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ORIGINAL ARTICLE

Photoprotective Activity of Medicinal Plants From the Caatinga Used as Anti-inflammatories

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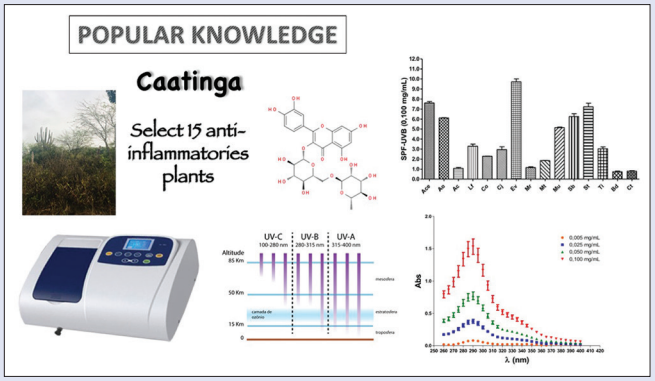
ABSTRACT

Background: Exposure to ultraviolet (UV) radiation may cause photoaging, unsightly marks, or dangerous lesions, such as carcinomas and/or melanomas. Sun filters are substances capable of absorbing, reflecting, or refracting UV radiation and thus protect the skin from direct exposure to sunlight. The current trend in the cosmetics industry, in Brazil, is to rationally explore local biodiversity as a way of developing products of natural origin, especially derived from plants. **Objective:** The present study aims to determine the *in vitro* sun protection factor (SPF) of 15 species from the Caatinga region used in popular medicine as anti-inflammatories. **Materials and Methods:** Samples of duly identified plant species were dried and ground and hydroethanolic extracts were obtained (80:20). Spectrophotometric analyses were carried out to determine the SPF, antioxidant activity, and quantification of secondary metabolites. *In vitro* calculation of SPF was based on the method developed by Mansur. **Results:** *Erythrina velutina* Willd. had the best SPF of 9.71 ± 1.29 at a concentration of 100 mg/L. **Conclusion:** The study showed that native species to the Caatinga used by the local population to treat inflammatory disorders have good photoprotective potential and could be used for pharmaceutical preparations to this end.

Keywords: Antioxidant activity, Caatinga, flavonoids, phytochemistry, sun protection factor

SUMMARY

- Medicinal plants from Caatinga used as anti-inflammatories have a photochemoprotective potential.
- *Erythrina velutina, Spondias tuberosa and Amburana cearensis* presented the best SPF values among the 15 species studied.
- *Erythrina velutina* was the only one to obtain an absorption peak at 290 nm, the same absorption region of UV type B.



Abbreviations used: ANOVA: Analysis of variance; AOA: Antioxidant activity; CC: Coumarin content; CE: Coumarin equivalent; DNA: Deoxyribonucleic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: Inhibitory concentration 50%; LEA-UFPE: Laboratory of Ecology and Evolution of Social-ecological Systems-Federal University of Pernambuco; RE: Rutin equivalent; SPF: Sun protection factor; TAE: Tannic acid equivalent; TFC: Total flavonoid content; TPC: Total phenolic content; TTC: Total tannin content; UFPE: Federal University of Pernambuco; UFRPE: Federal Rural University of Pernambuco; UV: Ultraviolet; UVA: Ultraviolet type A; UVB: Ultraviolet type B; UV-VIS: Ultraviolet-visible

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INTRODUCTION

Solar radiation is necessary for various biological processes in human beings, plants, and animals but may also cause serious damage to human skin, depending on the frequency and length of exposure and other factors such as intensity of radiation, latitude, and the sensitivity of the individual.[1] Such damage is mostly caused by the ultraviolet (UV) region of the electromagnetic spectrum, with types UV type A (320–400 nm) and UV type B (UVB) (290–320 nm) being responsible for burns, erythemas, edemas, and photoaging of skin and a causative factor in the development of skin cancer.[2]

The prevention of melanoma, for example, is an important public health measure, giving the rising increase in the occurrence of this malignant lesion in the population. Inadequate protection against solar rays can have a drastic effect on human health from a clinical point of view.[3-5] Despite the large number of skin cancer prevention campaigns, the number of people who expose themselves to the sun without protection is still relatively high.

