In vitro determination of the sun protection factor (SPF) and photochemoprotection potential of aqueous extract of banana peels (Musa sp.) against UV-B radiation

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Highlights
• Banana peels are sources of bioactive compounds for the pharmaceutical/cosmetic industries.
• The use of peels by the industry can add economic value to this by-product and reduce pollution.
• Aqueous extract (AE) of banana peels shows an unprecedented sun protection factor.
• AE modulates collagen synthesis and maintains the morphology of fibroblasts exposed to UV-B.
• Alternative methods to animal experiments were used.

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KEYWORDS
Musa sp.; Banana peels; Photochemoprotective; UV-B; Sun protection factor; Photoaging.

Abstract: Banana (Musa sp.) fruits have been investigated by the scientific community for their healing properties due to their composition in bioactive compounds. In this study, the aqueous extract (AE) of banana peels (Musa sp., cv. Prata Anã) was chemically characterized and investigated regarding its antioxidant and photochemoprotective potential against UV-B radiation in BALB/3T3 clone A31 mouse fibroblasts. The results showed the AE as a source of phenolic compounds, mainly gallic acid. Antioxidant activity of AE was demonstrated by its ability to scavenge DPPH free radicals and to reduce ferric-tripyridyltriazine complex. A sun protection factor (SPF) of up to 36.4 was determined through the Mansur equation. The AE revealed low cytotoxicity (96 ± 12% cell viability - 100 µg/mL) to mouse fibroblasts and the determination of the photochemoprotective effect revealed preservation of collagen biosynthesis in cells exposed to UV-B (38.6 ± 7%), also contributing to the maintenance of cellular and nuclear morphologies. Thus, the AE of banana peels seems to be an interesting candidate for pharmaceutical/cosmetic formulations to combat skin’s photoaging caused by UV-B radiation.

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Introduction

Human skin is the outer covering and the largest organ of the body. Although the skin fulfills numerous physiological functions, its composition changes with overexposure to environmental pollution and solar radiation (Lopes et al., 2017a). Excessive or repeated exposure to ultraviolet (UV) irradiation, particularly ultraviolet-B (UV-B), is thought to be responsible for most sunburn and damages to the skin (Hwang et al., 2013). UV-B and UV-A radiations have been demonstrated to produce reactive oxygen species (ROS) in the cells and skin, which cause DNA damage leading to gene mutation and abnormal cell proliferation (Ryoo et al., 2001). In addition, the oxidative stress resulting from this chronic exposure accelerates the activity of matrix metalloproteinases (MMPs) that degrade the collagen in the extracellular matrix (Hwang et al., 2014). Collagen performs a key role in maintaining the structure of the skin dermis (Sun et al., 2016) and its degradation has been considered the major cause of the photoaging (Bravo et al., 2017).

The use of topical photoprotectors has been related to the prevention of acute effects of sun radiation, especially sunburn (Schalka & Reis, 2011). In this sense, increasing research efforts in developing topical formulations containing plant’s phenolic compounds have been performed, due to their property of absorption spectrum in the UV region of the sun light (Velasco et al., 2008). Indeed, potent antioxidant polyphenols derived from plants can protect skin by preventing UV-induced cytotoxicity, especially caused by UV-B, demonstrating the importance of these metabolites for the development of preventative therapies against photoaging (Olteanu et al., 2014; Chan et al., 2014).

Peels are the main by-product of the banana processing industry, accounting for approximately 38% of the total fruit weight, being considered as a waste with low economic value (Vu et al., 2017). Plant food processing by-products pose a major problem for industry, but they are also promising sources of compounds that can be industrially used due to their favorable technological or nutritional properties (Schieber et al., 2001). Different biotechnological applications have been suggested for banana peels such as the production of ethanol and methane, animal feed, and water purification adsorbents (Emaga et al., 2007). However, studies targeting this biomass to the pharmaceutical and cosmetic industries are still scarce.

