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## BIOACTIVE COMPOUND CONTENTS AND ANTIOXIDANT AND PHOTOPROTECTIVE ACTIVITIES OF *Eugenia dysenterica* (MART.) DC.

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### ABSTRACT

*Eugenia dysenterica* (Mart.) DC. is a tree species native to the Brazilian Cerrado, popularly known as “cagaita” or “cagaiteira.” The species is part of the Cerrado bee flora, and its leaves and bark are used in folk medicine to combat heart problems and treat diarrheal diseases, diabetes, and jaundice. The present study aimed to evaluate the antioxidant activity and photoprotective potential and determine the content of total phenolic compounds in the ethanol and aqueous extracts of the leaves and bark of a specimen of *E. dysenterica*. The antioxidant activity was determined by the DPPH<sup>•</sup> and ABTS<sup>•+</sup> free radical scavenging assays, and the Sun Protection Factor (SPF) in vitro was evaluated by the spectrophotometric method. Phenols, flavonoids, and total tannins were measured by spectrophotometry in the visible region. The extracts showed high antioxidant activity and photoprotective potential. The results indicate these activities are possibly related to the considerable levels of phenolic compounds found in the samples. Therefore, *E. dysenterica* extracts are promising for use in phytocosmetic formulations with antioxidant and photoprotective properties.

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## INTRODUCTION

The development of cosmetic products containing natural active ingredients, especially those of plant origin, is a growing trend. Essential oils and plant extracts that have a sun protection factor (SPF) are increasingly being used in cosmetics as sunscreen lotions, as their proven antioxidant capacity and ability to absorb solar radiation can intensify the product's protection and/or neutralize free radicals produced in the skin after sun exposure (Ramos *et al.*, 2022). Several studies describe the action of antioxidants in photoprotection; some evaluated their action in preventing the development of cutaneous erythema by determining the SPF value, and others analyzed their protective effects against molecular damage caused by oxidative stress induced by ultraviolet radiation (UV) capable of causing photoaging and damage such as erythema, edema, and carcinogenicity. Antioxidants such as beta-carotene and vitamins C and E are active ingredients commonly included in photoprotective formulations (Nunes *et al.*, 2018; Ramos *et al.*, 2022). Among the main categories of secondary metabolites produced by plants, phenolic compounds such as flavonoids and tannins stand out as antioxidants. Plant extracts rich in polyphenols and their glycosides can provide a photoprotective effect as they have chemical structures related to those of synthetic chemical sunscreens. With a structure consisting of condensed aromatic rings with multiple hydroxyl groups, these compounds, associated with their antioxidant action,

effectively absorb UV-A and UV-B radiation (Radice *et al.*, 2016; Kostyuk *et al.*, 2018). Therefore, these plant extracts have been used in photoprotective formulations associated with UV filters, seeking to provide additional photoprotective qualities and also reduce some undesirable effects caused by the use of synthetic filters (Ayad *et al.*, 2023). *Camellia sinensis* (green tea) extract, for example, stands out for its antioxidant and photoprotective properties related to the production of flavonoids and tannins and is frequently present in pharmaceutical formulations (Lima *et al.*, 2023). *Eugenia dysenterica* (Mart.) DC., Mirtaceae, popularly known as “cagaita” or “cagaiteira,” is a native fruit tree found in almost the entire Brazilian Cerrado (Silva *et al.*, 2001). The species is part of the Cerrado bee flora, and its leaves and bark are used in folk medicine to combat heart problems and diarrhea and in the treatment of diabetes and jaundice, however, the ripe fruits, if consumed in excess, cause a laxative effect. It is used as an ornamental plant and as a source of cork, and its wood is used in civil construction (Scariot and Ribeiro, 2015). In the literature, studies describe high contents of phenolics, carotenoids, terpenes, and saponins in different extracts of *E. dysenterica* (Justino *et al.*, 2022; Silva *et al.*, 2019; Thomaz *et al.*, 2018; Santana *et al.*, 2018; Vitek *et al.*, 2016). Important biological activities, such as antioxidant, antimicrobial, antidiabetic, antileukemic, and high phytotoxic potential, are also described for extracts and/or isolated substances, mainly for the leaves of the species (Thomaz *et al.*, 2018, Vitek *et al.*, 2016; Ribeiro *et al.*, 2020). However, reports on the photoprotective activity of this plant are scarce. Seeking to contribute to the knowledge of the biological potential of this species, the

present study aimed to evaluate the antioxidant activity and the sun protection factor, as well as measure the levels of phenols, flavonoids, and total tannins in the ethanol and aqueous extracts of the leaves and bark of a specimen of *E. dysenterica* occurring in the southern region of the state of Mato Grosso do Sul.