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One way of avoiding these pathologies is to use sunscreen, the products that have the ability to protect basal and stratum spinosum cells,[6] usually topical preparations. The current commercial products are thus derived from synthetic substances, which are not only heavy duty but also exclusively photoprotective.[7]

Photochemical protection has emerged as a way of increasing the effectiveness of sunscreens. This involves the addition of isolated natural products, usually vegetable extracts with antioxidant and/or anti-inflammatory activity, to counter some of the damage done by solar radiation[8,9] either topically or orally.[10] The compounds derivatives by vegetable biosynthesis are as a source of biodegradable products, which have a less environmental impact and are of interest from a commercial point of view.

Various species of plant native to the Caatinga region contain phenolic compounds such as flavonoids,[11] whose absorption spectrum has two maximal peaks, one between 240 and 280 nm and another between 300 and 550 nm. It is, therefore, possible to use these plant species to develop photoprotective solar filter preparations since they are predominantly composed of such compounds.[12]

Phenolic compounds are easily found in nature, especially plants, in a diverse range of classes.[13,14] This group has anti-inflammatory and immunomodulatory properties and is capable of repairing deoxyribonucleic acid (DNA). Some studies suggest that the photoprotective effects and anti-photocarcinogenic properties of these metabolites are related to inhibition of inflammatory mediators induced by UVB.[15]

The present study aims to determine the photoprotective activity, *in vitro*, of 15 medicinal species from the Pernambuco Caatinga, selected using an ethnologically guided method, indicated for inflammatory disorders and containing phenolic compounds, in the search for promising alternative products with such properties.

MATERIALS AND METHODS

Materials

Ethanol (Vetec, 99.5%) was used as the solvent to extract the samples. To determine the total phenolic content (TPC) and total tannin content (TTC), anhydrous sodium carbonate (Vetec, 99.5%) and Folin–Ciocalteu phenol reagent (Fluka, 2N) were used. Glacial acetic acid (Merck, 100%), aluminum chloride hexahydrate (Honeywell, 99%), and pyridine (Vetec, 99%) were used to quantify the flavonoid content. Cloridric acid (Vetec 37%) and lead acetate II (Êxodo) were used to quantify the coumarin content (CC). For the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, DPPH (Aldrich, 95%) was used. Methanol (Vetec, 99.8%), ascorbic acid (Vetec, 99%), tannic acid (Vetec, 99%), rutin (Acros Organics, 97%), and 1,2-benzopyrone (Sigma-Aldrich, 99%) were used as standards. Weights were measured on a Shimadzu analytical balance (AX200), and absorbance readings were recorded using a Shimadzu UV-Visible (UV mini-1240) spectrophotometer.

Experimental methods

Botanical materials

The species were selected from a database formed by the survey of traditional knowledge, carried out by the application of the main techniques of ethnopharmacological data collection, such as free-list and semi-structured interviews, described in Araujo *et al*.[16] This database, previously developed by the Laboratory of Ecology and Evolution of Social-ecological Systems-Federal University of Pernambuco (UFPE), has already been used in several studies, among other indications, for anti-inflammatory purposes.[11,17-20]

The plant samples were collected in a semiarid part of the municipality of Altinho, in the Brazilian State of Pernambuco (08° 35'13.5" S and 36°05'34.6" W). All the species were indicated, at least three times, by the local population for the treatment of inflammatory disorders. The species, families, and parts used are listed in Table 1.

Vouchers of the selected species were identified at the Applied Ethnobotany Laboratory of UFPE with the aid of the keys for botanical identification and specialized bibliography. The vouchers of the species were deposited in the Professor Vasconcelos Herbarium collection, Federal Rural University of Pernambuco, in the Professor Geraldo Mariz Herbarium collection, UFPE and in the Agronomic Institute of Pernambuco.

Preparation and chemical characterization of extracts

The plant samples were cut and exposed to the ambient environment for 2 weeks to dehydrate. After drying, the samples were ground in a vertical Wiley type knife mill (Adamo 340) with standardized sieves, to obtain a 20 Mesh (1.2 mm) granulometry. Extraction was then carried out by maceration (48 h) at a proportion of 1:10 (m/v) with 80% ethanol (v/v) and the extracted liquid macerated twice more, giving a total of three macerations. The extracts were then filtered and evaporated under reduced pressure, at a temperature of 40 ± 5°C.

Determination of the total phenolic content and total tannin content

The TPC of the extracts was determined by the Folin–Ciocalteu method, and the residual phenolic content was determined by precipitation of casein followed by Folin–Ciocalteu method, where the TTC is the difference between the levels of total and residual phenols.[21] TPC and TTC were expressed as 1 mg of tannic acid per gram of sample (mg TAE/g). The samples were evaluated in triplicate. The calibration equation for tannic acid was $y = 0.047x + 0.127$ ($R^2 = 0.985$).