In the last few decades, banana (Musa sp.) has been evaluated by scientific and medicinal interests as an important source of bioactive compounds able to reduce ROS levels, such as flavonoids, anthocyanins, condensed tannins, and biogenic amine (Apriasari et al., 2014; Lopes et al., 2017b). A series of investigations developed in recent years have shown banana biomasses as sources of secondary metabolites with effective pharmacological activities both in vitro and in vivo biological models (Lopes et al., 2020). Importantly, banana peel has been claimed to promote wound healing when used topically in the Brazilian traditional medicine. Indeed, it has been reported the use of peels of ripe bananas to prepare a poultice which is wrapped around the lesion, reducing the pain and swelling (Pereira & Maraschin, 2015). However, literature lacks in information regarding the photoprotective effect of banana peels’ bioactive compounds on skin cells exposed to UV radiation. In this study, through in vitro methods the photochemoprotection potential of the aqueous extract (AE) of banana peels against damage caused by UV-B radiation in BALB/3T3 clone A31 mouse fibroblast cell line was investigated by determining its antioxidant activity and sun protection factor (SPF) property.

Material and methods

Plant material and extraction

Samples of banana peels (Musa sp., cv. Prata Anã) were collected (June 2015) from an ecologically managed orchard and provided by the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI - Criciúma County,
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28º40′39′′ S, 49º22′11′′ W, Santa Catarina State, southern Brazil). The banana samples were sanitized in running water and dried with paper towel. Then, the fruit peels were manually removed and dried (45 °C) until constant weight. The dry biomass was packed into polyethylene bags and stored at −20 °C. Dried banana peels samples (0.5 g) were added to 7.5 mL distilled water, homogenized in vortex and incubated (water bath, 37 °C, 30 min), followed by centrifuging and recovering of the supernatant as aqueous extract (AE).

UV-vis scanning spectrophotometry

The AE’s (1:9 in distilled water, v/v) chemical profiles were determined by UV-vis scanning spectrophotometry (Gold Spectrum Laboratories 53 spectrophotometer, BEL photonics, BR) on a spectral window from 200 nm to 800 nm (resolution 1 nm/data point) for identification of the maximum absorbance regions. All determinations were made in triplicate (n = 9).

Determination of the total phenolic and flavonoid contents

The total phenolic content (TFC) of the AE was measured spectrophotometrically, using the Folin-Ciocalteau reagent as previously described by Waterhause (2002). Quantification of the compounds used an external standard curve of gallic acid (15.625 - 1000 µg/mL; \( r^2 = 0.99; y = 0.00113x \)) and the results were expressed as mean ± standard deviation (SD) in mg gallic acid equivalents/g dry weight (mg GAE/g DW). All determinations were made in triplicate (n = 9). In its turn, the concentration of flavonoids in the AE followed the aluminum chloride colorimetric method (Woisky & Salatino, 1998). A standard curve of quercetin (2.5 - 15 µg/mL, \( r^2 = 0.99, y = 0.002899x \)) was built for purpose of calculation of the amounts of the metabolites of interest and the values were expressed as mean ± SD in mg quercetin equivalents/g dry weight (mg QE/g DW). All determinations were made in triplicate (n = 9).

Ultra high-performance liquid chromatography (UHPLC)

Aliquots (10 µL) of AE sample were analyzed on a Thermo Scientific UltiMate 3000RS Dual System (Thermo Fisher Scientific, San Jose, CA, USA) liquid chromatography, equipped with a C18 reverse phase column (Kinetex® 150 x 4.6 mm, 2.6 µm particle size), thermostated at 25°C, and a diode array detector (λ = 254, 270, 280, and 354 nm). The elution used acidified aqueous solution (0.1% trifluoroacetic acid - v/v, phase A) and acetonitrile (ACN - phase B) at 1 mL/min over 15 min, as follows: 0 - 5 min phase A 90%: phase B 10%; 5 - 10 min phase A 20%: phase B 80%; 10 - 15 min phase A 90%: phase B 10%. The retention times of analytical standards (10 µg/mL) solubilized in mobile phases (A: B, 1: 1, v/v) was adopted for the identification of the compounds of interest. The analytical quantification used an external standard curve of gallic acid (1 - 100 µg/mL, \( r^2 = 0.99, y = 0.344623 x \)), having as reference the integral of the area of the corresponding peaks expressed in µg/g dry peels. The values were expressed as mean ± SD of three consecutive injections (n = 3).

Antioxidant activity

For determination of the antioxidant activity, AE was previously prepared at 10 mg/mL concentration, followed by serial dilutions (10 - 0.31 mg/mL). The measurements of the antioxidant activity were performed by the methods of scavenging the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Ribeiro et al., 2008), and the ferric reducing antioxidant power (FRAP) (Pulido et al., 2000).

