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Original Article

Photoprotective Activity of Plants Used for Skin Disorders by the Native Population from the Brazilian Amazon Basin: A Preliminary Study

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ABSTRACT

Background: Sunscreens are essential in tropical countries with a high annual incidence of solar radiation. The incorporation of natural products in their formulations has great public acceptance and prevents several issues related to organic and inorganic filters, such as adverse reactions. The present study evaluates the antioxidant and photoprotective activities and the ability to inhibit tyrosinase of three plant species traditionally used by Amazonian inhabitants in the treatment of skin disorders. **Materials and Methods:** The antioxidant activity, the capacity to inhibit the enzyme tyrosinase, ratio UVA/UVB, critical wavelength, sun protection factor, and cellular viability was assessed in ethanolic extracts at 10% of *Chrysobalanus icaco, Kalanchoe pinnata*, and *Ayapana triplinervis* collected in the State of Para-Brazil. **Results:** *Chrysobalanus icaco* and *Kalanchoe pinnata* showed promising antioxidant activity. *Ayapana triplinervis* demonstrated considerable inhibition of the tyrosinase enzyme. Plants extracts had low protection against UVB radiation, but adequate UVA protection. Ayapana triplinervis presented the lowest cellular viability of keratinocytes and murine fibroblasts. **Conclusion:** The addition of these extracts to other natural products with high sun protection factors can provide the consumer with a natural sunscreen.

Keywords: Skin disorders, Photoprotection, Antioxidant, Depigmenting agent, *Chrysobalanus icaco*, *Kalanchoe pinnata*, *Ayapana triplinervis*.

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INTRODUCTION

The research and development of sunscreens with natural products are attractive for the cosmetic industry because there is good public acceptance allied with the concepts of environmental sustainability. Sunscreens prevent the acute and chronic harmful effects of solar radiation, such as burns, cracks, immune suppression, dermatitis, urticaria, hyperpigmentation, changes in elasticity and thickness, premature aging, and skin cancer.[1-3] Sunscreens absorb, reflect, and/or diffract sunlight. Some formulations have a narrow spectrum of action and provide a limited defense to the skin, whereas others have a broad spectrum and prevent the deleterious effects of long-term exposure to UVA radiation, such as aging and skin cancer.[4-6]

There are different mechanisms involved in skin damage caused by solar radiation, such as oxidative imbalance, inflammation, and alteration in pigmentation.[3,7-10] Thus, natural products with anti-inflammatory and antioxidant properties and, that modulate the activity of tyrosinase, a multi-cooper enzyme related to melanogenesis, are interesting to the cosmetic industry.[1-4,7] Natural products also prevent adverse reactions to organic and inorganic filters, such as acute or chronic allergic symptoms, endocrine disturbance, acne, and rosacea.[3,4,7,11,12]

The use of sunscreens is essential for tropical countries, such as Brazil, which shows a high annual incidence of solar radiation.[5-8] Several studies evaluated the photoprotective activity of different plant species, some with promising results. The species *Chrysobalanus icaco* L. (Chrysobalanaceae), *Kalanchoe pinnata* (Lam.) Pers. (Crassulaceae), and *Ayapana triplinervis* Vahl. (Asteraceae), have healing, astringent, and antiseptic properties, used for the treatment and prevention of skin disorders by traditional and non-traditional populations of the Amazon basin. These plant species have antioxidant activity that probably contributes to preventing oxidative damage produced by solar radiation.[13-16] However, no studies assessed in these plants the parameters of photoprotection, the ability to regulate the activity of tyrosinase enzyme, and the cytotoxicity in different skin cells.

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The present study aimed to evaluate total phenols and flavonoids levels, the antioxidant activity, cellular viability, the *in vitro* parameters related to photoprotection (sun protection factor, the critical wavelength, UVA/UVB ratio), and the ability to modulate tyrosinase enzyme of ethanolic extracts of *Chrysobalanus icaco* L., *Kalanchoe pinnata* (Lam.) Pers. and *Ayapana triplinervis* Vahl.

