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The Phytochemical Profile and Photoprotective Potential of Bark and Leaves of *Erythrina velutina* Willd.—A Medicinal Species from the Caatinga Region of Brazil

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Abstract

The use of medicinal plants as therapeutic and economic resources has been on the rise in recent years. In Brazil, however, increasing doubt is being cast on the quality of these products, owing to the prevalence of adulteration and fraud. Solar radiation can cause serious damage to human skin, as a result, mostly, of ultraviolet light, which is a cause of skin cancer. Photoprotective substances are capable of absorbing, reflecting, or refracting ultraviolet radiation and thus protecting skin from exposure to sunlight. The present study aimed to characterize samples and examine the phytochemical profile and photoprotective potential of bark and leaves of *Erythrina velutina* Willd. The samples underwent five extraction methods using 80% ethanol. The phenolic content was measured using spectrophotometry. Antioxidant activity was examined using the DPPH and the photoprotective properties of the plant extracts were assessed using the method developed by Mansur. There was a quantitative difference in some groups of metabolites, with higher levels of tannin in the bark and of flavonoids in the leaves. The latter showed greater DPPH free radical scavenging capacity than the bark, although higher levels of SPF were obtained from the bark, with no statistically significant differences between methods. The results indicate that *Erythrina velutina* Willd. has potential as a photoprotector.

Keywords

Phytochemical, Caatinga, Phytochemistry, Sun Protection Factor (SPF)

1. Introduction

Sunscreens are substances capable of absorbing, reflecting, or refracting ultraviolet radiation and thus protecting skin from direct exposure to sunlight [1]. The biological activity of a sun protector is assessed by way of its ability to protect the skin from erythema and edemas, reducing the risk of burns and basal or squamous cell carcinoma [2]. In Brazil, the National Cancer Institute (INCA) estimates that skin cancer is the most common form of cancer [3]. Solar radiation can cause serious damage to human skin [4], mostly as a result of exposure to the ultraviolet (UV) region of the electromagnetic spectrum, with types UVA (320 - 400 nm) and UVB (290 - 320 nm) being responsible for burns, erythema, edemas and premature ageing of the skin, and now implicated in the development of skin cancer [5].

At present, the sunscreen market is dominated by synthetic protection factors, which are powerful and possess only photoprotective properties [6] [7]. However, various plant products have been used to produce sunscreens, because there is a structural analogy between synthetic and natural sunscreens [8] [9].

Given the extensive exposure to solar radiation to which Caatinga plants are subject and the photoprotective properties of their phenolic compounds, there is a need for prospection studies of species from this biome, with a view to obtaining suitable phytocosmetic formulations. Various species of plants native to this biome have high concentrations of phenolic compounds such as flavonoids [10], which are substances capable of absorbing ultraviolet light. Their absorption spectrum has two peaks, one at 240 - 280 nm and another at 300 - 550 nm [11].

One of the species of Caatinga plant studied for its biological properties is *Erythrina velutina* Willd. (Fabaceae). Popularly known as *mulungu*, *canivete*, *sanaduí*, *mulungu-da-catinga*, *pau-de-coral*, or *sananduva*, this species is used in folk medicine, principally in the Northeast region of Brazil, as a local anesthetic, sudorific, sedative and pectoral emollient, although there is no scientific evidence of its safety or efficacy [12].

Mulungu has high levels of phenolic compounds, such as flavonoids [10], which are substances capable of absorbing ultraviolet light, whose absorption spectrum occurs at two peaks, one at 240 - 280 nm and another at 300 - 550 nm [11]. For this reason, the species may exhibit photoprotective chemical properties. Although numerous Caatinga species possess high levels of this group of compounds and it is possible to use these plants to produce sunscreens [10], there have been few studies of species from this biome. In view of this, the present study aimed to characterize samples and examine the phytochemical profile and photoprotective potential of bark and leaves of *Erythrina velutina* Willd. using five methods of extraction.

The great diversity of plant species makes it difficult to understand them all, *E. velutina* being an example. The aim of this study was to deepen knowledge about the plant species, carry out a comparative study between different extractive methods, interference in the compositions of extracts and quantification of secondary metabolites, antioxidant and photoprotective activity.

2. Materials and Methods

2.1. Materials

Ethanol (Vetec, 99.5%) was used as the solvent to extract the samples. TPC and TTC were determined using anhydrous sodium carbonate (Vetec, 99.5%) and Folin-Ciocalteu phenol reagent (Fluka, 2N). Glacial acetic acid (Merck, 100%), aluminum chloride hexahydrate (Honeywell, 99%) and pyridine (Vetec, 99%) were used to quantify the flavonoid content. Hydrochloric acid (Vetec 37%) and lead (II) acetate (Êxodo) were used to quantify the coumarin content and 2.2-diphenyl-1-picrylhydrazyl (Aldrich, 95%) for the DPPH assay. Methanol (Vetec, 99.8%), ascorbic acid (Vetec, 99%), tannic acid (Vetec, 99%), rutin (Acros Organics, 97%) and 1,2-benzopyrone (Sigma, 99%) were used as standards. Weights were measured on a Shimadzu analytical balance (AX200) and absorbance readings were recorded using a Shimadzu UV-Vis (UV mini-1240) spectrophotometer.