Determination of in vitro sun protection factor (SPF)

The in vitro SPF was determined according to the spectrophotometric method of Mansur (Kaur & Saraf, 2010). The AE was diluted in distilled water to the concentrations of 2000 - 0.1 µg/mL. The absorbance spectrum of the samples was recorded from 290 to 320 nm (5 nm interval) in quartz plates, using distilled water as compensation liquid in a microplate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA). Three determinations were made at each point, followed by the calculations using the Mansur’s equation (Mansur et al., 1986).

In vitro cell cultures analyzes

Cell viability - MTT assay

BALB/3T3 clone A31 mouse fibroblasts (Mus musculus) were obtained from the Bank of Cells of Rio de Janeiro (BCRJ). Cells were inoculated at a density 1×10⁴ cells/well into a 96-well plate containing DMEM culture medium, supplemented with 10% FBS, 3.7 g sodium bicarbonate, and antibiotics (penicillin/streptomycin, 10 mL/L), following incubation at 37 °C in a humidified atmosphere containing 5% CO₂ overnight. Afterwards, fibroblasts were exposed to fresh DMEM culture medium supplemented with 10% FBS containing 0.1 - 1000 µg/mL AE, except in control, where only fresh DMEM culture medium was used. Cells were treated for 1, 12, and 24 h and after each incubation time 10 µL MTT solution (5 mg/mL in PBS) were added to the culture medium and incubated (37 °C, 5% CO₂) for 3 h. Subsequently, the culture medium was removed and 100 µL dimethylsulfoxide were added into each well. The plates were kept on microplate shaker, at room temperature, for 20 min, protected from light. After homogenizing formazan crystals, the absorbance was recorded at 540 nm on a microplate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA). The percentage of cell viability was calculated and compared to negative control (100% cell viability), according to Mosmann (1983), with modifications.

Photochemoprotective potential of the AE

UV-B irradiation

A fluorescent lamp (UVP, EL Series, Model UVLM-28, CA, USA) with maximum UV-B emission peak at 302 nm was used as UV-B radiation source at 25 cm distance from the culture plate, without the cap, inside a laminar flow to guarantee the aseptic conditions regarding the fibroblast cell cultures. A radiometer (UV Meter T13.2.01, Tecstel, BR) equipped
with a UV-B sensor was used for radiation measurements. The BALB/3T3 fibroblasts cell were inoculated at 1x10⁴ cells/well into a 96-well plate (100 µL/well), following incubation (37 °C, 5% CO₂) for cell adhesion. The plates were exposed to UV-B radiation doses (40, 80, 160, and 320 mJ/cm²), following re-incubation (37 °C, 5% CO₂) in DMEM culture medium containing 10% FBS. After 24 h, the MTT colorimetric method was used to determine cell viability. The irradiation dose that caused a decrease by 50% in cell viability was used to evaluate the AE’s photochemoprotection potential (Oliveira et al., 2018).

**Cytoprotective effect**

BALB/3T3 fibroblasts cell were inoculated (1x10⁴ cells/well) into two 96-well plates (100 µL/well) and incubated (37 °C, 5% CO₂). After cell adhesion, the culture medium was replaced by a new one containing AE (0.1 - 100 µg/mL), following incubation for 1 h (37 °C, 5% CO₂). Afterwards, the medium was removed and PBS containing Ca²⁺ and Mg²⁺ was added to the wells. One of the microplates randomly chosen was exposed to UV-B radiation (160 mJ/cm²) as the other one remained in the dark under the same culture conditions. After the treatments, the buffer solution was removed and fresh culture medium containing 10% FBS added. The cell viability evaluation was performed by the MTT colorimetric method, 24 h later, according to Oliveira et al. (2018), with modifications.

**Collagen synthesis**

BALB/3T3 fibroblasts were inoculated (1x10⁴ cells/well) into two 96-well plates (100 µL/well) and incubated (37 °C, 5% CO₂) until reaching confluency. Further, the culture medium was removed and the wells washed with PBS containing Ca²⁺ and Mg²⁺. One of the plates was UV-B-irradiated (160 mJ/cm²), while the other one remained protected from light under the same culture conditions. Afterwards, cells were treated with AE (0.1 - 100 µg/mL) and in 24 h-intervals this procedure was repeated over a 72 h experimental time. At the end of the incubation period (72 h), the culture medium was removed and the cells washed with PBS containing Ca²⁺ and Mg²⁺ and fixed with 100 µL/well Bouin fluid (15 mL saturated aqueous solution of picric acid 1.3%, 5 mL formaldehyde 35%, 1 mL glacial acetic acid) for 1 h. The fixative solution was removed and the excess eliminated by washing in running water (10 min). The cells were then stained for 1 h with Sirius Red dye (1 mg/mL) in saturated picric acid solution (1.3%, 100 µL/well), followed by washing with 0.01 M HCl (100 µL/well). The collagen content was measured by extracting the dye with 0.1 M NaOH (100 µL/well), following the absorbance reading (Å = 550 nm) in a microplate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA) (Tullberg-Reinert & Jundt, 1999). The results were expressed as percentage of increase in collagen deposition in relation to the negative control (± SD), according to the equation: Increase in collagen synthesis (%) = Abs SAMPLE × 100 / Abs CONTROL.