MATERIALS AND METHODS

Collection of plants material and extract preparation

Plants were collected from January to July 2019 in different areas of the state of Pará, in the Brazilian Amazon basin. Each plant species was sampled once without considering seasonal variations. Aerial parts of *A. triplinervis* were collected in the municipality of Acara (1°32'68.4"S 48°23'98.4"W) and identified in the herbarium of Museum Emilio Goeldi, with exsiccate number MG123913. The leaves of *C. icaco* were collected at the campus of the Para Federal University in Belem (1°28'17"S 48°26'49"W), and identified in the herbarium of Museum Emilio Goeldi, with exsiccate number MG236136. The leaves of *K. pinnata* were collected in the National Forest of Tapajos in Santarem-PA (2°27'02.5"S 54°45'13.4"W) and identified in the herbarium of the Western Para Federal University with exsiccate number 00062.

The plants were washed with purified water oven-dried at 50°C, ground, and extracted by 48-hr maceration three times successively with ethanol 96°GL. Extracts of *Chrysobalanus icaco* (EECi), *Kalanchoe pinnata* (EEKp), and *Ayapana triplinervis* (EEAt) were concentrated at reduced pressure to dryness and stored at -10°C until analysis.

Total phenolic content

The total phenolic content was determined using Folin-Ciocalteu reagent in an alkaline medium (saturated sodium carbonate). The extracts were dissolved in ethanol (10 mg mL^{-1}, *n*=3). Aliquots of the sample solutions (50 µL) were mixed in test tubes with 250 µL of Folin-Ciocalteu reagent, 500 µL of 20% aqueous sodium carbonate, and 4.2 mL of water. The test tubes were incubated and protected from light at room temperature for 30 min, and then the absorbance of the solutions was read at 760 nm (UV Mini 1240, Shimadzu, Japan). The total phenolic content was determined by plotting the absorbance of plant extracts in a standard curve of Gallic acid (Sigma-Aldrich, USA) in concentrations from 25-700 µg mL^{-1}.[17]

Total flavonoid content

Each extract was dissolved in ethanol (10 mg mL^{-1}, *n*=3). In conical centrifuge tubes, 2.5 mL of the extract solutions were mixed with 1 mL of chloroform and 1.5 mL of water and then centrifuged for 3 min at 2.465×g at 25°C. The microplates were prepared by adding the following solutions, in this order: 99 µL of water; 25 µL of 8% methanolic aluminum chloride hexahydrate solution; 100 mL of pyridine: methanol (2:8, *v/v*) solution; 6 µL of glacial acetic acid; and 20 µL of the supernatant of the centrifuged plant extract solution (or 20 µL of each rutin solution). The plate was brought to stirring in a microplate shaker for 2 min and incubated in the dark for 15 min, always capped to prevent evaporation. After incubation, the absorbance was recorded in a microplate spectrophotometric microplate reader (SpectraCount Microplate Reader, Packard, USA) at a fixed wavelength of 405 nm. Rutin (Sigma-Aldrich, USA) was used as standard, and its aqueous solutions (2-30 µg mL^{-1}) were used to build up a standard curve for determining the flavonoid content of the extracts. The assays were realized in triplicate.[18]

Antioxidant activity *in vitro*

The antioxidant activity was assessed by DPPH Radical Scavenging Assay and by the ABTS radical scavenging assay

DPPH Radical Scavenging Assay

The antioxidant activity of extracts at concentrations of 0.5 to 1000 µg/ml was assessed by the DPPH radical (1,1-diphenyl-2-picrylhydrazyl). Briefly, each extract in a volume of 50 µl was transferred to a polypropylene tube and added to 150 µl of a freshly prepared DPPH solution (0.05 mM). The tube was mixed and kept in the dark for 30 min, at room temperature. The absorbance was recorded at 510 nm in a UV-VIS spectrophotometer (UV Mini 1240, Shimadzu, Japan®). A solution of 50 µl of ultra-pure water with 150 µl of the DPPH solution (0.05 mM) was used as blank and L-ascorbic acid in the same concentrations of the extracts as the positive control. The assay was performed in triplicate. The antioxidant activity was calculated following the formula:[19]

$$\text{DPPH inhibition (\%)} = [(AC - AS)/AC] \times 100$$

Where AC is the absorbance of a DPPH solution without a sample, AS is the absorbance of the sample, which is equal to the absorbance of the samples plus the DPPH (0.05 mM) excluding the blank absorbance.