## MATERIAL E METHODS

**Collection of plant material and preparation of ethanolic and aqueous extracts:** The plant material (leaves and bark) was collected in March 2023 in the municipality of Itaquiraí, Mato Grosso do Sul, Brazil. An exsiccata was deposited in the “Ernesto Vargas Batista” herbarium of the State University of Mato Grosso do Sul under number 05. Leaves and bark were air-dried and then ground. To obtain the ethanolic extract, 35.1 g leaves, and 23.44 g bark were exhaustively extracted with cold ethanol. The resulting extracts were filtered and concentrated under reduced pressure to a syrupy consistency. To obtain the aqueous extract, distilled water at 98 °C was added to the leaves (30.5 g) and bark (20.2 g), and after 30 minutes, filtered and subjected to freeze-drying.

**Quantification of phenols, flavonoids and total tannins:** The total phenol content was measured using the Folin-Ciocalteu method (Norren *et al.*, 2017). The quantification of flavonoids was performed by the colorimetric method with aluminum chloride (Masturi *et al.*, 20019). Total tannins were measured using the Folin-Denis method (Suliman *et al.*, 2007).

**Determination of in vitro antioxidant activity:** The antioxidant activity of the extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) free radical scavenging method (Salachna *et al.*, 2021). An aliquot of sample solutions (6.25 to 100 µg.mL<sup>-1</sup>) was added with DPPH<sup>•</sup> methanolic solution (40 µg.mL<sup>-1</sup>) and, after 30 minutes, the absorbances of the reaction mixtures were read in a Tecnal UV/vis spectrophotometer, at 515 nm. Another method used is based on the ability to capture the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS<sup>•+</sup>) cation (Salachna *et al.*, 2021). The previously prepared ABTS<sup>•+</sup> solution was added to the samples (6.25 to 50 µg.mL<sup>-1</sup>) and after 6 minutes the read at 734nm. From the absorbance values, the percentages of reduction of DPPH<sup>•</sup> and ABTS<sup>•+</sup> radicals were determined and calculated the IC<sub>50</sub> values obtained by extrapolation of the regression analysis. The positive control used was quercetin.

absorbance readings from 290 to 320 nm in a Tecnal UV-visible spectrophotometer with a 1 cm quartz cuvette, at a reading interval of 5 at 5 nm. The analysis was carried out in triplicate and the absorbance values obtained were submitted to the equation by Mansur *et al.* (1986) below to verify the SPF in vitro, considering the erythematogenic effect and radiation intensity (EE x I) measured by Sayre *et al.* (1979).

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

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

**Statistical analysis:** The results were expressed as mean ± standard deviation (n = 3) for each extract. The statistical treatment of the data was done by analysis of variance (ANOVA) and Tukey's test, using the program Sisvar 5.6. The p value < 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

The efficiency of the plant extraction process depends mainly on the solvent. The polarity of the target compound, as well as molecular affinity between solvent and solute and toxicity, are some of the factors that must be considered when selecting the solvent. In this study, ethanol and water were used for the extraction process. Thus, the yields of the ethanol extracts obtained were 12.5% (leaves) and 11.6% (bark), and the aqueous extracts were 9.1% (leaves) and 6.5% (bark). These results indicated that the use of alcohol was more efficient than water. The estimated quantification of total phenols, flavonoids, and tannins in the extracts was determined by interpolating the absorbances of the samples (extracts) against the calibration curves constructed with specific standards for each group (Table 1). Both solvents were efficient in extracting the compounds. In general, these compounds are polar and are usually used with low-toxic polar solvents, such as ethanol and water (Benmeziiane *et al.*, 2014). Comparing the leaf and bark extracts in solvents, the leaves had the highest levels of phenols, flavonoids, and tannins.

