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Photoprotective and antioxidant activities of leaves and bark of *Solanum paniculatum* L. (Solanaceae)

Atividades fotoprotetora e antioxidante de folhas e cascas de *Solanum paniculatum* L. (Solanaceae)

A. F. G. Silva[1*]; V. C. Pereira[2]; S. H. M. Aliendes[2]

[1]*Programa de Pós-Graduação em Biodiversidade e Sustentabilidade Ambiental, Universidade Estadual de Mato Grosso do Sul, 7998000, Mundo Novo-MS, Brazil*
[2]*Unidade de Mundo Novo/Laboratório de Química de Produtos Naturais, Universidade Estadual de Mato Grosso do Sul, 7998000, Mundo Novo-MS, Brazil*

**anafranciscagomesdasilva@gmail.com*
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Solanum paniculatum, commonly known as jurubeba-verdadeira, is a species native to Brazil with several uses in cooking and traditional medicine. This study aimed to conduct a preliminary phytochemical analysis and evaluate the photoprotective and antioxidant activities of leaf and bark extracts from a specimen of *S. paniculatum* found in the southern region of Mato Grosso do Sul. Qualitative analytical tests were performed on ethanolic and aqueous extracts to identify the main classes of secondary metabolites. Sun protection factor (SPF) was evaluated in vitro using the spectrophotometric method, and the antioxidant potential was determined using the free radical reduction methods DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) and ABTS$^{\bullet+}$ (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). Phenols, flavonoids, tannins, and total alkaloids were quantified using spectrophotometry in the visible region. Phytochemical analysis revealed the presence of alkaloids and tannins in extracts from both plant parts, while flavonoids, triterpenes, steroids, and purines were only detected in leaf extracts. In general, the extracts exhibited high levels of alkaloids and phenolic compounds, effectively eliminated free radicals, demonstrated significant antioxidant potential, and showed promise for use in photoprotective formulations. These results suggest that photoprotective and antioxidant activities are related to the presence of alkaloids and phenolic compounds quantified in this study.
Keywords: phytochemistry, free radicals, photoprotection.

Solanum paniculatum, conhecida popularmente como jurubeba-verdadeira, é uma espécie nativa do Brasil com diversos usos na culinária e na medicina tradicional. O presente estudo objetivou realizar a análise fitoquímica preliminar e avaliar as atividades fotoprotetora e antioxidante dos extratos das folhas e cascas de um espécime de *S. paniculatum* que ocorre na região sul de Mato Grosso do Sul. Os extratos etanólicos e aquosos foram submetidos a testes analíticos qualitativos para identificar as principais classes de metabólitos secundários. O Fator de Proteção Solar (FPS) foi avaliado in vitro pelo método espectrofotométrico e o potencial antioxidante foi determinado pelos métodos de redução dos radicais livres DPPH$^{\bullet}$ (2,2-difenil-1- picrilidrazila) e ABTS$^{\bullet+}$ (2,2'-azinobis-3-etilbenzotiazolina-6-ácido sulfônico). A quantificação de fenóis, flavonoides, taninos e alcaloides totais foi realizada por espectrofotometria na região do visível. Na análise fitoquímica, alcaloides e taninos foram identificados nos extratos de ambas as partes da planta, enquanto flavonoides, triterpenos e/ou esteroides e purinas foram detectados apenas nos extratos das folhas. De modo geral, os extratos apresentaram teores consideráveis de alcaloides e compostos fenólicos, foram eficazes na eliminação dos radicais livres, exibindo considerável potencial antioxidante e mostraram-se promissores para formulações fotoprotetoras. Os resultados sugerem que as atividades fotoprotetora e antioxidante estão relacionadas à presença de alcaloides e compostos fenólicos, quantificados neste estudo.
Palavras-chave: fitoquímica, radicais livres, fotoproteção.

1. INTRODUCTION

Natural products, especially plants, have great potential for human health and have been used for therapeutic purposes since ancient times [1]. Several studies have demonstrated that essential oils, plant extracts, and pure substances isolated from plants have important, proven biological properties, including antimicrobial, antitumor, photoprotective, and antioxidant activities [2].

Plant extracts rich in antioxidants, such as polyphenols, have been used in topical cosmetic formulations containing UV filters. The ability of these extracts to absorb solar radiation, combined with their antioxidant activity, enhances the final protection of the product. In addition, they neutralize free radicals produced in the skin after sun exposure. This makes them a good option for photoprotective products because of their preventive action against photoaging [3, 4].

