Amparo, T. R.; dos Santos, V. M. R.; do Nascimento, A. M.; de Souza, G. H. B.; Vasconcellos, W. A.; Vieira, P. M. A.; Andrade, A. L.; Preparation of glass-ionomer cement containing ethanolic Brazilian pepper extract (*Schinus terebinthifolius* Raddi) fruits: chemical and biological assays. *Scientific Reports* **2020**, *10*, 22312. [Crossref] [PubMed]
20. De Azevedo, A. S.; Seibert, J. B.; Amparo, T. R.; Antunes, A. S.; Sousa, L. R. D.; Souza, G. H. B.; Teixeira, L. F. M.; Vieira, P. M. A.; Santos, V. M. R.; Nascimento, A. M.; Nascimento, A. M.; Chemical constituents, antioxidant potential, antibacterial study and photoprotective activity of Brazilian corn silk extract. *Food Science and Technology* **2022**, *42*, e98421. [Crossref]
21. Brand-Williams, W.; Cuvelier, M. E.; Berset C.; Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology* **1995**, *28*, 25. [Crossref]
22. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C.; Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* **1999**, *26*, 1231. [Crossref] [PubMed]
23. Valverde, T. M.; Soares, B. N. G. S.; do Nascimento, A. M.; Andrade, A. L.; Sousa, L. R. D.; Vieira, P. M. A.; Santos, V. R.; Seibert, J. B.; de Almeida, T. C. S.; Rodrigues, C. F.; de Oliveira, S. R. M.; Martins, F. S.; Ferreira Júnior, J. G.; dos Santos, V. M. R.; Anti-inflammatory, antimicrobial, antioxidant and photoprotective investigation of red propolis extract as sunscreen formulation in polawax cream. *International Journal of Molecular Sciences* **2023**, *24*, 5112. [Crossref] [PubMed]
24. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R.; New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute* **1990**, *82*, 1107. [Crossref] [PubMed]
25. Ferreira, P. M. P.; Costa, P. M.; Costa, A. M.; Lima, D. J. B.; Drumond, R. R.; Silva, J. N.; Moreira, D. R. M.; Oliveira Filho, G. B.; Ferreira, J. M.; Queiroz, M. G. R.; Leite, A. C. L.; Pessoa, C.; Cytotoxic and toxicological effects of phthalimide derivates on tumor and normal murine cells. *Anais da Academia Brasileira de Ciências* **2015**, *87*, 313. [Crossref] [PubMed]
26. Ratty, A. K.; Sunamoto, J.; Das, N. P.; Interaction of flavonols with 1.1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase-1. *Biochemical Pharmacology* **1988**, *37*, 989. [Crossref] [PubMed]
27. Basnet, P.; Matsuno, T.; Neidlein, R.; Potent free radical scavenging activity of propol isolated from Brazilian propolis. *Zeitschrift für Naturforschung C* **1997**, *52*, 828. [Crossref] [PubMed]
28. Burmistrov, S. O.; Oparina, T. I.; Prokopenko, V. M.; Arutiunian, A. V.; Antioxidant activity of the blood serum in pregnant and nonpregnant women: a comparison of various detection methods. *Klinicheskaja Laboratornaja Diagnostika* **1997**, *11*, 14. [Crossref] [PubMed]
29. Schulze-Kaysers, N.; Feuereisen, M. M.; Schieber, A.; Phenolic compounds in edible species of the Anacardiaceae family - a review. *RSC Advances* **2015**, *5*, 73301. [Crossref]
30. Almeida, W. A. S.; Antunes, A. S.; Penido, R. G.; Correa, H. S. G.; do Nascimento, A. M.; Andrade, A. L.; Santos, V. R.; Cazati, T.; Amparo, T. R.; de Souza, G. H. B.; Freitas, K. M.; dos Santos, O. D. H.; Sousa, L. R. D.; dos Santos, V. M. R.; Photoprotective activity and increase of SPF in sunscreen formulation using lyophilized red propolis extracts from Alagoas. *Revista Brasileira de Farmacognosia* **2019**, *29*, 373. [Crossref]
31. Sousa, L. R. D.; Amparo, T. R.; de Souza, G. H. B.; Ferraz, A. T.; Fonseca, K. S.; de Azevedo, A. S.; do Nascimento, A. M.; Andrade, A. L.; Seibert, J. B.; Valverde, T. M.; Braga, S. F. P.; Vieira, P. M. A.; dos Santos, V. M. R.; Anti-*Trypanosoma cruzi* potential of vestitol isolated from lyophilized red propolis. *Molecules* **2023**, *28*, 7812. [Crossref] [PubMed]
32. International Organization for Standardization; *ISO 10993-5:2009: Biological Evaluation of Medical Devices - Part 5: Tests for in Vitro Cytotoxicity*; Geneva, 2009. [Link]