**Morphological analysis**

To evaluate nuclear morphological alterations, BALB/3T3 fibroblasts were inoculated (5x10⁴ cells/well) into 6-well plates on 2x2 mm coverslips precoated with poly-L-lysine solution. After the cell adhesion, the culture medium was removed and a new one containing AE (0.1 - 100 µg/mL) was added, following 1 h-incubation. Afterwards, the culture medium was removed and PBS containing Ca²⁺ and Mg²⁺ was added to the wells. One of the microplates randomly chosen was exposed to UV-B radiation (160 mJ/cm²) as the other one remained in the dark under the same culture conditions. Finally, the buffer solution was removed, fresh culture medium containing 10% FBS was added and cells were prepared for nuclear and cellular morphological analysis.

**Nuclear morphometric analysis (NMA)**

After 24 h incubation, fixed cells (PFA 4% in PBS, 2 h) were further DAPI stained in 0.1% PBS (v/v) for 2 min, at room temperature. The samples were analyzed by confocal microscopy (DMI6000 B, Leica Microsystems, GER). Nuclei images were quantified using the Software Image J 1.50i (National Institutes of Health, Bethesda, USA) and the plugin “NII_Plugin” (available at http://www.ufrgs.br/labsinal/NMA/). Data were presented as a plot of Area versus Nuclear Irregularity Index (NII), which separates nuclei considering their morphometric phenotype. The percentages of normal, small regular, large, and regular or irregular nuclei were determined as described by Filippi-Chiela et al. (2012).

**Cell morphology**

After 24 h incubation, cells were fixed with modified Karnovsky’s solution (Karnovsky, 1965) for 30 min, at room temperature. Subsequently, the samples were dehydrated in increasing concentrations of ethanol (50, 70, 80, 90, and 100%, 10 min each), fixed in stubs with carbon tape and dried by adding hexamethyldisilazane, followed by sputter deposition with gold (100 sec, in a current of 40 mA) in 30 - 40 nm-thickness (EM SCD 500, Leica Microsystems, GER). The micrographs were recorded and analyzed by a scanning electron microscope (JSM-6390LV, Jeol, Tokyo, JNP).

**Statistical analysis**

The data were collected, summarized, and evaluated for the normality and homogeneity of their variances, followed by statistical analyzes performed by one-way ANOVA and Dunnett’s or Tukey’s post hoc tests for the mean comparison (p < 0.05, significance level). The statistical analyzes were performed using the algorithms of the GraphPad Prism 6 statistical program (GraphPad Software Inc., San Diego, CA, USA). The results were expressed as mean ± SD. Additionally, linear regressions models were built using scripts written in R language.

**Results and discussion**

**Chemical profile and antioxidant potential analyzes**

The AE of banana peels was analyzed by UV-vis scanning spectrophotometry, recording the absorbances over a spectral window from 200 to 800 nm. It is well known that phenolic compounds typically show absorption peaks in the UV region, i.e., 270 - 315 nm (Melo et al., 2012) and the spectroscopic profiles of the studied samples revealed absorbances spread over a 270 - 400 nm spectral window, with maximum values around 280 nm, indicating that the aqueous extraction protocol adopted was able to recover those secondary metabolites.
from banana peels. Banana pulp is rich in many nutrients, as banana peels have been reported as important sources of phytochemicals (Bennett et al., 2010). In previous studies, Sundaram et al. (2011) found a wide range of phenolics (0.17 to 9.99 mg of GAE/g DW) in M. paradisica peels at different ripening stages, using chloroform and ethyl acetate as solvents for ripe and unripe peels, respectively. In comparison to those findings, the extraction protocol herein adopted demonstrated a satisfactory recovery yield of phenolics from banana peels (i.e., 10.10 ± 0.37 mg GAE/g DW), mainly considering that the extractions were carried out with distilled water, providing greater safety to the final product that would be organic solvent-free. In a second step, the total content of flavonoids was measured in the AE, revealing to be those secondary metabolites a minor fraction (0.52 ± 0.04 mg QE/g DW) of the phenolics present in banana peels.