Solanaceae Juss is a subcosmopolitan plant family comprising 98 genera and approximately 2,500 species [5, 6]. In Brazil, 36 genera and approximately 500 species are distributed across all phytogeographic domains, notably the Atlantic Forest, Amazon region, and Cerrado [7]. Several species are of great economic relevance and have food and medicinal uses, such as *Solanum lycopersicum* (tomato), *Solanum tuberosum* (potato), and *Nicotiana tabacum*, which is cultivated worldwide for tobacco production [8].

Solanum paniculatum L. is a Solanaceae native to Brazil and is popularly known as jurubeba-verdadeira. This shrub can reach up to 2 meters in height and has alternate, petiolate, sinuous, and sublobed leaves; small, bluish or violet flowers; and globose and glabrous fruits. True jurubeba is commonly found in pastures, perennial crops, orchards, vacant lots, and along roadsides [9].

The species is widely used in traditional medicine, where the leaves, flowers, roots, and fruit are prepared for various purposes, including treating anemia, digestive disorders, liver problems, and as healing agents [10, 11]. *S. paniculatum* is included in the National List of Medicinal Plants of Interest to SUS (RENISUS), which comprises plants with potential for the study and development of products for the Unified Health System [12].

Chemical and biological studies on *S. paniculatum* have reported the presence of phenolic compounds, alkaloids, and saponins, as well as significant biological properties, such as antioxidant, hepatoprotective, cytotoxic, antibacterial, and larvicidal activities [13-17].

However, studies on the photoprotective potential of this species are limited. Thus, this study aimed to conduct a preliminary phytochemical analysis and evaluate the photoprotective and antioxidant activities of ethanolic and aqueous extracts from the leaves and bark of a *S. paniculatum* specimen from the southern region of the state of Mato Grosso do Sul.

2. MATERIAL AND METHODS

2.1 Chemicals

The reagents used in the tests were of analytical grade. Gallic acid, anhydrous sodium carbonate, aluminum chloride, ferric chloride, Folin-Denis reagent, tannic acid, hydrochloric acid, and 1,10-phenanthroline were purchased from Dinâmica® (Brazil). Potassium persulfate was obtained from Neon (Brazil), and yohimbine.HCl from Purifarma (Brazil, with identity confirmed by UV spectroscopy). Folin-Ciocalteu reagent, ABTS - 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH - 2,2-diphenyl-1-picrylhydrazyl, and quercetin were purchased from Sigma-Aldrich (Brazil).

2.2 Collection of plant material

Plant material (leaves and bark) was collected in June 2023 near the Forest Garden of the municipality of Mundo Novo, state of Mato Grosso do Sul, Brazil (23°55'44" S and 54°17'25" W). An exsiccate was deposited in the "Ernesto Vargas Batista" herbarium of the State University of Mato Grosso do Sul under number 315. The project was registered on the SisGen platform under number A908622.

2.3 Preparation of extracts

The leaves (134.6 g) and bark (34.1 g) were air-dried and ground in a Wiley mill. Half of the samples were extracted using 99.8% PA ethanol. The resulting extracts were filtered and concentrated under reduced pressure at 40 °C using a Quimis® rotary evaporator. This process yielded 6.50 g of ethanolic leaf extract and 1.01 g of bark extract. For the aqueous extracts, distilled water at 98 °C was added to the leaves and the bark. After 30 minutes, the material was filtered and lyophilized. This process yielded 6.86 g of leaf extract and 2.29 g of bark extract.

2.4 Phytochemical analysis

Preliminary phytochemical analysis of the crude ethanolic and aqueous extracts was performed according to classical methodology, through qualitative chemical reactions specific to each class of secondary metabolites [18]. Alkaloids were tested using Bouchardat, Dragendorff, Wagner, and Mayer reagents; flavonoids using the Shinoda reaction; triterpenes and/or steroids using the Lieberman-Burchard reaction; tannins using ferric chloride; and saponins using the foam index after shaking the solution and neutralizing it with sodium carbonate. Sesquiterpene lactones and other lactones were analyzed using hydroxylamine hydrochloride and ferric chloride reagents, and purines were analyzed using hydrogen peroxide.