2.2. Botanical Materials

The biological materials were collected with support from a local productor in an area of Caatinga in the State of Pernambuco, in the Carão community (08˚35'13.5"S and 36˚05'34.6"W), located in the rural zone of Altinho, a municipality in the Central Agreste district of Pernambucano, 163.1 km from Recife, with an area of 454.486 km^2 and a semi-arid hot climate [13]. The species was identified by Dr. Reinaldo Farias de Paiva Lucena and a voucher specimen was deposited in the Professor Geraldo Mariz Herbarium collection, UFPE under the number 46,180. The species to be studied, *E. velutina*, was selected based on a preliminary survey in which the sun protection factor (SPF) of 15 plants was measured. All the species had been reported at least three times by the local population as being used to treat inflammation [14]. The samples—bark and leaves—of the plant were collected in July and August 2015. The study and the collection of the material were registered in the Ministry of the Environment's National Genetic Heritage and Associated Traditional Knowledge Management System under ABA5112.

2.3. Preparation of Samples

The plant samples were cut and exposed to the ambient environment for two weeks to dehydrate. After drying, the samples were ground in a vertical Wiley type knife mill (Adamo 340).

2.4. Physicochemical Characterization of Samples

Physicochemical characterization of samples of leaves and bark was carried out as a way of classifying the samples in terms of particle size, degree of purity and moisture content.

2.5. Sieve Analysis

Twenty-five grams of powdered plant material were passed through previously calibrated sieves, with mesh sizes of 850, 600, 425, 250, 150 and 90 μm. Sieving was carried out at 60 vibrations per second for 15 min in a Bertel® apparatus. The data were analyzed using retention and passage curves. Analysis was conducted in triplicate and the results expressed as mean ± standard deviation [15].

2.6. Loss of Mass on Desiccation

One gram of plant material, weighed in calibrated weighing bottles, was placed in a heating chamber for 2 h, at a temperature of 105˚C ± 2˚C. After cooling in a desiccator, the bottles were weighed and put back in the chamber for a further 30 min. This procedure was repeated until the weight of the samples remained constant [15]. The results were expressed as percentage loss of mass, by way of mean ± standard deviation for three measurements, this variation shouldn't be bigger than 2.5% [16].

2.7. Total Ash

Three grams of plant material were transferred to previously calcinated, cooled and weighed porcelain crucibles and incinerated in a furnace at 450˚C for 2 h. The percentage of ash in relation to initial plant material was calculated [10]. The results were expressed as mean ± standard deviation for three measurements, should be the valor between 2% - 20% [16].

2.8. Obtaining Extracts

The samples of bark and leaves underwent five different extraction methods—maceration, decoction under reflux, turbo-extraction, ultrasound-assisted, and microwave-assisted—using 80% 1:10 (g/mL) hydroethanolic solution as a solvent according previous studies from the research group [14], considering the potential to swelling the from the vegetal material, expanding the superficial area between solvent and vegetal matrix. The plant samples underwent three macerations and the solvent was replenished every 48 h. Decoction was carried out in a heating mantle for 30 minutes counting from the point at which the solvent began to boil. Turbo-extraction was performed using an industrial liquidizer for 30 minutes (six five-minute cycles, with intervening two-minute intervals). Unique® UltraCleaner 1400A model sonication for 60 minutes at a fixed warming rate of (60˚C ± 5˚C) was used to obtain the extracts. In microwave-assisted extraction, potency and extraction time were calculated using the physical formula that relates the quantity of heat to potency ($Q = m \times c \times \Delta T$) to obtain the boiling point of the solvent used.

The extracts were filtered and subjected to evaporation under reduced pressure, at a temperature of 40˚C ± 5˚C. The extracts were suspended in distilled water and subjected to lyophilization (FTS Systems, TDS-00209-A model). The samples were frozen in a Ultrafreezer at a final temperature of −70˚C and dried under internal pressure of 420 mmHg for 96 hours.

The choice of extractive techniques was guided by the comparison between traditional and modern extraction methods, choosing methods already consolidated in the literature, of lower cost and easy to carry out.

2.9. Phytochemical Prospection

Analyses were carried out using CAMAG® HPTLC (High performance thin layer chromatography) equipment, comprising Automatic TLC Sampler 4, Automatic development chamber ADC2 and TLC Visualizer modules connected to WINCATS (version 1.4.4.6337) software. The extraction samples and standards were applied 10 mm from the base, in 8.5-mm-wide bands, and 14 mm from the edge, with a distance of 10 mm between bands and a constant application rate under a flow of nitrogen gas.

2.10. Determination of the Total Phenolic (TPC) and Total Tannin Content (TTC)

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, where the TTC is the difference between the levels of total and residual phenols [17]. In alkaline medium, the total phenols reduce the mixture of phosphotungstic and phosphomolybdic acids that are present in the Folin-Ciocalteu phenol reagent in blue colored tungsten and molybdenum oxides, the color intensity is proportional to the concentrations of phenolic compounds [17]. TPC and TTC were expressed as one milligram of tannic acid per gram of sample (mg TAE/g). The samples were evaluated in triplicate. The calibration equation for tannic acid was $y = 0.047x + 0.127$ ($R^2 = 0.985$).