UHPLC analysis of the AE were performed and the analytes identified using a library of phenolic compounds by comparing their retention times and UV spectra with those of analytical standards and reported in literature. Additionally, co-chromatographic analyzes were performed to confirm the identification of the compounds. The AE samples investigated presented as the major compound gallic acid (1.44 ± 0.01 mg/g DW), with minor amounts of galocatechin, epigallocatechin, and chlorogenic acid. Phenolic compounds are widely distributed in the plant kingdom and have been reported as secondary metabolites with high antioxidant activity (Khoo et al., 2016). It has been documented that these compounds may act as reducing agents, free radical scavengers, metal chelators, or even as singlet oxygen deactivateors (Ferrera et al., 2016). UV-B radiation causes skin disorders through the generation of free radicals and, therefore, the continuous administration of these phytochemicals has been suggested as a possible way to enhance antioxidant capacity and improve cell’s defenses against UV-B radiation (Leerach et al., 2017). This is of great value for this study, since phenolic compounds are present in the AE, mainly in their acidic form, i.e., gallic acid. Additionally, it has been argued that the antioxidant activity determined by a single method may underestimate the antioxidant potential of an extract, reflecting its ability to inhibit only one class of oxidant compounds (Trabelsi et al., 2012). Thus, in this study, the AE’s antioxidant property in different concentrations (0.31 - 10 mg/mL) was evaluated both by the DPPH% and FRAP methods, which have been reported to provide reproducible results in plant matrices (Delgado-Andrade et al., 2005).

The DPPH assay measures the scavenging activity through a mechanism of donation of an electron or a hydrogen by the antioxidant molecule to that synthetic radical (Oliveira et al., 2018). AE exhibited a DPPH free radical scavenging activity varying from 14.26 ± 0.2 to 79.36 ± 0.7%, as shown in Figure 1A.

The increase in the antioxidant activity of the AE was significant (p < 0.01) in a concentration dependent manner (r²=0.84, y=6.891x + 20.49). In this method, the IC 50 value is defined as the amount of sample required to decrease the initial absorbance by 50% at 515 nm and the AE presented an IC 50 = 3.15 ± 0.02 mg/mL DW. However, other researchers have found better results in comparison to the ones herein described where, for instance, the aqueous and ethanolic extracts of Musa sapientum presented IC 50 values of 54.11 μg/mL and 19.10 μg/mL, respectively (Dahham et al., 2015).

Darsini et al. (2012) analyzed peel extracts of several varieties of Musa acuminata Colla using a series of solvents for extraction of bioactive compounds with antioxidant activity. In that study, the methanolic extract (500 μg/mL) of the Grand Naine and Gruesa varieties showed a DPPH radical scavenging activity of 44 ± 8% and 43 ± 5%, respectively. However, the aqueous extracts of those genotypes showed no significant sequestering activity (3 ± 0.1, Grand Naine and no activity, Gruesa). Thus, it is hypothesized that the choice of solvent, as well as the species/cultivar analyzed may influence the recovery of extracts with high content of bioactive compounds and antioxidant activity. DPPH is a stable radical with low decay rate and reactivity with most compounds. Therefore, only strong reducing reagents are capable of reacting with that radical in a stoichiometric mode (Santos et al., 2007), underlining the importance of radical scavenging activity found with AE.

The antioxidant capacity of AE was also investigated by the FRAP method. This method is based on the reduction of the ferric ion (Fe³⁺ - TPTZ) to the ferrous form (Fe²⁺ - TPTZ), resulting in a blue-colored complex in the presence of antioxidants (Mazzarino et al., 2018). The reducing power of the AE was linearly proportional (r² = 0.99%, y = 33.69x - 34.77) to the sample concentration (p < 0.05) as determined by the

Figure 1. Antioxidant activity of the aqueous extract of banana peels (0.31 - 10 mg/mL). (A) Scavenging activity on DPPH radicals and (B) reducing power of Fe²⁺. Data are presented as mean ± SD of three independent experiments (n = 9). The letters represent statistical difference (p < 0.05, ANOVA followed by the Tukey’s test).
regression model and Tukey’s test. As shown in Figure 1B, AE reducing ability was up to 3.07 ± 0.05 mM at 10 mg/mL. Importantly, the increase in AE reducing power indicates the presence of electron donors’ compounds able in reacting with free radicals converting them into more stable products. Such a property may be related, for instance, to the redox potential of hydroxyl groups of phenolic compounds and to the structural relationships between different parts of your chemical structure (Darsini et al., 2012). The FRAP assay findings corroborate the results found in the DPPH assay, confirming the antioxidant potential of AE samples, suggesting it can be used as a biomass for biological studies against free radical damage such as that produced by UV-B radiation.