2.5 Quantification of phenols, flavonoids, tannins and total alkaloids

The total phenol content was determined using the Folin-Ciocalteu method [19]. Folin-Ciocalteau reagent and 7.5% Na_2CO_3 were added to 1 mL of each sample (1 $mg.mL^{-1}$). After 1 hour and 30 minutes, the absorbance was measured at 750 nm in a Tecnal UV-5100 spectrophotometer. The dosage was obtained by interpolating the absorbance against a gallic acid calibration curve (15.625 to 250 $\mu g.mL^{-1}$), and the results were expressed in mg of gallic acid equivalent per g of sample (mg $GAE.g^{-1}$).

Flavonoid quantification was performed using the colorimetric method with aluminum chloride [20]. $AlCl_3$ to 10% and distilled water were added to 1 mL of each sample (1 $mg.mL^{-1}$). After 30 minutes, the absorbance was measured at 420 nm using a Tecnal UV-5100 spectrophotometer. The total flavonoid content was determined using a quercetin standard curve (15.625 to 250 $\mu g.mL^{-1}$). The results were expressed in mg of quercetin equivalents per g of sample (mg $QE.g^{-1}$).

Total tannin content was determined using the Folin-Denis method with modifications [21]. Folin-Denis reagent, 7.5% Na_2CO_3, and distilled water were added to 1 mL of each sample (1 $mg.mL^{-1}$). After 40 minutes, the absorbance was measured at 725 nm using a Tecnal UV-5100 spectrophotometer. The total tannin content was determined using a calibration curve with tannic acid (15.625 to 250 $\mu g.mL^{-1}$). The results were expressed as mg of tannic acid equivalent per g of sample (mg $TAE.g^{-1}$).

Total alkaloid content was measured using the 1,10-phenanthroline method [22]. $FeCl_3$ in hydrochloric acid and 1,10-phenanthroline were added to each sample solution (1 $mg.mL^{-1}$). After 30 minutes in a hot water bath (70ºC), the absorbance was measured at 510 nm using a Tecnal UV-5100 spectrophotometer. Alkaloid content was determined using a yohimbine.HCl standard curve (62.5 to 500 $\mu g.mL^{-1}$). The results were expressed as mg of yohimbine equivalent per g of sample (mg $YE.g^{-1}$).

2.6 Antioxidant activity

The antioxidant activity of the samples was evaluated using the stable free radical scavenging method with 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) [23]. A 1 mL aliquot of the sample solutions (12.5 to 100 $\mu g.mL^{-1}$) was added to 2 mL methanolic DPPH solution (40 $\mu g.mL^{-1}$). After

30 minutes, the absorbance of the reaction mixtures was measured at 515 nm using a Tecnal UV-5100 spectrophotometer.

Another common method is based on the sample's ability to scavenge the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) cation [23]. $ABTS^{\bullet+}$ was produced by reacting a 7 mM ABTS solution with a 140 mM potassium persulfate solution at room temperature in the dark for 16 hours. The $ABTS^{\bullet+}$ (2 mL) solution was added to 1 mL of the sample (12.5 to 100 $\mu g.mL^{-1}$) and after 6 minutes, the absorbance was measured at 734 nm in a Tecnal UV-5100 spectrophotometer.

According to the equation below, the percentages of reduction of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals and the IC_{50} values (inhibitory concentration required to reduce the radicals by 50%) were determined by extrapolating the regression analysis. Quercetin was used as the positive control and was prepared under the same conditions as the samples.

$$\% \text{ Reduction (Radical)} = [A_{control} - A_{sample}/A_{control}] \times 100$$

Where: $A_{control}$ is the initial absorbance of the radicals, and A_{sample} corresponds to the absorbance of the radicals in the medium after reacting with the sample.

2.7 Determination of Sun Protection Factor (SPF)

The UVB-SPF of the extracts was determined using the method proposed by Mansur et al. (1986) [24]. This method determines the absorption characteristics of sunscreen agents based on the spectrophotometric analysis of diluted solutions.

Extract solutions (50, 200, 500, and 1,000 $\mu g.mL^{-1}$) were subjected to spectral scanning in the range of 280 to 400 nm at 5-nm intervals using a Tecnal UV-5100 spectrophotometer with a 1-cm quartz cuvette, to verify absorption in the ultraviolet A and B regions (UVA and UVB). The absorbance values obtained at 290 and 320 nm were subjected to the equation of Mansur et al. (1986) [24] to calculate the in vitro UVB-SPF, considering the erythemogenic effect (EE) and radiation intensity (I), as measured by Sayre et al. (1979) [25].

$$SPF = CF \times \sum_{290}^{320} EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda)$$

Where: CF = Correction factor, which is 10 (based on the measurement of the 8% homosalate standard, whose SPF is 4). EE = Erythemogenic effect of radiation of wavelength λ. I (λ) = Intensity of sunlight at wavelength λ. Abs (λ) = Spectrophotometric absorbance reading of the sample solution at wavelength λ.