2.11. Determination of Total Flavonoid Content (TFC)

The TFC of the extracts was estimated using a colorimetric method based on the reaction of aluminum ion (Al^{3+}) with flavonoid molecules in the sample, establishing the stable flavonoid-Al^{3+} complex, yellow in color, whose intensity is proportional to the concentration of flavonoids. This reaction promotes a bathochromic shift and an intensification of its absorptions, which can be quantified without being influenced by other phenolic compounds present in the sample [18]. The results were expressed as one milligram of rutin for each gram of sample (mg RE/g). The samples were evaluated in triplicate. The rutin calibration equation was $y = 0.026x + 0.020$ ($R^2 = 0.997$).

2.12. Determination of Coumarin Content (CC)

CC was determined using the colorimetric assay described by Osório and Mar-

tins [19] with some adjustments. The results were expressed as one milligram of coumarin (1,2-benzopyrone) per gram of sample (mg EC/g). The samples were evaluated in triplicate. The coumarin calibration equation was $y = 0.022x + 0.005$ ($R^2 = 0.994$).

2.13. Evaluation of Antioxidant Activity (AOA)

The free-radical scavenging activity (DPPH) assay was performed in triplicate, based on the method described by Araujo *et al.* [20]. The absorbance readings were used as the basis for the EC_{50}, which represents the concentration of extract or ascorbic acid (positive control) required to reduce the initial concentration of DPPH by 50%. The EC_{50} was calculated using a graph in which the sample concentrations (μg/mL) or positive control were displayed on the x-axis and the percentage of DPPH remaining (% $DPPH_{REM}$) on the y-axis, to generate a first order curve and its equation.

The % $DPPH_{REM}$ was calculated according to the following formula:

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

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

2.14. Determination of Maximum Absorption Wavelength and Sun Protection Factor (SPF) *in Vitro*

For determining 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 used as a blank [21]. The SPF was calculated using the equation developed by Mansur *et al.* [22] (**Table 1**):

Table 1. Normalized product function used in the calculation of SPF.

Wavelength (nm)	EE x 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 – erythema effect spectrum; I – Solar intensity spectrum.

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

where: EE(λ)—erythemal effect spectrum; I(λ)—solar intensity spectrum; Abs(λ)—absorbance of sunscreen product; CF—correction factor (=10). The values of EE × I are constants. They were determined by Sayre *et al.* [23] and are shown in Table 1.

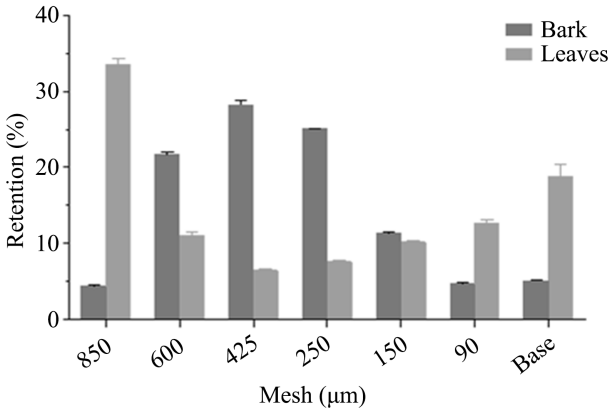
2.15. Statistical Analysis

The Shapiro-Wilk test confirmed the normality of the data obtained. Data were expressed as means ± standard deviation and were analyzed using one-way ANOVA followed by the Tukey test. A Pearson correlation test was used to compare the figures for phenolic content both with one another and with the antioxidant and SPF tests of the samples. A p-value < 0.05 was considered significant. BioEstat 5.3 software was used to perform statistical analysis and GraphPad Prism 5 to plot graphs.

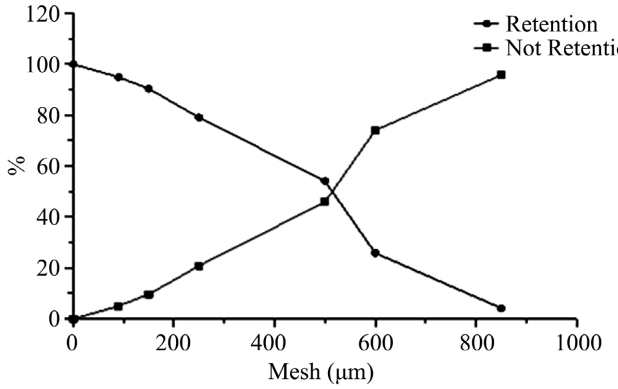
3. Results and Discussions

Graphs 1-3 show the results obtained by sieve analysis. The graph of particle size distribution (Graph 1) shows that most of the particles of bark were retained by sieves of 425, 250 and 600 μm with mean percentages ± standard deviation of 28.16 ± 0.73; 25.02 ± 0.04 and 21.64 ± 0.43 in relation to total initial material, respectively. Material from the leaves presented a distinct retention profile, with more particles retained by the 850 μm sieve, with mean percentages ± standard deviation of 33.51 ± 0.81 and 18.80 ± 1.54 in relation to total initial material, respectively.