**In vitro sun protection factor (SPF)**

The SPF is the most important variable to quantify the effectiveness of a sunscreen, being universally accepted. The method is based on determining the minimum erythematous dose (MED), defined as the smallest amount of energy required for triggering the erythema in areas of protected and unprotected skin (Schakal & Reis, 2011). To be effective in preventing sunburn and other skin damage, a sunscreen product should have a wide range of absorbance between 290 and 400 nm as previously detected by UV-vis scanning spectrophotometry. The *in vitro* SPF is useful for screening test during product development as a first experimental approach in respect to the *in vivo* SPF assay (Kaur & Saraf, 2010). Due to its absorption in the UV region and its hydrophilic character, the AE was evaluated by UV spectrophotometry applying Mansur mathematical equation and the SPF values found according to the AE concentrations are shown in Table 1.

According to the ANVISA’s protection levels classification in Brazil (Brasil, 2012), as of 1.25 mg/mL the AE showed sunscreen activity in a concentration-dependent manner ($r^2 = 0.99, y = 3.620x + 1.342$), reaching the highest SPF of 36.4 at 10 mg/mL. Thus, these findings indicate that AE might be considered as an alternative to synthetic chemical filters, important constituents of photoprotective creams and lotions. Although there are no methodologies yet officially approved in Brazil for the determination of SPF, ANVISA determines that reports of efficacy tests, performed *in vivo* according to the Food and Drug Administration (FDA) or The European Cosmetic Toiletry and Perfumery Association (COLIPA) methodologies, shall be provided to enable the registration of photoprotector products (Brasil, 2012). *In vitro* testing has been thought as an experimental approach to identify new natural compounds with UV-filter properties, optimize combinations of old ones, and pre-screen protective formulas prior to the *in vivo* testing in humans (Santos et al., 1999). Importantly, according to Ferrari et al. (2007), *in vitro* and *in vivo* methodologies have demonstrated a good correlation in their findings and, up to date, no reports have been found regarding sunscreen factor studies of AE of banana peels. Therefore, these results contribute in an unprecedented way for technological innovations in the fields of cosmetology and pharmaceutical.

**Cell viability assessment**

To assess cytotoxicity by measuring cell viability, BALB/3T3 fibroblasts were exposed to AE at increasing concentrations (0.1 - 1000 µg / mL DW) for 1, 12, and 24 h. The results shown in Figure 2A suggest that AE has no cytotoxic effect until 100 µg/mL, except after 24 h exposure (79 ± 15% cell viability, $p < 0.05$) when compared to negative control (100 ± 3%). In addition, exposure of fibroblasts for 12 h (89 ± 8%) and 24 h showed significant cytotoxicity compared to the 1 h-treated group (96 ± 12%, $p < 0.05$) for the concentration of 100 µg, demonstrating that AE at exposure times longer than 1 h may present cytotoxicity and/or that it may have its beneficial effects in the first moments of exposure. Interestingly, there appears to be a stimulus to fibroblasts growth when exposed to AE at 0.1 and 0.3 µg/mL for 1 h, which may be related to an increase in cell proliferation.

Figure 2B shows a significant decrease in cell viability ($p < 0.001$) in a dose dependent manner ($r^2 = 0.93, y = - 0.3749x + 101.9$), following exposure of cells to irradiation at 80 mJ/cm². The 120 mJ/cm² dose decreased cell viability by 49.5 ± 18.7% when compared to negative control ($p < 0.001$). However, to ensure that future trials achieved a 50% decrease in cell population, the 160 mJ/cm² dose was selected (57.7 ± 18.6%).

Following the trend of using natural compounds in the production of cosmetics, several researches have been developed aiming at verifying the photoprotective action of plant extracts and oils containing flavonoids, tannins, alkaloids, and polyphenols (Ferrari et al., 2007). Polyphenols have an important property concerning strong free radical neutralization and exhibit the ability to modulate multiple cell pathways. Recently, their skin photoprotective effects have gained considerable attention (Pérez-Sánchez et al., 2016).