33. Paino, I. M. M.; Gonçalves, F. J.; Souza, F. L.; Zucolotto, V.; Zinc oxide flower-like nanostructures that exhibit enhanced toxicology effects in cancer cells. *ACS Applied Materials & Interfaces* **2016**, *8*, 32699. [Crossref] [PubMed]
34. Sousa, L. R. D.; Azevedo, M. L. S.; Rocha, D. F.; Andrade, A. L.; Amparo, T. R.; dos Santos, O. D. H.; Seibert, J. B.; Pereira, L. R.; Vieira, P. M. A.; Carneiro, C. M.; Barboza, A. P. M.; Neves, B. R. A.; Sales Júnior, P. A.; Murta, S. M. F.; Novack, K. M.; dos Santos, V. M. R.; Trypanocidal activity and increased solubility of benznidazole incorporated in PEG 4000 and its derivatives. *Journal of the Brazilian Chemical Society* **2021**, *32*, 1162. [Crossref]
35. De Oliveira, D. M.; Menezes, D. B.; Andrade, L. R.; Lima, F. C.; Hollanda, L.; Zielinska, A.; Sanchez-Lopez, E.; Souto, E. B.; Severino, P.; Silver nanoparticles obtained from Brazilian pepper extracts with synergistic anti-microbial effect: production, characterization, hydrogel formulation, cell viability, and *in vitro* efficacy. *Pharmaceutical Development and Technology* **2021**, *26*, 539. [Crossref] [PubMed]
36. Fernandes, S. G.; Gala, K.; Khattar, E.; Telomerase inhibitor MST-312 and quercetin synergistically inhibit cancer cell proliferation by promoting DNA damage. *Translational Oncology* **2023**, *27*, 101569. [Crossref] [PubMed]
37. Zhaorigetu; Farrag, I. M.; Belal, A.; Badawi, M. H. A.; Abdelhady, A. A.; Galala, F. M. A. A.; El-Sharkawy, A.; El-Dahshan, A. A.; Mehany, A. B. M.; Antiproliferative,

apoptotic effects and suppression of oxidative stress of quercetin against induced toxicity in lung cancer cells of rats: *in vitro* and *in vivo* study. *Journal of Cancer* **2021**, *12*, 5249. [Crossref] [PubMed]

38. Seregheti, T. M. Q.; Pinto, A. P. R.; Gonçalves, M. C.; Antunes, A. S.; Almeida, W. A. S.; Machado, R. S.; Silva, J. N.; Ferreira, P. M. P.; Pessoa, C.; Santos, V. M. R.; Nascimento, A. M.; Antiproliferative and photoprotective activities of the extracts and compounds from *Calea fruticosa*. *Brazilian Journal of Medical and Biological Research* **2020**, *53*, e9375. [Crossref] [PubMed]
39. Villas-Bôas, S. G.; Smart, K. F.; Sivakumaran, S.; Lane, G. A.; Alkylation or silylation for analysis of amino and non-amino organic acids by GC-MS? *Metabolites* **2011**, *1*, 3. [Crossref] [PubMed]
40. Saraji, M.; Ghambari, H.; Suitability of dispersive liquid-liquid microextraction for the *in situ* silylation of chlorophenols in water samples before gas chromatography with mass spectrometry. *Journal of Separation Science* **2015**, *38*, 3552. [Crossref] [PubMed]
41. Oliveira, M. B. S.; Valentim, I. B.; Rocha, T. S.; Santos, J. C.; Pires, K. S. N.; Tanabe, E. L. L.; Borbely, K. S. C.; Borbely, A. U.; Goulart, M. O. F.; *Schinus terebenthifolius* Raddi extracts: From sunscreen activity toward protection of the placenta to Zika virus infection, new uses for a well-known medicinal plant. *Industrial Crops and Products* **2020**, *152*, 112503. [Crossref] [PubMed]
42. Nunes, A. R.; Rodrigues, A. L. M.; Queiróz, D. B.; Vieira, I. G. P.; Câmara Neto, J. F.; Calixto Júnior, J. T.; Tintino, S. R.; Morais, S. M.; Coutinho, H. D. M.; Photoprotective potential of medicinal plants from Cerrado biome (Brazil) in relation to phenolic content and antioxidant activity. *Journal of Photochemistry and Photobiology B: Biology* **2018**, *189*, 119. [Crossref] [PubMed]
43. Wu, C.; Bian, Y.; Lu, B.; Wang, D.; Azami, N. L. B.; Wei, G.; Ma, F.; Sun, M.; Rhubarb free anthraquinones improved mice nonalcoholic fatty liver disease by inhibiting NLRP3 inflammasome. *Journal of Translational Medicine* **2022**, *20*, 294. [Crossref] [PubMed]
44. Verebová, V.; Benes, J.; Stanicova, J.; Biophysical characterization and anticancer activities of photosensitive phytoanthraquinones represented by hypericin and its model compounds. *Molecules* **2020**, *25*, 5666. [Crossref] [PubMed]