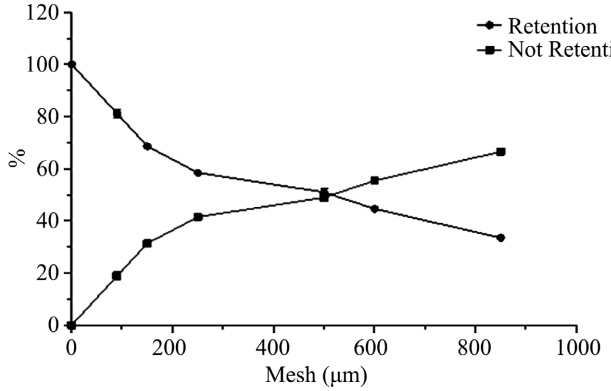
The sieve retention results were different for bark and leaves in terms of mean particle size. The two different samples, however, presented a similar result, (Graph 2 and Graph 3), with particle sizes (mean diameter) ± standard deviation of 521.66 μm ± 1.44 for bark samples and 518.67 μm ± 25.32 for leaves. The samples of bark and leaves of *E. velutina* were thus classified as coarse powders, since they did not pass through the 850 μm sieve in their entirety, with 33.51% ± 0.81% of leaves and 4.28% ± 0.31% of bark retained.



Graph 1. Particle size distribution for bark and leaves of *Erythrina velutina* Willd.



Graph 2. Retention and not retention curves in the meshes for bark of *Erythrina velutina* Willd.



Graph 3. Retention and not retention curves in the meshes for leaves of *Erythrina velutina* Willd.

The results obtained for desiccation loss tests and total ash content are reported in **Table 2**. The results for the specific humidity of samples were similar with mean percentages of 10.97 ± 0.13 and 10.32 ± 0.20, respectively. As for total ash content, the samples of *E. velutina* bark presented lower quantities, with mean percentages of 4.94 ± 0.14, while the leaves presented mean percentages of 7.01 ± 0.20. Furthermore, no extraneous matter, such as twigs, sand, plastic, stone, seeds or insects was found in the samples [24].

Qualitative chemical characterization of the leaves and bark of *E. velutina*, using the five different extraction methods, is outlined in **Table 3**. **Figure 1** shows a chromatogram identifying phenolic compounds in the extracts of leaves and bark of this plant species. Analysis of the extracts obtained by maceration, ultrasound, microwave, turbo-extraction and decoction showed, for all methods and in both parts of the species, phenolic compounds, flavonoids, coumarins, alkaloids, triterpenes mono/sesqui/diterpenes, anthracene derivatives, and condensed and hydrolyzable tannins, with no influence of the method on the extraction of these compounds. Anthocyanins and naphthoquinones were found only in the leaves, and anthraquinones were not detected in the plant parts examined here.

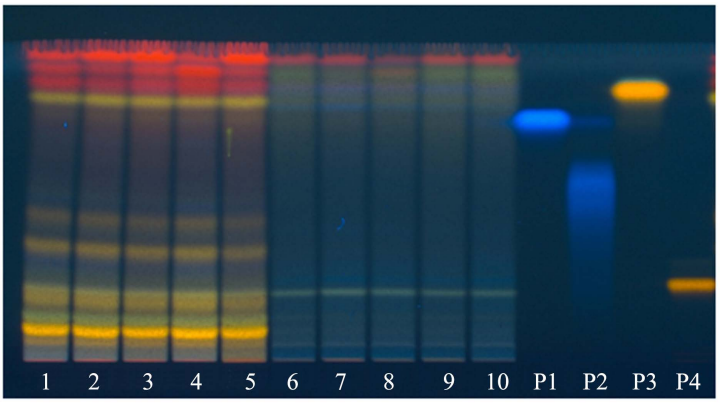


Figure 1. Chromatogram for identification of Phenolic Compounds in leaves extracts (left) and barks (right) of *Erythrina velutina* Willd. Subtitle: 1 = leave ultrasound, 2 = leave microwave, 3 = leave turbo-extraction, 4 = leave maceration, 5 = leave decoction, 6 = bark microwave, 7 = bark ultrasound, 8 = bark maceration, 9 = bark turbo-extraction, 10 = bark decoction, P1 = Gallic acid, P2 = Tannic acid, P3 = Quercetin, P4 = Rutin.

Table 2. Results of assays for loss of mass on desiccation and total ash content in leaves and bark of *Erythrina velutina* Willd.

Analysis	Leaves	Bark
Total Ash Content (%)	7.01 ± 0.20	4.94 ± 0.14
Moisture Content (%)	10.97 ± 0.13	10.32 ± 0.20

Source: Author.

Table 3. Phytochemical characterization of extracts of leaves and bark of *Erythrina velutina* Willd. Using different extraction methods.