**Photochemoprotection potential**

Fibroblasts are important cellular components in the dermis, responsible for the synthesis of collagen and elastic fibers, among other components of the cellular matrix that form the main body of the dermal stratum (Oliveira et al., 2018). In this scenario, the prevention and protection of fibroblasts from photoinduced damage is relevant. To evaluate the AE photoprotective potential, BALB/3T3 fibroblasts were treated for 1 h with 0.1, 1, 10, and 100 µg AE/mL and irradiated with 160 mJ/cm². Figure 3A shows that UV-B

<table>
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<th>SPF</th>
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<tr>
<td>10</td>
<td>36.40 ± 1.23</td>
<td>30 - 50 SPF High</td>
</tr>
<tr>
<td>5</td>
<td>21.00 ± 2.57</td>
<td>15 - 29.9 SPF Moderate</td>
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*Protection level classification according to the RDC n° 30 1/6/2012 issued by the National Health Surveillance Agency, ANVISA.*
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radiation induced toxicity in fibroblasts, an effect not inhibited by the AE administration.

Von Atzingen et al. (2011) observed an increase in collagen fiber concentration when describing the effect of a gel containing unripe Musa sapientum green peel extract in the treatment of lesions by excision model in rats. Furthermore, it is reported in the literature that UV radiation can promote damage to fibroblasts, with consequent suppression of procollagen production and fiber degradation in the dermis, leading to changes in photoaging (Oliveira et al., 2018).

No data have been found in the literature evaluating the photochemoprotective effect of banana peel AE on the collagen synthesis of fibroblasts exposed to UV-B radiation so far. Thus, in a follow-up experiment, it was evaluated the effect of AE on collagen synthesis in BALB/3T3 fibroblasts previously exposed to UV-B radiation (160 mJ/cm²). After 72 h exposure, the irradiated cell population (UV-B group) showed a significant reduction in collagen synthesis by up to 72.3 ± 5% when compared to the NC (p < 0.001), corroborating literature reports on cell disorders resulting from UV radiation exposure (Bravo et al., 2017; Hwang et al., 2013) (Figure 3B). The AE treatment showed a significant increase (p < 0.05) in collagen synthesis at 1 µg/mL and 10 µg/mL (37.6 ± 5 and 38.6 ± 7%) in relation to the UV-B group (27.7 ± 5%). It is well known that oxidative stress caused by UV radiation can lead to the activation of genes that increase the expression of MMPs and the degradation of collagen, such as AP-1 (Hwang et al., 2014). Thus, it is suggested that AE may regulate any of these mechanisms and protect the synthesis of collagen in BALB/3T3 fibroblasts exposed to UV-B radiation. However, further studies are needed to determine these assumptions.

The morphological traits of a cell and its physiology are closely related. According to its genetic profile, a cell has its shape related to the function it performs. In continuity, the influence of AE on the protection of the morphology of BALB/3T3 fibroblasts exposed to UV-B radiation was evaluated. Fibroblasts were treated with AE (0.1 - 100 µg/mL) for 1 h and then irradiated with UV-B (160 mJ/cm²), following incubation...
with DAPI for 2 min at room temperature and visualization of the nucleus' morphology by fluorescence microscopy (Figure 4A). Images were captured and analyzed by nuclear morphometric analysis (NMA) (Figure 4B). Morphometric

![Figure 4A](image1.png)