2.8 Statistical Analysis

In this study, the quantification of phenols, flavonoids, tannins, and alkaloids, as well as the evaluation of antioxidant activity, were performed in triplicate, and the results were expressed as mean ± standard deviation (n = 3). The data obtained were tested using one-way ANOVA followed by Tukey's test, using Sisvar 5.6 software. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

Solid-liquid extraction with solvents such as ethanol and water is a common method for obtaining non-volatile bioactive products [26]. In the present study, the yields of the ethanolic extracts were 9.70% for leaves and 5.94% for bark. The yields of the aqueous extracts were 10.24% for the leaves and 13.43% for the bark. Water was more efficient than alcohol, suggesting that the chemical constituents are predominantly slightly more polar compounds.

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A preliminary phytochemical profile analysis of the ethanolic and aqueous extracts of *S. paniculatum* was performed to identify the main secondary metabolite groups [18]. The tests revealed the presence of alkaloids and tannins in both plant parts. Flavonoids, triterpenes, and/or steroids were identified only in the leaf extracts. These results corroborate those of previous studies on this species [27]. In contrast, sesquiterpene lactones, other lactones, purines, and saponins were not detected in any of the analyzed extracts.

The quantitative analysis determined the total levels of phenols, flavonoids, tannins, and alkaloids by interpolating the absorbances of the samples against the calibration curves constructed with specific standards for each group of compounds (Table 1). In general, both extracts had high levels of these compounds.

The ethanolic bark extract produced significantly higher levels of phenols (87.68 ± 4.02 mg GAE.g^{-1}) and tannins (103.72 ± 5.73 mg TAE.g^{-1}). Alkaloid levels were statistically similar between the bark and leaves at 568.38 ± 5.92 and 555.37 ± 11.93 mg YE.g^{-1}, respectively. However, flavonoids were recorded at a lower concentration (14.54 ± 0.71 mg QE.g^{-1}).

The aqueous extract of the leaves had higher flavonoid (27.40 ± 4.15 mg QE.g^{-1}), tannin (95.35 ± 0.97 mg TAE.g^{-1}), and alkaloid (584.37 ± 2.51 mg YE.g^{-1}) contents, but lower phenol content (49.95 ± 2.19 mg GAE.g^{-1}) (Table 1).

Table 1. Contents of phenols, flavonoids, tannins, total alkaloids, and IC_{50} of extracts from the leaves and bark of Solanum paniculatum.

Sample	Phenols mg GAE.g^{-1}	Flavonoids mg QE.g^{-1}	Taninns mg TAE.g^{-1}	Alkaloids mg YE.g^{-1}	IC_{50} (µg.mL^{-1})	
					DPPH$^{\bullet}$	ABTS$^{\bullet+}$
Extracts ethanolic						
Leaves	60.82 ± 4.69^{b}	40.97 ± 2.38^{a}	75.62 ± 3.54^{a}	555.37 ± 11.93^{a}	77.69 ± 7.37^{b}	59.98 ± 1.41^{b}
Bark	87.68 ± 4.02^{a}	14.54 ± 0.71^{b}	103.72 ± 5.73^{b}	568.38 ± 5.92^{a}	54.66 ± 2.24^{a}	27.01 ± 1.94^{a}
Extracts aqueous						
Leaves	49.95 ± 2.19^{b}	27.40 ± 4.15^{a}	95.35 ± 0.97^{a}	584.37 ± 2.51^{a}	47.21 ± 3.11^{b}	29.03 ± 2,44^{b}
Bark	67.39± 1.23^{a}	18.78 ± 3.05^{b}	52.91 ± 8.37^{b}	425.27 ± 14.53^{b}	115.76 ± 1.75^{a}	75.16 ± 2.24^{a}
Quercetin					4.07 ± 1.43	2.79 ± 0.19

Values expressed as mean ± standard deviation (n = 3). Means followed by different letters in the same column differ from each other ($p < 0.05$).

The use of natural products to prevent skin damage caused by ultraviolet (UV) radiation is on the rise. Continuous exposure to this radiation, particularly UVA (400–320 nm) and UVB (320–290 nm) radiation, is one of the main external factors contributing to the generation of free radicals. Excessive free radicals can adversely affect biomolecules and trigger diseases, such as skin cancer [27].