Determination of total flavonoid content

The TFC of the extracts was estimated using a colorimetric method based on the formation of a flavonoid–aluminum complex.[22] The results were expressed as 1 mg of rutin per each gram of sample (mg RE/g). The samples were evaluated in triplicate. The rutin calibration equation was $y = 0.026x + 0.020$ ($R^2 = 0.997$).

Table 1: Medicinal species used to treat inflammatory processes in the municipality of Altinho/PE

Scientific name	Popular name	Family	Used part
Amburana cearensis (Allemao) A.C. Sm.	Imburana açú	Fabaceae	Bark
Anacardium occidentale L.	Caju-roxo	Anacardiaceae	Bark
Anadenanthera colubrina (Vell.) Brenan	Angico	Fabaceae	Bark
Boerhavia diffusa L.	Pega pinto	Nyctaginaceae	Root
Cedrela odorata L.	Cedro	Meliaceae	Bark
Cereus jamacaru DC.	Mandacaru	Cactaceae	Root
Crateva tapia L.	Trapiá	Capparaceae	Bark
Erythrina velutina Willd.	Mulungu	Fabaceae	Bark
Libidibia ferrea Mart.	Jucá	Fabaceae	Bark
Maytenus rigida Mart.	Bom nome	Celastraceae	Bark
Mimosa tenuiflora (Willd.) poir.	Jurema lisa	Fabaceae	Bark
Myracrodruon urundeuva Allemao	Aroeira	Anacardiaceae	Bark
Schinopsis brasiliensis Engl.	Baraúna	Anacardiaceae	Bark
Spondias tuberosa Arruda	Umbu	Anacardiaceae	Bark
Tabebuia impetiginosa (Mart. ex DC.) Standl.	Pau d'arco roxo	Bignoniaceae	Bark

Medicinal species used to treat inflammatory processes in the municipality of Altinho - State of Pernambuco, Brazil

Determination of the coumarin content

The CC was determined using the colorimetric assay described by Osório and Martins[23] with some adjustments. The results were expressed as 1 mg of coumarin (1,2-benzopyrone) per gram of sample (mg CE/g). The samples were evaluated in triplicate. The coumarin calibration equation was $y = 0.022x + 0.005$ ($R^2 = 0.994$).

Evaluation of antioxidant activity

The free-radical scavenging activity (DPPH) assay was performed in triplicate, based on the method described by de Sousa Araújo *et al.*[24] Based on the absorbance readings, the inhibitory concentration 50% (IC_{50}) was obtained, which represents the concentration of extract or ascorbic acid (positive control) required to decrease the initial concentration of DPPH by 50%. To calculate the IC_{50}, a graph was prepared where the sample concentrations (μg/mL) or positive control was displayed in the abscissa, and the percentage of DPPH remaining (%$DPPH_{REM}$) was placed in the ordinate, obtaining a first-order curve and its equation. The %$DPPH_{REM}$ was calculated according to the following formula:

$$\%DPPH_{REM} = ([DPPH]_{T=t}/[DPPH]_{T=0}) \times 100$$

where $[DPPH]_{T=t}$ corresponds to the concentration of DPPH after reaction with the extract and $[DPPH]_{T=0}$ is the initial concentration of DPPH, that is, 40 μg/mL (100 μmol/L).

Determination of the maximum absorption wavelength and sun protection factor in vitro

For the determination of the maximum absorption wavelength (λ_{max}), the dried extracts were diluted in absolute ethanol, obtaining concentrations of 0.005, 0.025, 0.050, and 0.100 mg/mL. Subsequently, spectrophotometric scanning was performed at wavelengths between 260 and 400 nm, with intervals of 5 nm. The readings were performed using 1 cm quartz cell, and ethanol was used as the blank.[25] Sun protection factor (SPF) was calculated according to the equation developed by Mansur *et al.*[26] [Table 2] as follows:

$$FPS = FC.\sum_{290}^{320}.EE(\lambda).\ I(\lambda).\ abs(\lambda)$$

where EE (λ) is the erythemal effect spectrum; I (λ) is the solar intensity spectrum; abs (λ) is the absorbance of sunscreen product; and CF is the correction factor (=10). The values of EE × I are constants. They were determined by Sayre *et al.*[27] and are showed in Table 2.