**Figure 4.** Nuclear morphometric analysis (NMA). (A) BALB/3T3 fibroblasts were stained with DAPI to detect nuclear morphology. Cells treated with aqueous extract (AE) of banana peels (0.1 - 100 µg/mL for 1 h) and irradiated with UV-B (160 mJ/cm²) were photographed and analyzed. White arrows indicate normal nucleus. Red arrows indicate large and regular nucleus. Yellow arrows indicate irregular nucleus (630 ×). (B) Data were presented as a plot of Area versus Nuclear Irregularity Index (NII), which separates nuclei considering their morphometric phenotypes. Letters represent normal (N), larger and regular (LR), irregular nuclei (I), large and irregulars (LI), small and regular (SR), and small (S) nuclei. (C) Data shown represent the mean ± SD of three independent experiments (n = 9). Letters indicate statistical difference between the groups (p < 0.05, ANOVA followed by Tukey’s test).
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Analysis of nuclei size and eventual irregularities showed that AE increased the percentage of normal nuclei (N) by 87.4% ± 3.6% and 89.0% ± 6.0% with 10 µg and 100 µg/mL, respectively, when compared to the UV-B group (78.8 ± 6%) (p < 0.05, Figure 4C). The cells that presented small and regular (SR) and small nuclei (S) in the UV-B group, represented by apoptotic and mitotic ones, respectively, are not shown in Figure 4C, since they comprised less than 1% of the cell population. Although not prominent effect of the AE has been detected, two considerations are important regarding the large and regular (LR) group, related to senescent cells: (i) there was an increase in senescent nuclei (LR) in the UV-B group compared to the NC group; (ii) a depletion of senescent nuclei (LR) was observed when administered AE. Senescence is a condition in which cells are unable to proliferate. This is a stress response and can be induced by multiple mechanisms including telomere shortening and DNA damage (Schneider et al., 2018). This may be a mechanism that explains the results found so far according to Xiao et al. (2018), that postulate that external stimuli, i.e., UV light, can accelerate skin senescence. Thus, it is suggested that AE may be able to regulate molecular inducers of senescence and, consequently, interfere in the cell cycle of fibroblasts exposed to UV-B radiation.

BALB/3T3 fibroblasts were also analyzed as to their cellular morphology by scanning electron microscopy (SEM). In Figure 5 it is possible to identify an increase in cell volume in UV-B-treated

Figure 5. Cell morphological analysis by scanning electron microscopy (SEM) of BALB/3T3 fibroblasts exposed to AE (1 - 100 µg/mL) and to UV-B radiation (160 mJ/cm²) (Magnifications 500× - column 1, 1000× - column 2, and 2000× - column 3). Red arrows indicate lamellipodia. Yellow arrows indicate filopodia.
cells when compared to NC group. These data corroborate the findings of the nuclear morphology assay, where large regular nuclei, possibly represented by senescent cells, were detected. In addition to the typical fusiform fibroblast morphology, the NC also showed cytoplasmic extensions compatible with the cell type under analysis, such as lamellipodia (red arrows) and filopodia (yellow arrows). These protrusions are typical of cells undergoing cell migration. In contrast, in the UV-B group these phenotypes were disturbed. Apparently, UV-B radiation interferes with this mechanism. To date, and to the best of our knowledge, several studies have attempted to reveal the molecular mechanisms that regulate cell migration; however, they remain largely uncertain (Tashiro & Imoto, 2016). From the SEM images of fibroblasts treated with 1 µg/mL AE small alterations have been detected, regarding mainly to the induction of filopodia. At concentrations of 10 and 100 µg/mL, a cell volume compatible with cells found in NC was observed, reinforcing the results of nuclear morphology analysis. In addition, it can be speculated that at these doses, cell communications were more constant, given the greater formation of lamellipodia and filopodia.

Conclusions

The investigation of the chemical profile of the banana peels AE (Musa sp., cv. Prata Anã) showed that it was possible to recover the majoritarian secondary metabolites from that biomass, i.e., phenolic compounds, in a majorly way gallic acid. Additionally, it was shown that AE protects the BALB/3T3 fibroblasts against UV-B radiation damage, possibly due to the content and synergistic effect of the phenolic compounds, among other components of that chemical matrix. Cells exposed to UV-B radiation, when treated with AE kept collagen synthesis and revealed a more preserved morphology, as evidenced by the nuclear and cell membrane integrity and their cytoplasmic extensions. It is hypothesized that the maintenance of morphology may be related to the preservation of collagen and its interaction with adhesion proteins and extracellular matrix, and to molecular mechanisms that control cell cycle arrest or senescence, observed in deleterious processes caused by UV-B radiation, a subject that deserves further investigation. Due to the scarcity of data in the literature on the photochemoprotective potential of the banana peels AE and the findings herein described, new assays are suggested to elucidate the mechanism of action of the extract in BALB/3T3 fibroblasts exposed to UV-B radiation. Taken together, our results suggest that AE of banana peels (Musa sp., cv. Prata Anã) presents a prominent sun protection activity and hence it can be a candidate for future research on cosmetic product formulations to sun protection and preventing photoaging. In fact, the experimental strategy herein adopted is thought to be an interesting approach to gain scientific insights to subsidize further biotechnological applications of that biomass with a certain economic value added to it.

Conflict of interests

The authors declare no conflicts of interest.

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References


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