Group of Secondary Metabolites	EXTRACTS									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
General Alkaloids	+	+	+	+	+	+	+	+	+	+
Anthocyanins	+	+	+	+	+	−	−	−	−	−
Anthraquinone	−	−	−	−	−	−	−	−	−	−
Phenolic Compounds	+	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+	+
Anthracene derivatives	+	+	+	+	+	+	+	+	+	+
Flavonoids	++	++	++	++	++	+	+	+	+	+
Monoterpenes/Diterpenes/Sesquiterpenes	+	+	+	+	+	+	+	+	+	+
Naphthoquinones	+	+	+	+	+	−	−	−	−	−
Condensed Tannins	+	+	+	+	+	++	++	++	++	++
Hydrolyzable Tannins	+	+	+	+	+	+	+	+	+	+
Triterpenes/Steroids	+	+	+	+	+	+	+	+	+	+

Source: Author. 1 = leaf/ultrasound, 2 = leaf/microwave, 3 = leaf/turbo-extraction, 4 = leaf/maceration, 5 = leaf/decoction, 6 = bark/microwaves, 7 = bark/ultrasound, 8 = bark/maceration, 9 = bark/turbo-extraction, 10 = bark/decoction.

Total phenolic, tannin, flavonoid and coumarin content in the leaves and bark using the five different extraction methods are given in Table 4. Decoction of *E. velutina* bark was the method that presented the highest total phenolic and tannin content, with mean quantities of 251.03 ± 14.11 and 159.76 ± 15.34 mg EAT/g, respectively. This is statistically different from the other extracts of bark and leaves. The results obtained from decoction of the leaves are also worth noting, since they show figures close to those for extract of bark extracted using the same method (207.10 ± 20.02 and 118.46 ± 11.85 mg EAT/g, respectively). As for flavonoid content, the decoction of the leaves achieved the largest quantity, with 127.13 ± 5.33 mg ER/g, a figure statistically equivalent to that for leaves but different from that for other methods used with either leaves or bark. As for the coumarin content of different extracts, the ultrasound method used on leaves achieved mean quantities of 423.93 ± 17.58 mg EC/g, which is statistically different from the other methods with either leaves or bark of *E. velutina*.

DPPH free radical scavenging capacity, represented by the 50% effective concentration (EC_{50}), is expressed as mean ± standard deviation in Graph 4 and Table 5. Raw leaf extract using the decoction method presented the greatest free radical DPPH scavenging capacity, with the lowest EC_{50} of 291.96 ± 25.73 μg/mL. However, this is no different statistically from the extract obtained from leaves of *E. velutina* by maceration, with a mean EC_{50} of 340.33 ± 30.70 μg/mL, as shown in Table 5.

The sun protection factors (SPFs) for concentrations of 0.005, 0.025, 0.050 and 0.100 mg/mL are expressed as mean ± standard deviation in Table 5. Analysis of *E. velutina* extraction methods for the parts studied showed the hydroethanolic extract of bark obtained by maceration at a concentration of 0.100 mg/mL to have the best absolute SPF of 9.57 ± 0.67, but this was not statistically significant

Table 4. Total phenolic, tannin, flavonoid and coumarin content (mg/g), expressed as mean ± standard deviation, of leaves and bark of *Erythrina velutina* Willd., using different extraction methods.

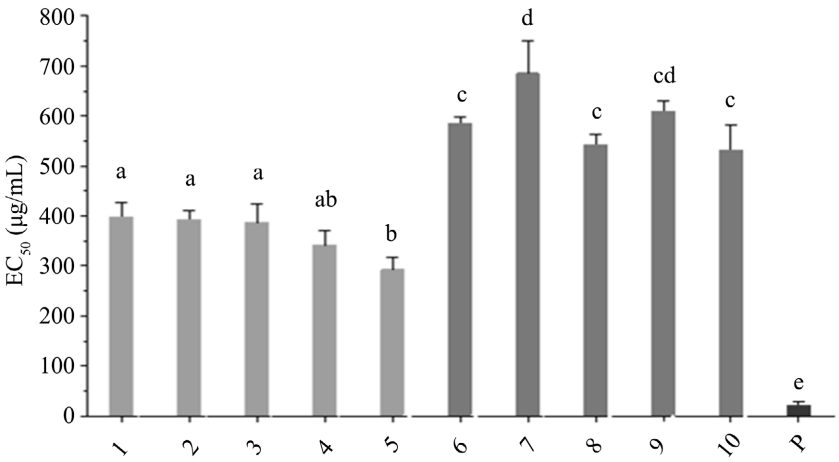
Plant Part/Extraction Method	TF (mg EAT/g)	TAN (mg EAT/g)	FLA (mg ER/g)	COUM (mg EC/g)
Leaf/Ultrasound	103.73 ± 4.34 ade	20.43 ± 1.93 ac	100.53 ± 5.11 a	423.93 ± 17.58 a
Leaf/Microwave	113.60 ± 6.94 ab	13.84 ± 1.23 a	99.26 ± 9.76 a	250.43 ± 15.65 b
Leaf/Turbo-extraction	98.88 ± 8.30 ad	19.08 ± 3.14 a	94.58 ± 1.44 a	270.66 ± 24.96 bc
Leaf/Maceration	122.76 ± 4.45 b	28.04 ± 2.15 ah	119.33 ± 10.42 be	281.77 ± 19.55 c
Leaf/Decoction	207.10 ± 20.02 c	118.46 ± 11.85 b	127.13 ± 5.33 b	257.91 ± 15.34 bc
Bark/Ultrasound	90.97 ± 5.94 df	34.78 ± 1.06 ch	65.08 ± 6.31 c	174.46 ± 13.28 d
Bark/Microwave	117.68 ± 8.56 eb	101.06 ± 7.90 d	48.34 ± 4.68 d	182.23 ± 16.32 de
Bark/Turbo-extraction	79.72 ± 7.86 f	37.96 ± 3.68 eh	68.38 ± 5.43 c	198.36 ± 18.84 de
Bark/Maceration	115.54 ± 9.84 eb	65.47 ± 6.23 f	69.15 ± 5.82 c	191.26 ± 3.73 de
Bark/Decoction	251.03 ± 14.11 g	159.76 ± 15.34 g	114.29 ± 2.91 e	203.37 ± 9.27 e