ABTS radical scavenging assay

The antioxidant activity of plant extracts at concentrations of 125 to 2000 µg/ml was determined by ABTS radical cation (2,2'-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid) decolorization assay. ABTS·+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. ABTS·+ solution was diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After a total of 88 µl of plant extracts was added to 3920 µl of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. A standard curve with Trolox (6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2h-chromene-2-carboxylic acid) in concentrations from 100 µM a 2000 µM was used as a positive control. The

inhibition of absorbance at 734 nm, in percentage, was calculated using the formula:[20]

$$ABTS^{\cdot+} \text{ scavenging effect } (\%) = [(AB-AA)/AB] \times 100$$

Where AB is the absorbance of ABTS radical in methanol; AA is the absorbance of ABTS radical with the sample/Trolox. The assay was performed in triplicate and the mean value was recorded.

Photoprotection assays *in vitro*

There were determined the sun protection factor (SPF), the critical wavelength, and the ratio UVA/UVB. The minimum SPF acceptable by the Brazilian Regulatory Agency regulatory is six.[21] The critical wavelength indicates the spectrum of protection of the extracts, sunscreen products with values above 370 nm are considered of the large spectrum. The ratio UVA/UVB determines the range of spectrum blocked by sunscreen.[4-7,11,22] The ratio of UVA/UVB of each extract was classified according to the UK Boots Star® Rating (BSR) which measures the percentage of UVA that is absorbed compared to UVB. Values ranging from 0.0 to 0.59 are unacceptable; 0.6 to 0.79 (three stars), 0.8 to 0.9 (four stars) and above 0.9 (five stars).[23]

Each extract was incorporated at 10% in a neutral cosmetic lotion following the procedure proposed by Polonini *et al.* (2013).[24] Resveratrol at 15% was the standard. Samples were accurately and quickly weighed (1.3 $mg.cm^{-2}$) in polymethylmethacrylate (PMMA) plates (n=3) and protected from light exposure in a dark chamber at room temperature (≈20°C) for 15 min. The transmittance was measured from 290 to 450 nm, at 1 nm intervals, at nine different sites of each plate, using a transmittance analyzer (UV2000S, Labsphere, USA®). A blank of glycerin (15µL) was applied over coated PMMA plates as recommended by the European Association of Cosmetics and Perfumery (COLIPA).[25] The transmittance was recorded for each sample and the software Labsphere Transmittance Analyzer SPF Report® calculates the SPF, the critical wavelength (λc), and the ratio UVA/UVB.

Tyrosinase activity reaction assay

The assay was based on the procedure validated by Lima *et al.* (2013), with modification.[26] Briefly, a total of 10 µl of tyrosinase (125 U/ml) obtained from mushrooms (Sigma-Aldrich, USA) were added to 96-well microplates, 70 µl of pH 6.8 buffer solution, and 60 µl of the plant extract at 350 µg/ml with DMSO 25%. The negative control was DMSO 2.5%, and the positive control was a solution of kojic acid at 17.5 µg/ml in DMSO 25%. A total of 70 µl of an aqueous solution of L-tyrosine was added to the mixture. The absorbance was measured at 492 nm (T_0) in a microplate spectrophotometer reader (Perkin Elmer®). The microplates were incubated at (30±1)°C for 60 min in the first screening (T_1), and for 60 min in the second screening (T_2). Optical densities were registered in a computer coupled to the spectrophotometer reader. Inhibitory activity was obtained according to the formula: IA%=[(C-S)/C] x 100, where IA%=Inhibitory activity; C=negative control absorbance; S=sample or positive control absorbance (absorbance at time T_1 or T_2 minus the absorbance at time T_0), and each parameter was a mean of 6 measures.