Statistical analysis

The Shapiro–Wilk test confirmed the normality of the data obtained. Data were expressed as means ± standard deviation and were analyzed using one-way analysis of variance followed by the Tukey's test. A Pearson correlation test was used to compare the phenolic content among themselves and with the antioxidant and SPF tests of the samples. $P < 0.05$ was considered statistically significant. BioEstat 5.3 software was used to perform statistical analysis and GraphPad Prism 5 to plot graphs.

Table 2: Normalized product function used in the calculation of sun protection factor

Wavelength (nm)	EE × I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

EE: Erythemal effect spectrum; I: Solar intensity spectrum

RESULTS

Myracrodruon urundeuva presented the highest total phenol content (497.07 ± 12.13 mg TAE/g) and was statistically different from *Schinopsis brasiliensis*, *Anadenanthera colubrina*, *Cedrela odorata*, *Mimosa tenuiflora*, and *Anacardium occidentale*. In terms of tannin content, *A. occidentale* presented 460.85 ± 15.90 mg TAE/g, which was not statistically different from *C. odorata*, *M. urundeuva*, and *Spondias tuberosa* [Table 3]. It is worth noting that there was a positive correlation between total phenolic compounds and tannins ($r = 0.9315$ and $P < 0.0001$).

In the TFC and CC, *Amburana cearensis* (150.77 ± 11.10 mg RE/g and 461.40 ± 33.84 mg CE/g) and *Erythrina velutina* (257.14 ± 11.95 mg RE/g and 433.33 ± 14.29 mg CE/g) contained notably high quantities in bark samples.

Analysis of the antioxidant capacity of the extracts of the species under study followed an adapted version of the classification proposed by Gomes de Melo *et al.*[28] according to which the plants are classified in terms of activity into three groups: good (IC_{50} <44.34 μg), average (44.34 μg/mL <IC_{50} <103.46 μg/mL), and poor (IC_{50} >103.46 μg/mL).

Using this standard classification, Table 3 shows that, of all the species studied, *M. urundeuva* (Aroeira) showed the greatest capacity for capturing the DPPH free radical, with lowest IC_{50} for 16.46 ± 0.41 μg/mL, a result similar to that for the ascorbic acid (14.78 ± 1.40 μg/mL), used as a positive control. Despite the higher level of antioxidant activity (AOA), there was no statistically significant difference between Aroeira and the other eight species: *A. occidentale*, *A. colubrina*, *Libidibia ferrea*, *C. odorata*, *Maytenus rigida*, *M. tenuiflora*, *S. brasiliensis*, and *S. tuberosa*.

According to the correlation test, the AOA may be associated with total phenolic compound and tannin content ($r = -0.8066$, $P = 0.0015$, and $r = -0.7815$; $P = 0.0027$, respectively). The negative association derives from the fact that the greater the quantity of these compounds, the higher the level of free-radical capture activity. There was also no correlation between this activity and flavonoids or coumarins.

E. velutina (Mulungu) has the highest SPF (9.71 ± 0.30) compared to the other species, as can be seen in Figure 1. In the group of plants studied, photoprotection was correlated positively with flavonoids ($r = 0.8534$ and $P = 0.0146$) and coumarins ($r = 0.8737$ and $P = 0.0010$), suggesting a possible contribution of these groups of metabolites to the activity under investigation.

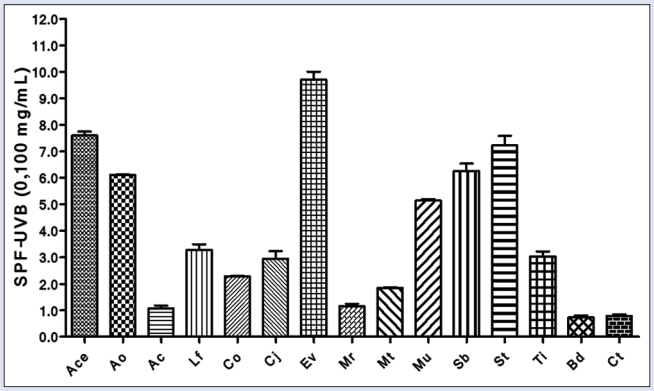

Figure 1: Sun protection factor-ultraviolet type B values at a concentration of 0.100 mg/mL for the species studied. Sun protection factor-ultraviolet type B = ultraviolet type B sun protection factor, Ace = *A. cearensis*, Ao = *A. occidentale*, Ac = *A. colubrina*, Lf = *L. ferrea*, Co = *C. odorata*, Cj = *C. jamacaru*, Ev = *E. velutina*, Mr = *M. rigida*, Mt = *M. tenuiflora*, Mu = *M. urundeuva*, Sb = *S. brasiliensis*, St = *S. tuberosa*, Ti = *T. impetiginosa*, Bd = *B. diffusa* and Ct = *C. tapia*