Source: Author. TP = Total Phenolic, TAN = Tannins, FLA = Flavonoids, COUM = Coumarins. Identical letters in the same column indicate no statistically significant difference according to ANOVA (followed by Tukey), $p < 0.05$.

Table 5. EC_{50} (μg/mL) and SPF at concentrations of 0.005, 0.025, 0.050 and 0.100 mg/mL, expressed as mean ± standard deviation, for leaves and bark of *Erythrina velutina* Willd using different extraction methods.

Plant Part/Extraction Method	EC_{50} (μg/mL)	FPS			
		0.005 mg/mL	0.025 mg/mL	0.050 mg/mL	0.100 mg/mL
Leaf/Ultrasound	397.25 ± 29.96 a	0.63 ± 0.06 ab	1.58 ± 0.15 a	2.71 ± 0.20 a	5.07 ± 0.36 a
Leaf/Microwave	392.13 ± 18.62 a	0.57 ± 0.02 a	1.69 ± 0.06 ac	3.01 ± 0.17 a	5.58 ± 0.46 a
Leaf/Turbo-extraction	385.53 ± 38.15 a	0.52 ± 0.02 ad	1.52 ± 0.14 a	2.73 ± 0.28 a	5.17 ± 0.45 a
Leaf/Maceration	340.33 ± 30.70 ab	0.70 ± 0.07 b	1.76 ± 0.06 acd	3.19 ± 0.13 a	6.10 ± 0.23 a
Leaf/Decoction	291.96 ± 25.73 b	0.56 ± 0.02 ae	1.57 ± 0.16 a	2.85 ± 0.29 a	5.80 ± 0.50 a
Bark/Ultrasound	584.58 ± 14.30 c	0.39 ± 0.04 c	2.06 ± 0.21 b	4.24 ± 0.43 b	8.44 ± 0.85 b
Bark/Microwave	684.91 ± 65.80 d	0.39 ± 0.03 c	2.34 ± 0.24 bc	4.74 ± 0.50 b	8.45 ± 0.35 b
Bark/Turbo-extraction	541.84 ± 21.66 c	0.54 ± 0.05 cd	2.29 ± 0.02 b	4.64 ± 0.10 b	9.29 ± 0.25 b
Bark/Maceration	608.85 ± 22.02 cd	0.42 ± 0.04 ad	2.39 ± 0.20 b	4.90 ± 0.35 b	9.57 ± 0.67 b
Bark/Decoction	532.14 ± 49.96 c	0.44 ± 0.04 cde	2.16 ± 0.20 bd	4.32 ± 0.44 b	8.65 ± 0.85 b

Source: Author. EC_{50} = 50% Effective Concentration, SPF = sun protection factor. Identical letters in the same column indicate no statistically significant difference according to ANOVA (followed by Tukey), $p < 0.05$.



Graph 4. Values EC50 (μg/mL) expressed as mean ± standard deviation, for leaves and bark of Erythrina velutina Willd., using different extraction methods and Ascorbic acid. 1 = leave ultrasound, 2 = leave microwave, 3 = leave turbo-extraction, 4 = leave maceration, 5 = leave decoction, 6 = bark ultrasound, 7 = bark microwave, 8 = bark turbo-extraction, 9 = bark maceration, 10 = bark decoction e P = Ascorbic acid. Identical letters indicate no statistically significant difference according to ANOVA (followed by Tukey), $p < 0.05$.

different from other methods using bark. The maceration extract at the same concentration was also found to have the best SPF for the leaves of 6.10 ± 0.23, but this again was statistically equivalent to the other methods.

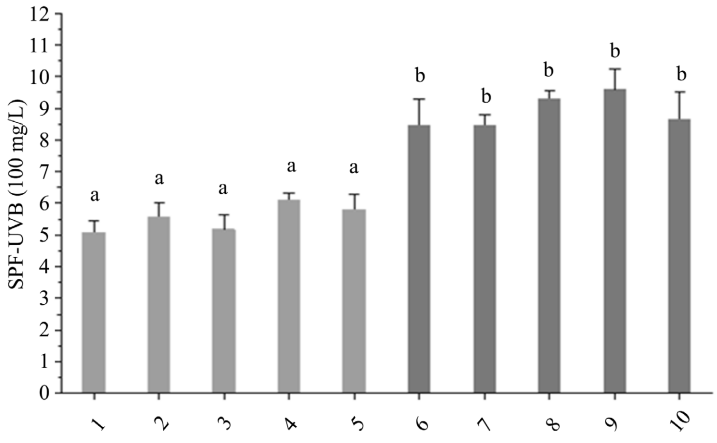
Comparison of leaves and bark showed all methods obtaining better results for bark extract than for leaves, with a statistically significant difference between the plant parts studied in terms of in vitro photoprotective activity at concentrations of 0.050 and 0.100 mg/mL. For the bark and leaf extraction methods used

for this species, there was no correlation between the quantity of metabolites and photoprotective activity.