Cellular viability

The cellular viability of ethanolic extracts of the plants was tested in murine fibroblast (L929) and epidermal keratinocyte (human HaCaT cell line), purchased from the European Collection of Cell Cultures (Salisbury, UK®). The cells were grown in Dulbecco's modified Eagle's Medium (DMEM High Glucose) supplemented with 10% fetal calf serum (FCS), 1% of L-glutamine, and 1% of penicillin/streptomycin and incubated at 37°C in an 8.4% CO_2 atmosphere. Cell cultures were passaged once per week. All experiments were performed with cultures at approximately 80% of confluence for both cells.[27]

The effects of ethanolic extracts on the viability and proliferative activity of cultured L929 and HaCaT cells were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay that evaluates the mitochondrial NADH-dependent dehydrogenase activity, which is proportional to both cell viability and proliferation rates of cells.

A total of 5×10^4 cells/well were seeded in single wells of 96-well plates and upon reaching the desired confluence, were incubated with concentrations of plant extracts from 7.8125 to 1000 µg/ml for 24 hr under the same conditions. Cells with the culture medium containing DMSO at 0.5% were the negative control. The supernatants containing free-floating dead cells were removed at the end of the incubation period and replaced with a fresh culture medium containing MTT at a final concentration of 5 mg/ml. The cell layers were further incubated for another three hours. Then, culture supernatants were removed, and the cells adherent to the plate surface were collected in 100% DMSO and incubated for 15 min at 37°C to finalize the reaction and dissolve formazan crystals. The absorbance of formazan formed was recorded at 570 nm in a microplate reader. The assays were performed in triplicate. The following formula was used to estimate cell viability:[27]

$$\% \text{ Cell viability} = (\text{absorbance of treated cells} / \text{absorbance of control cells}) \times 100$$

IC_{50} was determined based on the percentage of viable cells versus the different concentrations of plant extracts.

Data analysis

Data are presented as mean (standard deviation) or as median and range. One-way analysis of variance with Bonferroni post-test or Mann-Whitney-U test compared the variables of the study. The level of significance accepted was 5%.

RESULTS

The concentrations of flavonoids and total polyphenols and the antioxidant activity of *C. icaco (*EECi*), K. pinnata* (EEKp), and *A. triplinervis (*EEAt*)* are in Table 1.

The EECi presented the highest content of total polyphenols and *EEKp* of flavonoids. Moreover, *EECi* presented the lowest IC_{50} for both DPPH and ABTS radical scavenging assays. Other hand, EEAt presented the highest IC_{50} by both methods and no mensurable concentrations of total flavonoid. The ethanolic extracts of plants at 10% (m/m) showed SPF ≤ 2. The critical wavelength of plant extracts was above 370 nm. The UVA/UVB ratios of EECi and EEAt at a concentration of 10% (m/m) were above 0.8, which allows a classification of four stars, and EEKp of 0.699, received three stars. Critical wavelength and UVA/UVB ratios of ethanolic extracts were higher than the resveratrol at 15% (Table 2).

In vitro photoprotective parameters: Sun Protection Factor (SPF), Critical Wavelength (λc), and UVA/UVB ratio of Ethanol Extracts of *Chrysobalanus icaco* (EECi), *Kalanchoe pinnata* (EEKp) and *Ayapana triplinervis* (EEAt), at 10% (m/m). Standard substance Resveratrol 15% (m/m).

The assay of inhibition of tyrosinase enzyme revealed that the EEAt showed inhibitory activity of 87.9% and 64.94% at 60min and 120 min. The EECi demonstrated an inhibitory activity of 78.75% at 60 min, which dropped off to 42.12% after 120 min. EEKp presented inhibitory activity below 40% at 60 and 120 min. There were no statistical differences between EEAt and kojic acid at 17.5%, but EECi showed an inhibitory activity like kojic acid

Table 1: Total polyphenols, flavonoids and the antioxidant activity of Ethanolic Extracts of *Chrysobalanus icaco* (EECi), *Kalanchoe pinnata* (EEKp), and *Ayapana triplinervis* (EEAt).