Plant extracts with photoprotective and antioxidant properties are increasingly used in cosmetic formulations because of their ability to neutralize free radicals and absorb UV radiation, thereby reducing oxidative damage to skin cells [28, 29].

The molecular structures of the active ingredients present in some plants are comparable to the base structures of some of the main synthetic sunscreens. Organic filters, which are composed of organic molecules, are described as UVA and UVB filters because they can absorb radiation at wavelengths between 290 and 400 nm.

UV spectrophotometry is commonly used to evaluate the SPF of compounds, particularly those of plant origin. In this context, spectrophotometric absorption profiles of *S. paniculatum* extracts were initially obtained. Different intensity bands were observed in the UVA and UVB regions of the spectra. The maximum absorption wavelengths were 290 and 350 nm for the ethanolic leaf and bark extracts, respectively. For the aqueous extracts, the maximum absorption values were observed at 280 nm for the leaves and 290 nm for the bark (Figure 1).

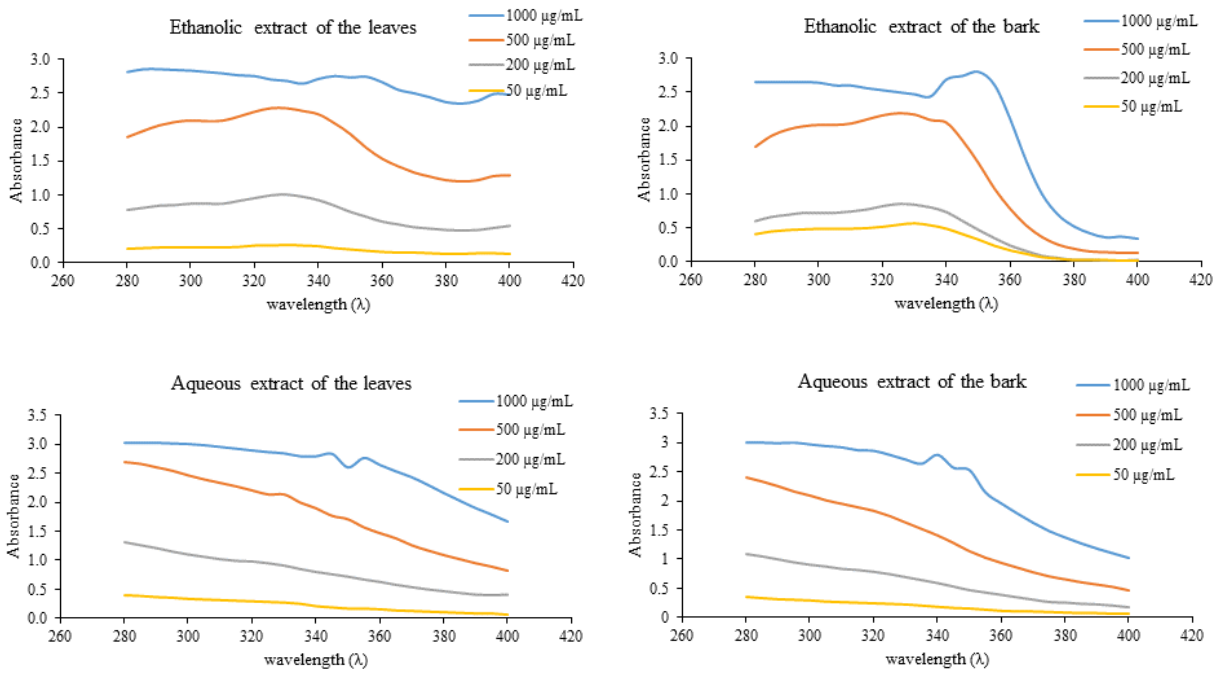

Figure 1: Spectroscopic absorption profiles of the ethanolic and aqueous extracts of Solanum paniculatum leaves and bark.

The in vitro SPF evaluation method developed by Mansur et al. (1986) [24] is rapid and accessible. It correlates well with in vivo methods because it relates the absorbance of the substance to the erythemogenic effect of radiation and light intensity at specific wavelengths between 290 and 320 nm, which correspond to the UVB region [3].

Thus, the SPFs were calculated within the wavelength range characteristic of the UVB region (Table 2), which is considered the region with the highest incidence during the day, where people are exposed for the longest time, and is mainly responsible for skin lesions, such as burns, deep pigmentation, premature aging, and skin cancer [30].