The peak absorption of compounds found in plant samples is a factor determining the best photoprotective properties. Bark of *E. velutina* presented an absorption peak in the 290 nm band (Graph 4), while the leaves showed a peak in the 270 nm band (Graph 5). This result is of fundamental importance, as the absorption peak for the bark of the species lies in the UVB radiation band (290 - 320 nm), thus increasing the SPF. It is therefore likely that the compounds found in the bark are different from those in the leaves, in terms either of the composition of the class of metabolites or the quantity of any one compound responsible for the greatest level of absorption in the UVB region.

Granulometric analysis of raw plant materials is of fundamental importance, because it standardizes the size of their particles, a factor that is directly related to the homogeneity and replicability of extraction methods [25] [26]. Furthermore, this kind of test helps to characterize the plant sample [27] and the distribution determines the surface area of contact between the sample and the solvent used to obtain the plant extract, which is a preliminary factor determining both the extraction method chosen and the appropriate solvent to use [28].

Although the samples of bark and leaves of *E. velutina* have been classified as coarse powders in the Brazilian Pharmacopoeia [15], it was found that the granulometric distribution of the bark presented better results than the leaves, owing to the higher percentage retention in the medium-sized mesh of sieves of 600, 500 and 250 μm. The leaves, however, produced particles that were mostly larger or very small, with a small quantity of medium-sized particles, suggesting greater likelihood of agglomeration of smaller particles with larger ones and and a smaller contact surface in the leaf extract compared to the bark samples.



Graph 5. Values SPF-UVB expressed as mean ± standard deviation, for leaves and bark of *Erythrina velutina* Willd., using different extraction methods, in the concentration of 100 mg/mL. 1 = leave ultrasound, 2 = leave microwave, 3 = leave turbo-extraction, 4 = leave maceration, 5 = leave decoction, 6 = bark ultrasound, 7 = bark microwave, 8 = bark turbo-extraction, 9 = bark maceration, 10 = bark decoction e P = Ascorbic acid. Identical letters indicate no statistically significant difference according to ANOVA (followed by Tukey), $p < 0.05$.

The moisture content is an important factor in evaluating the efficiency of the process of drying and storing plant samples, with smaller content representing high efficiency and greater stability of the chemical compounds that make up the plant tissue [21] [29]. The results obtained show a reduced risk of microbiological proliferation and greater chemical stability of samples of bark and leaves of E. velutina. This is due to the fact that a moisture content above the established thresholds is associated with a greater risk of the development of micro-organisms [30] and generates more enzyme activity, with reactions such as oxidation and hydrolysis of the compounds present in the plant species [31].

The total ash content found in the samples of leaves of *E. velutina* was greater (7.01 ± 0.20) than that found in bark (4.94 ± 0.14). Although the Brazilian Pharmacopea does not stipulate a common upper limit for total ash content [31], various monographs suggest limits of between 2% and 20% [16]. The mean values obtained in the present study for total ash content, both in the leaves and the bark, thus lie within the acceptable upper limits reported in the literature, not exceeding 8%.

In terms of the phytochemical results obtained, it is worth noting that the extraction methods involving heating (decoction and ultrasound) presented the best results for the two different parts of the plant species studied for the extraction of compounds, measured principally by total phenolic compound and tannin content. When the chemical composition of the extracts produced by the five methods used for leaves of *E. velutina* and the five used for bark was examined, there was found to be a positive correlation only between total phenolic compounds and tannins ($r = 0.8662$; $p = 0.0012$), with no correlations among the other secondary metabolites tested.

It is worth considering the influence of the extraction method on the composition of the extracts, since the raw plant material base, pre-treatment and extraction are key parameters. It is worth standardizing these, because many factors influence the final composition, including the extraction method used [15]. In the case of extraction using ultrasound, in addition to the heating, the collapse of cavitation bubbles is another factor that may influence the quality of the extraction of compounds.

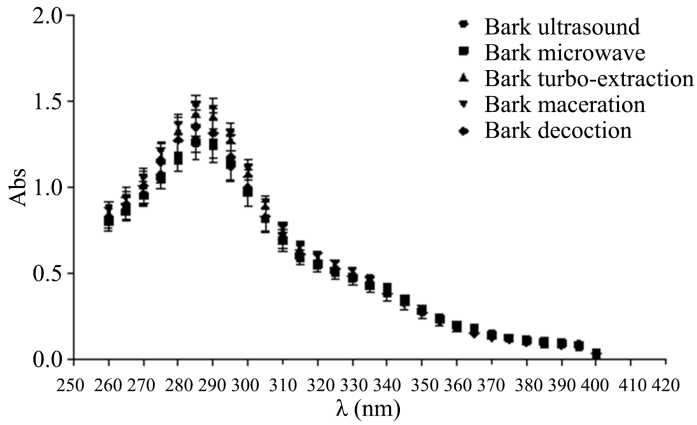
Another important point to bear in mind in analysis of the results is the presence of greater tannin content in the samples of bark and of flavonoids and coumarins than in the leaves of *E. velutina*, which corroborates findings in other studies. Araújo *et al.* [13] found that, in medicinal Caatinga species, tannins are present in greater proportions in samples of bark. This occurs because the bark and its seams are the parts of the plant that are most likely to be attacked by predators, such as herbivores and insects, and tannins protect them against these [33].