Plant extract	Assay			
	Concentration (mg/g±S.D)+		Antioxidant activity (IC_{50} in µg/mL)	
	Total polyphenols (EAG)	*Flavonoids (EQT)*	*DPPH*	*ABTS*
EECi	234.4±3.01[a]	131.2±2.31[a]	8.56±1.80*	3.95±0.40
EEKp	196.8±2.37[b]	137.7±2.81[a]	24.50±4.56*	8.95±1.77*
EEAt	14.7±0.19[c]	-	109.49±9.10*	34.11±6.27*
Ascorbic Acid[#]	-	-	1.50±1.44	-
Trolox[#]	-	-	-	2.39±0.69

+ Results expressed as mean and standard deviation; EAG = equivalent to gallic acid; EQT = equivalent to quercetin; DPPH (2,2-difenil-1-picrilhidrazil); reference standard; different letter in the same column indicates statistical difference among the samples ($p<0.05$).

Table 2: *In vitro* photoprotective parameters: Sun Protection Factor (SPF), Critical Wavelength (λc), and UVA/UVB ratio of Ethanol Extracts of *Chrysobalanus icaco* (EECi), *Kalanchoe pinnata* (EEKp) and *Ayapana triplinervis* (EEAt), at 10% (m/m). Standard substance Resveratrol 15% (m/m).

Sample	SPF	λc (nm)	UVA/UVB
EECi	1.0±0.0	384	0.807
EEKp	2.0±0.1	386	0.696
EEAt	2.0 ±0.1	389	0.870
Resveratrol	7.0 ± 1.7	362	0.460

Table 3: Tyrosinase enzyme inhibitory activity (IA) of the ethanolic extracts of *Ayapana triplinervis* (EEAt), *Chrysobalanus icaco* (EECi) and Kalanchoe *pinnata* (EEKp) at 10%. Kojic acid at 17.5µg/ml (AK) is the standard.

Sample	IA 60 min. (%)	IA 120 min (%)
EEAt	87.90 ± 4.51	64.94 ± 2.20
EECi	78.73 ± 6.41	42.12 ± 7.05*
EEKp	34.99 ± 13.40*	29.87 ± 9.48*
AK	90.21 ± 4.38	69.13 ± 1.94

*Statistical difference compared to the positive control (Kojic Acid), $p < 0.01$.

Table 4: Mean Inhibitory Concentration (IC_{50}) of Ethanol Extracts of *Chrysobalanus icaco* (EECi), *Kalanchoe pinnata* (EEKp), and *Ayapana triplinervis* (EEAt) in Murine Fibroblasts (L929) and Human Keratinocytes (HaCat) cells.

Plant extract	IC_{50} (µg/ml)	
	L929	HaCat
EECi	136.40±13.91*	216.20±50.60*
EEKp	> 500*	201.20±21.22*
EEAt	74.72±5.22	76.06±7.94

*Statistical difference to EEAt ($p<0.05$).

only at 60min. Finally, EEKP presented inhibitory activity lower than the kojic acid in both periods. (Table 3).

The cellular viability of murine fibroblast (L929) and human keratinocytes exposed to the plant extracts are shown in Table 4. The mean IC_{50} of EECi was 136.4 μg/ml and 216.2 μg/ml, respectively. The mean IC_{50} of EEKp in human keratinocytes was 201.2 μg/ml and in murine fibroblast was above 500 μg/ml. EEAt presented the lowest cellular viability when compared to the other plant extracts, with a mean IC_{50} of 74.72 μg/ml and 76.06 μg/ml for murine fibroblasts and human keratinocytes.