**Table 1. Contents of phenols, flavonoids, total tannins and IC<sub>50</sub> of extracts from the leaves and bark of *Eugenia dysenterica***

Sample	Phenols mg GAE.g <sup>-1</sup>	Flavonoids mg QE.g <sup>-1</sup>	Tannins mg TAE.g <sup>-1</sup>	IC <sub>50</sub> (µg.mL <sup>-1</sup> )	
				DPPH <sup>•</sup>	ABTS <sup>•+</sup>
<i>Extracts ethanolic</i>					
Leaves	270.41 ± 5.00 <sup>a</sup>	33.40 ± 0.76 <sup>a</sup>	267.71 ± 9.29 <sup>a</sup>	5.36 ± 0.24 <sup>b</sup>	1.96 ± 0.19 <sup>b</sup>
Bark	240.44 ± 1.62 <sup>b</sup>	18.78 ± 0.79 <sup>b</sup>	211.30 ± 8.83 <sup>b</sup>	8.84 ± 0.41 <sup>a</sup>	4.64 ± 0.06 <sup>a</sup>
<i>Extracts aqueous</i>					
Leaves	245.27 ± 5.71 <sup>a</sup>	11.59 ± 0.36 <sup>a</sup>	199.06 ± 3.27 <sup>a</sup>	18.55 ± 2.49 <sup>b</sup>	7.02 ± 0.25 <sup>b</sup>
Bark	234.09 ± 2.53 <sup>b</sup>	6.40 ± 0.14 <sup>b</sup>	208.45 ± 9.06 <sup>a</sup>	35.19 ± 0.83 <sup>a</sup>	14.46 ± 0.28 <sup>a</sup>
Quercetin				4.07 ± 1.43	2.79 ± 0.19

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

**Table 2. SPF of ethanolic and aqueous extracts of *Eugenia dysenterica* in different concentrations**

Sample	SPF		
	50 µg.mL <sup>-1</sup>	200 µg.mL <sup>-1</sup>	500 µg.mL <sup>-1</sup>
<i>Extracts ethanolic</i>			
Leaves	3.43 ± 0.010	14.62 ± 0.060	27.31 ± 0.100
Bark	3.20 ± 0.019	12.74 ± 0.020	24.46 ± 0.090
<i>Extracts aqueous</i>			
Leaves	2.55 ± 0.020	9.61 ± 0.070	23.22 ± 0.062
Bark	2.61 ± 0.009	10.49 ± 0.087	23.97 ± 0.080

**Determination of Sun Protection Factor (SPF) Ultraviolet B (UV-B):** The UV-B SPF was determined using the in vitro spectrophotometric method proposed by Mansur *et al.* (1986). Thus, solutions of the extracts (50, 200 and 500 µg.mL<sup>-1</sup>) were subjected to

In the aqueous extract, there was no significant difference in total tannin levels. There are several methods for evaluating in vitro antioxidant activity proposed in the literature. In general, these assays differ concerning the mechanism of action, target species, and reaction conditions, and given the complexity of the composition of

plant extracts, the results are more reliable if the antioxidant activity is determined by at least two different methods (Norren *et al.*, 2017). Thus, ethanol and aqueous extracts of *E. dysenterica* leaves and bark were evaluated using DPPH<sup>•</sup> and ABTS<sup>•+</sup> assays. Both scavenge radicals; however, in the ABTS method, the activity of substances with a hydrophilic and lipophilic nature can be measured, a limiting factor in the DPPH assay, which has little reproducibility for hydrophilic substances (Munteanu and Apetrei, 2021). The extracts showed similar profiles in the production of IC<sub>50</sub> values with high radical scavenging capacity, with the cationic radical ABTS<sup>•+</sup> being more reactive than DPPH<sup>•</sup> (Table 1). The ethanolic and aqueous leaf extracts were significantly more active, the IC<sub>50</sub> of the ethanolic extract ( $5.36 \pm 0.24$  and  $1.96 \pm 0.19 \mu\text{g}\cdot\text{mL}^{-1}$ ) were close to the quercetin standard (Table 1). Bark extracts inhibited radicals less (higher IC<sub>50</sub> values), demonstrating less potential as sources of free radical scavenging substances. Studies describe that the antioxidant activity of vegetables is related to the presence of phenolics and considering, mainly their reducing properties and chemical structure, these compounds are free radical scavengers and are very efficient in preventing and/or reducing oxidative stress (Stagos, 2019; Sousa *et al.*, 2007). Thus, the high antioxidant potential of *E. dysenterica* can be attributed to the high levels of phenolics and this action was significantly higher in leaf extracts, and in general, these extracts presented higher levels of phenols, flavonoids and tannins. Considering the total phenols content, it is possible that the main phenolic constituents are tannins, and the radical scavenging capacity of the extracts is attributed mainly to these compounds. Flavonoids were found in the lowest concentrations in all extracts. In previous studies, similar results were reported for *E. dysenterica* leaf extracts, recording considerable levels of phenolic compounds and high antioxidant potential investigated by the DPPH method (Ávila *et al.*, 2016; Prado *et al.* 2014; Takao *et al.*, 2014).