Table 3: Total phenol, tannin, flavonoid, and coumarin content (mg/g), inhibitory concentration for reduced absorbance of 50% (μg/mL), and sun protection factor (100 mg/L) expressed as mean±standard deviation for Caatinga species

Scientific name	TPC (mg TAE/g)	TTC (mg TAE/g)	TFC (mg RE/g)	CC (mg CE/g)	IC_{50} (μg/mL)	SPF (0,100 mg/mL)
Amburana cearensis	216.63±17.92[a]	186.31±17.79[f]	150.77±11.10[d]	461.40±33.84[a]	332.89±21.32[c,f]	7.61±0.14[g]
Anacardium occidentale	488.43±6.58[b]	460.85±15.90[c]	ND	109.41±3.72[c]	23.34±0.65[b]	6.12±0.02[b]
Anadenanthera colubrina	492.09±3.57[b]	331.24±3.52[d,g]	ND	ND	16.66±1.32[b]	1.08±0.09[e]
Boerhavia diffusa	ND	ND	ND	ND	1014.86±63.08[e]	0.73±0.07[e]
Cedrela odorata	477.78±28.24[b,e]	447.53±28.69[c]	31.80±2.37[c]	34.85±2.62[b]	19.43±0.95[b]	2.28±0.02[c]
Cereus jamacaru	ND	ND	69.11±6.05[e]	64.62±1.69[be]	576.36±44.46[a,c]	2.95±0.29[f]
Crateva tapia	ND	ND	ND	ND	13158.01±1207.78[d]	0.79±0.06[e]
Erythrina velutina	186.28±12.51[a]	147.88±13.83[a]	257.14±11.95[a]	433.33±14.29[a]	838.05±78.77[a,e]	9.71±0.30[a]
Libidibia ferrea	370.33±35.28[c]	370.33±35.28[b]	13.41±1.30[b]	53.79±3.47b[e]	27.53±0.54[b,f]	3.29±0.21[f]
Maytenus rígida	382.43±8.33[c]	296.20±9.37[d]	42.20±1.87[c]	ND	49.71±4.13[b,f]	1.17±0.08[e]
Mimosa tenuiflora	478.46±11.62[b,e]	379.50±17.28[b]	ND	38.80±3.75[b]	24.78±0.35[b]	1.85±0.02[c]
Myracrodruon urundeuva	497.07±12.13[b]	441.66±30.52[c]	13.33±1.26[b]	117.43±7.28[c]	16.46±0.41[b]	5.15±0.06[d]
Schinopsis brasiliensis	493.88±13.23[b]	367.12±21.35[b,g]	ND	ND	19.69±0.77[b]	6.26±0.28[b]
Spondias tuberosa	452.56±29.33[e]	452.1±7.23[c]	ND	187.72±3.04[d]	24.98±0.43[b]	7.22±0.36[g]
Tabebuia impetiginosa	107.22±10.46[d]	79.78±6.28[e]	ND	87.72±8.04[c,e]	348.41±16.84[c]	3.03±0.19[f]

Values followed by the same letter in column are not statistically different according to ANOVA followed by the Tukey's test (P<0.05). TPC: Total phenolic content; TTC: Total tannin content; TFC: Total flavonoid content; CC: Coumarin content; IC_{50}: Inhibitory concentration for reduced absorbance of 50%; SPF: Sun protection factor; ND: Not detected; ANOVA: Analysis of variance