DISCUSSION

Sunscreen is essential for tropical countries, such as Brazil, where the rate of skin diseases is high in the population. The non-melanoma skin cancer is the malignancy most prevalent, representing approximately 30% of cases of cancer with about 176.940 cases diagnosed in 2020.[28] Incorporation of natural resources into sunscreens is in line with the concept of environmental sustainability as it can provide cosmetics with adequate cost-effectiveness to consumers, which allows for the popularization of these compounds, contributing to a decrease in the rate of skin cancer.[1,3,29,30]

The antioxidant activity is an important property of sunscreens because the UVA radiation contributes to the production of reactive oxygen species in the layers of the skin, including the dermis promoting the oxidation of lipids and proteins, accompanied by depletion of enzymes and molecules responsible for antioxidant defenses. The anti-inflammatory response also is affected. There is an upregulation of the antioxidant response to preserve the oxidative equilibrium. The oxidative imbalance is associated with changes in the production of melanin, premature photo-aging, suppression of immunologic functions, necrosis of endothelial cells, damage to dermal blood vessels, and skin cancer.[2,4-6,9,10]

A natural product candidate for incorporation into sunscreens formulations must present robust antioxidant activity. Compounds such as polyphenols (flavonoids, tannins), carotenoids, anthocyanins, fixed and volatile oils from vegetables, fruits and, plants parts are incorporated with success into sunscreens formulations because of their antioxidant property.[1,3,30,31] In the study, the concentrations of flavonoids and total phenols were high in the ethanolic extracts of *C. icaco* and *K. pinnata,* but *A. triplinervis* showed low levels of total phenols and no mensurable concentrations of flavonoids were found in the ethanolic extract.

The antioxidant activity of the ethanolic extracts of *C. Icaco* and *K. pinnata* showed low IC_{50} by both *in vitro* methods of analysis when compared to *A. triplinervis*, which can be related to phenolic compounds that also dissipate the energy emitted by solar radiation and provide natural protection to plants against sunlight.[15,32,33] Previous studies reported the correlation between antioxidant activity and the concentrations of phenolic compounds in plants.[24,31,32-34]

C. icaco and *K. pinnata* have phenolic compounds, with a similar concentration of flavonoids, which corroborate other studies,[13,14,34,35] but *A. triplinervis* contains polyphenols, with low concentrations of flavonoids, but with elevated levels of coumarins, which have as precursors the phenolic compounds,[15,16,36] which justifies the high IC_{50} when compared with other plants studied. However, *A. triplinervis* has antioxidant activity *in vivo*, characterized by increased levels of reduced glutathione and in the activities of antioxidant enzymes such as catalase and superoxide dismutase, accompanied by decreased lipid peroxidation.[36,37]

The SPF is the most popular parameter to evaluate the effectiveness of sunscreen. This factor estimates the protection against erythema and sunburn caused by UVB radiation. The Food and Drugs Administration (FDA) proposes that all sunscreens products with SPF ≥ 15 and a UVAI/UV ratio of 0.7 or higher are classified as broad spectrum. The FDA also states that only broad-spectrum sunscreens with an SPF of 15 or higher can reduce the risk of skin cancer and premature skin aging.[4,22] Non-broad-spectrum sunscreens and broad-spectrum sunscreens with an SPF between 2 and 14 can merely prevent sunburn.[4,7,11,22] In the study, the SPF was low (range, 1-2) in the ethanolic extracts, which can solely limit sunburns.

Critical wavelength and the ratio of UVA/UVB were determined in the plant extracts to assess if they give protection against UVA radiation. The critical wavelength was above the minimum acceptable of 370nm, with the highest value in *A. triplinervis*, followed by *K. pinnata* and *C. icaco*.[4,11,22] The UVA/UVB ratio of the extracts classified according to the UK Boots Star® Rating received three (*K. pinnata*) and four stars (*C. icaco* and *A. triplinervis*), which allow for the classification as superior and maximum.[23] Thus, the plant extracts provided adequate protection against UVA radiation.