Antioxidant products are of fundamental importance in emerging photoprotective pharmaceutical preparations and the relation between antioxidant activity and photoprotection has thus been extensively studied [6] [34]. Evidence of this has been provided by Wu *et al.* [35], who showed that products with anti-

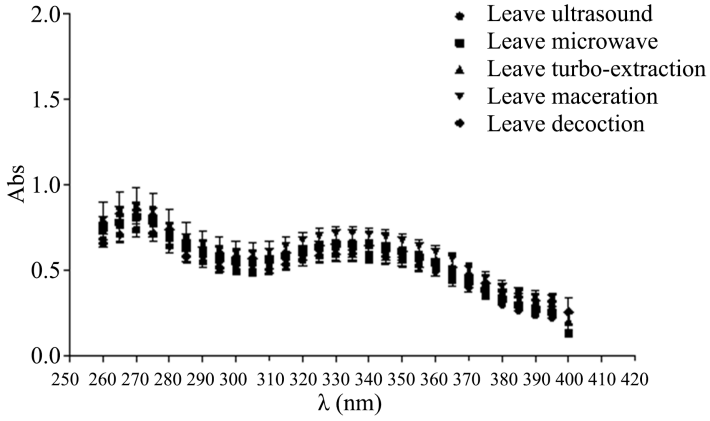
oxidant compounds reduced skin erythema up to 72 h after exposure, whilst those with only photoprotectors did not.

A growing number of studies are being carried out into the photoprotective properties of Caatinga species. Oliveira Júnior *et al.* [36] obtained good SPF results from a study of a species native to the Caatinga (*Neoglaziovia variegata*) which tested samples of leaves obtained by extraction using four different solvents and found a high SPF for chloroform and ethyl acetate extracts.

Although there was a statistically significant difference between the SPF for leaves and bark of *E. velutina*, no such difference was found for extraction methods used, since all the bark extracts presented statistically equivalent figures, as did all the leaf extracts. It would thus appear that, different from the part of the plant used, the method employed had no influence on the activity under study, as shown in the Graph 6 and Graph 7. The choice of method used should therefore be guided by other inherent factors, such as the time taken to accomplish extraction and, above all, the operational costs.



Graph 6. Scanning spectrum of absorbances, 260 and 400 nm, for bark of *Erythrina velutina* Willd., in the concentration of 100 mg/mL, using different extraction methods. Abs = absorbances, λ = Wavelength (nm).



Graph 7. Scanning spectrum of absorbances, 260 and 400 nm, for leaves of *Erythrina velutina* Willd., in the concentration of 100 mg/mL, using different extraction methods. Abs = absorbances, λ = Wavelength (nm).

4. Conclusions

The tests used to evaluate the quality of plant material indicate satisfactory parameters for granulometry, moisture content, total ash content, and the presence of extraneous matter in the leaves and bark of the species compared to the specifications laid out in the pharmacopoeias available. The extraction method did not influence the chromatographic profile in the parts used but the bark differed from the leaves and there were quantitative differences for some groups of metabolites, depending on the part. Higher tannin content was found in the bark and more flavonoids in the leaves, as expected. The leaves of the species presented greater DPPH free radical scavenging capacity, while the bark had a higher sun protection factor (SPF). The latter, however, was not influenced by the extraction method for the quantities found. Development of a future photoprotection formula will thus require selection of a simple more economically viable method, such as maceration.

The results obtained indicate that different parts of *Erythrina velutina* Willd. have potential photoprotective properties. These can be exploited more fully using processes that lead to an increase in the metabolites responsible.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Ethical Approval

Not applicable.

Informed Consent

Not applicable.

Authors' Contributions

Bruno de Almeida Andrade and Patricia Maria Silva Neri-Cruz contributed in collecting plant sample and identification. Bruno de Almeida Andrade, Allan Jonathan Chernichiarro Corrêa, Ana Klarissa Soares Gomes, Patricia Maria Silva Neri-Cruz, Aline Michaele de Saint-George Albuquerque Cheron, and Valérium Thijan Nobre de Almeida e Castro running the laboratory work, analysis of the

data and drafted the paper. Bruno de Almeida Andrade, Allan Jonathan Chernichiarro Corrêa, Décio Henrique Araújo Salvadôr de Mello, Cláudia Sampaio de Andrade Lima and Ricardo Yara contributed to chromatographic analysis. Bruno de Almeida Andrade and Elba Lúcia Cavalcanti de Amorim designed the study. Elba Lúcia Cavalcanti de Amorim supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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