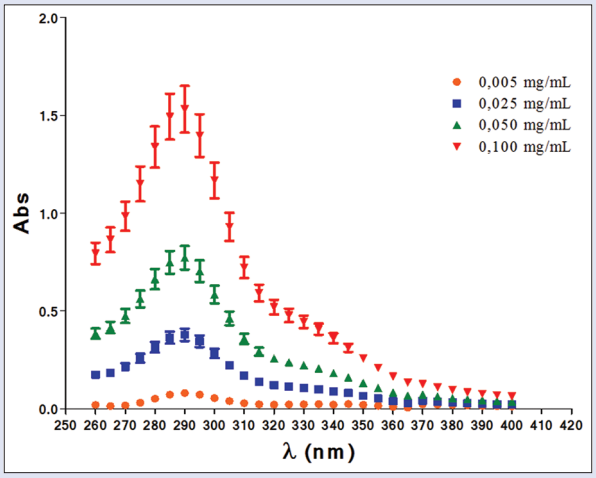

Figure 2: Absorbance of *Erythrina velutina* Willd. at concentrations of 0.005, 0.025, 0.050, and 0.100 mg/mL. Abs = absorbance, λ = wavelength

Another notable feature of *E. velutina* was a maximum absorption peak at 290 nm, as can be seen in Figure 2, while the other species studied had peaks below this wavelength.

This result is of fundamental importance, as the absorption peak for the species lays in the UVB radiation band (290–320 nm), justifying the use of this extract in photoprotection products.

DISCUSSION/CONCLUSION

The results for quantitative determination showed the presence of total phenols and tannins, as expected, since almost all samples obtained were bark, a part of the plant in which tannins are responsible for the protection against attack by predators, such as herbivores and insects. The results of the present study also corroborate the findings of Monteiro *et al.*[29] who found *M. urundeuva* to provide the best results.

A higher content of flavonoids and coumarins was found in the barks, in addition to the high exposure to solar radiation of plants in this biome; these species are deciduous, causing metabolites commonly found in larger quantities in leaves and fruit to be displaced to other parts of the plant.[30] These results corroborate the finding of secondary metabolites in the bark of *A. cearensis*[31,32] and the *Erythrina* genus.[33]

Phenolic compounds are the main group of antioxidants of plant origin, possibly owing to their chemical structure, which makes them capable of deactivating reactive oxygen species. Flavonoids are among the most important of these metabolites since, apart from eliminating radicals induced by UV, they provide protection from UV radiation.[34,35]

Various studies have associated AOA primarily with the presence of phenolic compounds in plants since these have reductive properties and their chemical structures are found to contain resonance, which enables the formation of relatively stable intermediaries.[36,37]

Studies show the effectiveness of phenolic compounds in combating inflammation, oxidative stress, DNA damage, and suppression of the immune response induced by UV radiation. These mechanisms, in association with photoprotection, contribute to the anti-photocarcinogenic action of these compounds.[9]

Many studies have focused on the importance of antioxidants in photoprotection.[9] According to Damiani *et al.*,[38] most commercially available sunscreen formulations do not provide full protection, especially against effects considered chronic, such as skin aging and photocarcinogenesis. Plant extracts rich in phenolic compounds, such as tannins and flavonoids, are thus being used in photoprotection formulas along with UV filters, as it has been shown that these extracts possess antioxidant properties and are capable of absorbing UV radiation, resulting in a product that provides greater protection.[39-45]

In Brazil, sunscreens in cosmetic products are regulated by RDC nº 30/12,[46] which sets a minimum SPF of 6 and provides the recommended SPF for different skin phototypes: less sensitive (6 ≤SPF ≤14.9), sensitive (15 ≤SPF ≤29.9), very sensitive (30 ≤ SPF ≤ 50), and extremely sensitive (50 <SPF <100).

Photoprotection can also be evaluated by determining the percentage inhibition of erythemas caused by solar radiation. Martorana *et al.*[47] found inhibition of around 65% of skin erythemas for formulas containing 2% *Pistacia vera* L seed extract.

There are still few studies of the photoprotective properties of Caatinga plants, although species from other regions of Brazil and other countries have been studied more thoroughly. Research carried out by Oliveira Junior *et al.*[34] obtained good SPF results for one species native to the Caatinga (*Neoglaziovia variegata*), testing leaf samples obtained from extraction using four different solvents and finding a high SPF for chloroform and ethyl acetate extracts.

The present study showed that species from the Caatinga region commonly used by the local population to treat inflammatory disorders have potential photoprotective properties. The metabolites related to photoprotective activity were flavonoids and coumarins, the result for the latter being one not yet reported in the literature.

Plant extracts are increasingly being used to protect the skin from ageing caused by exposure to the sun, and the results obtained for *E. velutina* Willd are thus especially interesting. Other species also showed good potential for photoprotection and antioxidant properties and could be used in future studies to develop skin protection products.

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Conflicts of interest

There are no conflicts of interest.

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