The inhibition of tyrosinase is an important property of natural compounds as it is associated with the depigmenting activity of cosmetic products.[3,38,39] *A. triplinervis* presented an inhibitory activity like Kojic Acid at 60- and 120-min. *C. icaco* too showed significant inhibition of tyrosinase activity at 60 min, which dropped off after 120 min, suggesting a degradation of the extract. *K. pinnata* presented the lowest capacity of enzyme inhibition. In Indonesia, *A. triplinervis* is used for cutaneous hyperpigmentation, a property that could be associated with enzymatic inhibition by 7-metoxycoumarin, already reported in melanoma C16 cells.[40]

The assessment of cellular viability demonstrated that *A. triplinervis* have an IC_{50} significantly lower than the ethanolic extracts of *C. icaco* and *K. pinnata* for murine fibroblasts

and human keratinocytes. However, the low cytotoxicity of methanolic extract of *A. triplinervis* was demonstrated in human fibroblasts WS1.[40] The low cytotoxicity of *Kalanchoe pinnata* was shown in T cell lines H-9[35] and macrophages of the bone marrow of mice.[34]

The ethanolic extracts of the study plants showed adequate UVA protection, which is important to prevent the deleterious effects of long-term exposure to sunlight. However, the UVB protection was unsatisfactory, which could be improved either by determining the SPF of isolated components or by incorporating these substances in natural compounds with SPF that attend the recommendations of regulatory agencies, such as resveratrol (SPF 19), kaempferol (SPF 24.9), apigenin (SPF 28.8), and caffeic acid (SPF 28.8) at 7%.[1-3,31] These associations already were tested, for example, resveratrol associated with extracts of *Olea europaea, Hibiscus sabdariffa*, and *Rubusidaeus* sp., attained an SPF of 46 and UVA/UVB ratio of 0.75 whereas resveratrol alone has an SPF of 10 and UVA/UVB ratio of 0.65.[41] Alternatively, the extracts could be incorporated into organic or inorganic compounds. For instance, the extract of fruits from *Passiflora edulis* associated with titanium dioxide increased the SPF from 15.48 to 18.75 e 18.99 in formulations of plant extract at 0.1 and 0.3%.[42] Further studies are required to evaluate the indirect photoprotection of these extracts after oral uses, as reported with *Polypodium leucotomos*, and related to its antioxidant propriety.[43]

The inhibition of the tyrosinase enzyme by *A. triplinervis* is another property that can be used in sunscreens formulations to improve protection against the deleterious effects of UV radiation.

CONCLUSION

The ethanolic extracts of plants provided adequate protection against UVA radiation *in vitro*, but low protection against UVB radiation. The addition of natural compounds with the required SPF can provide a natural sunscreen of a broad spectrum to protect the skin against sunlight. Extract of *A. triplinervis* also inhibited tyrosinase. The cell viability demonstrated that *A. triplinervis* have an IC_{50} significantly lower than the ethanolic extracts of *C. icaco* and *K. pinnata*. The *in vitro* tests performed in the study suggest that these extracts are candidates for incorporation into natural sunscreens formulations and recommend further *in vivo* studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UVA: Ultraviolet A; **UVB:** Ultraviolet B; **EECi:** Ethanolic extract of *Chrysobalanus icaco* **EEKp:** Ethanolic extract of *Kalanchoe pinnata*; **EEAt:** Ethanolic extract of *Ayapana triplinervis* (EEAt); **DPPH:** 1,1-diphenyl-2-picrylhydrazyl; **ABTS:** radical cation (2,2'-azino-di-(3-ethylbenzothiazoline)-6-sulfonic acid; **SPF:** Sun Protection Factor; **DMSO:** Dimethyl Solfoxide; **FCS:** Fetal calf Serum; **DMEM:** Dulbecco's Modified Eagle's Medium; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **NADH:** Nicotinamide adenine dinucleotide; **FDA:** Food and Drugs Administration; **IC_{50}:** The concentration of drug required for 50% inhibition.

SUMMARY

Several mechanisms are involved in the damage caused by solar radiation on human skin, such as imbalance oxidative, inflammation, and alteration of skin pigmentation. Sunscreens formulations with natural compounds that present such properties are beneficial to protect the skin. These activities were tested in the ethanolic extracts of *C. icaco, K. pinnata,* and *A. triplinervis., C. icaco, K. pinnata* demonstrated the presence of phenolic compounds and flavonoids, antioxidant activity, and a broad spectrum of action against UVA radiation. *A. triplinervis* presented an interesting inhibition of tyrosinase, a broad spectrum of action, low content of phenols compounds and the lowest cellular viability for murine fibroblast and human keratinocytes.

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