According to ANVISA (2012) [31], a product is considered suitable for use in photoprotective cosmetics when it has an SPF of at least 6.0. Thus, *S. paniculatum* extracts at concentrations of 200, 500, and 1,000 µg.mL^{-1} proved promising for photoprotective formulations, as they exceeded the minimum value required for photoprotection (SPF ≥ 6).

The SPF values of the extracts were directly proportional to their concentrations; that is, the higher the concentration, the higher the SPF. Concentrations of 500 and 1,000 µg.mL^{-1} exhibited SPF values greater than 20. This behavior is similar to that observed in other plant species, which also have higher SPF values [32, 33].

Table 2: SPF of ethanolic and aqueous extracts of Solanum paniculatum at different concentrations.

Sample	SPF (Sun Protection Factor)			
	50 µg.mL^{-1}	**200 µg.mL^{-1}**	**500 µg.mL^{-1}**	**1,000 µg.mL^{-1}**
		Ethanolic extract		
Leaves	2.31	8.73	20.92	28.10
Bark	4.90	7.29	20.21	26.12
		Aqueous extract		
Leaves	3.21	10.58	24.11	29.77
Bark	2.74	8.72	20.24	29.44

Because of the complex composition of plant extracts, the results are more reliable when the antioxidant activity is determined using at least two methods [19]. Thus, the extracts of

S. paniculatum were evaluated using in vitro DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging assays [23]. The results are expressed as the IC_{50} value (Table 1), which indicates the concentration required to reduce free radicals by 50%.

The extracts effectively eliminated DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, demonstrating similar IC_{50} values. The ethanolic bark extract exhibited the greatest antioxidant effectiveness, with IC_{50} values of 54.66 ± 2.24 and 27.01 ± 1.94 μg.mL^{-1}. The aqueous leaf extract also demonstrated significant effectiveness, with IC_{50} values of 47.21 ± 3.11 and 29.03 ± 2.44 μg.mL^{-1}. However, both had IC_{50} values higher than that of the quercetin standard (Table 1). The ethanolic extract of the leaves and the aqueous extract of the bark exhibited lower antioxidant potential because they reduced the concentration of radicals to a lesser extent.

Previous studies also have reported significant antioxidant activity in ethanolic extracts of *S. paniculatum* leaves and bark, as assessed by DPPH and ABTS assays, which is possibly related to the presence of phenolic compounds [27]. Ribeiro et al. (2007) [34] recorded considerable activity when analyzing the ethanolic and aqueous extracts of the leaves. Martins et al. (2021) [13] investigated the influence of temperature and drying on the leaves. They quantified the levels of total phenolic compounds and alkaloids and assessed their antioxidant activity. However, their results were inferior to those of the present study.

Plant-derived compounds, such as phenolic compounds and alkaloids, can be used in dermatological products to prevent skin cancer and other dermatological pathologies caused by the sun and/or free radicals [35, 36]. Due to their antioxidant properties, these compounds effectively provide UV protection because their chemical structures are similar to those of synthetic sunscreen chemicals [3, 4].

Furthermore, phenolic compounds are often associated with the antioxidant activity of plant extracts, mainly because of their reducing properties and chemical structure [37]. Pazinatto et al. (2022) [38] attributed the antioxidant activity of three *Beta vulgaris rubra* extracts to the presence of phenolic and flavonoid compounds (38). Another study showed that the polyphenol tannic acid is an effective antioxidant in different in vitro assays and is comparable to standard antioxidant compounds such as BHA and BHT [39].

Studies have shown that some alkaloids are as effective as or more effective than standard antioxidants [40, 41]. Gan et al. (2017) [42] suggest that total phenols and alkaloids are responsible for the antioxidant potential of a Brassicaceae specimen and that the effect of alkaloids is greater than that of phenols in the analyzed samples.

Similar to other plant species, whose antioxidant and photoprotective activity is mainly attributed to the phenolic compounds and alkaloids in their composition [43, 44]. Tannins and alkaloids, in particular, may be primarily responsible for the observed antioxidant potential in the ethanolic and aqueous extracts of *S. paniculatum* leaves and bark and are directly related to the significant SPF values.

4. CONCLUSIONS

Ethanolic and aqueous extracts from the leaves and bark of *S. paniculatum* exhibited photoprotective and antioxidant activities. These effects may be related to the presence of alkaloids and phenolic compounds, which are known antioxidants with photoprotective properties. This species shows promise for use in phytocosmetic formulations, encouraging continued research to isolate and characterize the substances responsible for these activities and to confirm its use as a natural photoprotective agent.

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