UV radiation, particularly UV-B, is most responsible for skin lesions, such as burns, deep pigmentation, premature aging, and skin cancer (Sgarbi *et al.*, 2007). In evaluating the photoprotective activity in vitro, after reading the absorbances of wavelengths established between 290 and 320 nm (UV-B spectrum), the values found were applied to the equation referenced by Mansur *et al.* (1986) to determine the SPF and are listed in Table 2. In accordance with RDC 30, of June 1, 2012, of the National Health Surveillance Agency (ANVISA), which approves the MERCOSUR technical regulation on sunscreens in cosmetics and provides other measures, assigning technical regulation applied to products and cosmetics intended for sun protection of the skin, the minimum sun protection factor (SPF) has a value of 6 (six) (Brasil, 2012). Based on the analysis of SPF values found at concentrations of 50, 200, and 500  $\mu\text{g}\cdot\text{mL}^{-1}$  of extracts from the leaves and bark of *E. dysenterica*, promising results were found regarding its use in photoprotective formulations for concentrations of 200 and 500  $\mu\text{g}\cdot\text{mL}^{-1}$  since these samples exceeded the minimum value of 6 for photoprotection. The ethanolic extract samples presented the highest values of UV-B photoprotective activity, with emphasis on the concentration of 500  $\mu\text{g}\cdot\text{mL}^{-1}$  with an SPF of  $27.31 \pm 0.100$  for the leaves and  $24.46 \pm 0.090$  for the bark. Even more, there was a correlation between the extract concentration evaluated and the SPF found. In all cases, the higher the extract concentration, the higher the SPF. Similarly, other plant species showed the same behavior. For example, the ethanol and aqueous extracts of *Rhaphiodon echinus*, at the highest concentrations (500 and 1,000  $\mu\text{g}\cdot\text{mL}^{-1}$ ), showed a photoprotective effect > 23 SPF (Medeiros *et al.*, 2020; Medeiros *et al.*, 2021).

As well as the ethanol extract of *Praxelis clematidea*, at concentrations of 500 and 1,000  $\mu\text{g}\cdot\text{mL}^{-1}$ , exhibited SPF values of 15.17 and 20.33, respectively, with possible therapeutic application in dermatological preparations (Simão *et al.*, 2019). The results found in this study suggest that phenolic compounds are mainly responsible for the high antioxidant potential demonstrated by *E. dysenterica* extracts and are directly related to FPS values. The action of antioxidants directly impacts the photoprotection, as they act to retain the effects of oxidative stress related to exposure to ultraviolet radiation (Medeiros *et al.*, 2021; Dunaway *et al.*, 2018). The relationship

between antioxidants and SPF opens up ways to prevent photoaging and diseases related to oxidative processes, such as skin cancer. Furthermore, it has been reported that microemulsions containing aqueous leaf extract of *E. dysenterica* may be promising systems in dermatological treatments that require antioxidant action (Nunes *et al.*, 2018). Moreira *et al.* (2017) describe that a mixture based on ethanolic leaf extract shown cellular regenerative effects against damage induced by UV-A exposure and inhibition of enzymes related to dermatological disorders, without relevant toxic effects and considering these results support its potential use in cosmetic/pharmaceutical products. In conclusion, the present study reveals that *E. dysenterica* extracts are sources of phenolic compounds and have the potential to be used in phytocosmetic formulations with antioxidant and photoprotective activities. Nevertheless, more studies are needed to confirm them as an innovative alternative for use as a natural product for photoprotection.

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