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Antioxidant Activity and Determination of Phenolic Compounds, Total Flavonoids and Hispidulin in *Baccharis erioclada* DC.

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HIGHLIGHTS

- Crude extract showed higher antioxidant activity.
- The flavonoid hispidulin was found in the crude extract.
- 47.98 mg/g of flavonoids were quantified in the crude extract.
- In the crude extract were quantified 160.66 mg/g of the total phenolics.

Abstract: Phenolic compounds are frequently found in the specific tissues in the leaves and stems of *Baccharis species*. However, only a few species of the genus have been studied in recent years, including *B. erioclada*. This study evaluates the *in vitro* antioxidant potential of *B. erioclada* crude extract and its fractions. Phytochemical studies, including quantification of total phenolics and flavonoids have also been carried out. Crude extract presented higher antioxidant capacity since it showed lower values of IC50 or greater reductive capacity compared to the fractions. The total phenolic compounds and flavonoids in the

crude extract were quantified as 160.66 mg/g and 47.98 mg/g, respectively. Hispidulin showed 27,2296 g/100 g of dried extract. The present study evidenced that *B. erioclada* extracts are promising sources of antioxidant compounds.

Keywords: antioxidant; Asteraceae; broom; phenolics.

INTRODUCTION

Oxidative stress and free radicals can promote diseases, such as cardiovascular, cancers, atherosclerosis, inflammatory processes and type 2 diabetes mellitus. An antioxidant substance inhibits the oxidation process and protects biological systems against the damaging effects of reactions that promote oxidation of the macromolecules or cell structures [1,2]. Several studies have been performed to investigate compounds with antioxidant properties from natural resources [3,4]. Phenolic compounds with high antioxidant properties, such as flavonoids and tannins, have been found in many plants belonging to different families [5,6].

The genus *Baccharis* L. in the Asteraceae family comprises about 442 species distributed from Argentina to the United States. In Brazil, the genus is represented by 185 species [7]. Several species of *Baccharis* are used in folk medicine as analgesic, antidiabetic, anti-inflammatory, antitrypanosomal, antimalarial, digestive, diuretic, insecticidal and spasmolytic [8–11].

Previous studies have indicated the presence of phenolic compounds in the specific tissues in the leaves and stems of *Baccharis* species [12–18]. A recent review of the genus *Baccharis* (33 species) has documented 139 compounds. Most of them are flavonoids, such as quercetin, kaempferol, apigenin, naringenin and aromadendrin, and phenolic acids, such as artepillin C, drupanin, ferulic acid, caffeic acid and dicaffeoylquinic acid. However, only a few species, such as *B. dracunculifolia* DC., *B. crispa* Spreng. (synonym *B. trimera*), *B. articulata* (Lam.) Pers., *B. uncinella* DC., *B. salicifolia* (Ruiz &Pav.) Pers., and *B. gaudichaudiana* DC., have been most studied in recent years [10]. Few studies have been conducted on the chemical profile of *B. erioclada* DC. (“lageana broom”) essential oil [15,19].

The objectives of this study were to evaluate *in vitro* antioxidant potential and quantify total phenolic compounds and flavonoids of the crude extract and its fractions of *B. erioclada*.

MATERIAL AND METHODS

Plant Material

Aerial parts of *Baccharis erioclada* were collected in Campos Gerais, Ponta Grossa, Paraná, Southern Brazil (coordinates 25°08’S and 50°27’W) in the summer of 2013. The plants were identified by taxonomist Dr. Gustavo Heiden (EMBRAPA ClimaTemperado/Rio Grande do Sul), and the vouchers were registered at the herbarium of the State University of Ponta Grossa, under the number ICN 20412. Access to the botanical material was approved and licensed by the Conselho de Gestão do Patrimônio Genético (CGEN) registered under number 02001001-165/2013-47. The collected plant materials were sorted, shade-dried and cut into small pieces.

Extraction and isolation

The dried aerial parts (100 g) were extracted with ethanol (96°GL, 500 mL) for 7 days. The solvent was changed every 2 days, obtaining a crude extract (CE). This extract was lyophilized and stored at 4 °C. Part of the CE extract (50 g) was partitioned in a chromatographic column at pressure. Fractions were obtained using analytical grade hexane (FHex), chloroform (FCl), ethyl acetate (FEa) and methanol (FMe). The fractions were concentrated under pressure and stored at 4 °C. During partitioning, a yellow precipitate was observed in the ethyl acetate fraction. The precipitate was filtered off and analyzed by Nuclear Magnetic Resonance (NMR).

Identification

The precipitate was filtered and and submitted to spectrometric analysis for structural elucidation. For this, NMR 1H-13C was performed, using the Bruker DPX 600 NMR spectrometer, operating at 4.7 Tesla, observing the ^{1}H and 13C nuclei at 200.13 and 50.62 MHz, respectively. The 1H and 13C chemical shifts were expressed in ppm and referenced to the tetramethylsilane (TMS) signal, internal reference, at 0.00 ppm. The data were compared with the literature. The analysis was carried out in the NMR Center of the Universidade Federal do Paraná.

Antioxidant assays *in vitro*

DPPH (1,1-diphenyl-2-picrylhydrazyl)

Five methanol solutions were prepared in different concentrations (5 to 200 µg.mL^{-1}) for each sample [20,21]. In a test tube, 2.5 mL of the sample to be tested was added along with 1 mL of 0.03 mmol.mL^{-1} of DPPH methanolic solution. For each sample, a blank was prepared with 2.5 mL of this solution and 1 mL of methanol PA. In parallel, a control with 2.5 mL of methanol PA and 1 mL of DPPH solution was done. After 30 min of reaction, the spectrophotometer readings were taken at 518 nm, corresponding to the maximum absorption of the radical under study. Rutin and vitamin C were used as standards. All analyzes were performed in triplicate.The percentage of antioxidant activity (AA%) was calculated using the formula below:

$$AA\% = \frac{100 - [(abs\ sample - abs\ blank)]}{Abs\ negative\ control}$$

The sample concentration values required to exercise 50% of the antioxidant activity (IC_{50}) were calculated on the graph. The x-axis represents the concentration of the sample, and the y-axis represents the mean of the AA% of the samples tested; each sample had 5 different concentrations. Thus, the equation of the graph line, of type y = ax + b, serves as the basis for determining the IC_{50} value [20].

Reduction power (complex Prussian blue)

The evaluation of the reducing power was performed asYen and Chen [22] (1995) with modifications. 1 mL of sample, 2.5 mL of phosphate buffer (0.2 mol.L^{-1}) and 2.5 ml of ferricyanide solution (1%) were added to a test tube, mixed and incubated in an oven at 50 °C for 20 min. Subsequently, 2.5 mL of trichloroacetic acid (10%) was added to the mixture. 2.5 mL of this mixture was transferred to another test tube. 2.5 mL of ultrapure water and 0.5 mL of 1% FeCl3 (m/v) were added to this test tube. The reading was carried out at 700 nm.

TBARS

For this assay, 0.1 mL of 0.3% ethanol solution of the CE, FHex, FCl and FEa samples was added to 0.4 mL of ultrapure water, 0.5 mL of egg yolk solution 5% (m/v) previously prepared in 0.55% sodium dodecyl sulfate (SDS) solution, 50 µL of a 0.035% solution of 2,2'-azo-bis-2-amidinopropane (ABAP), 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.4% TBA (thiobarbituric acid) also prepared in 0.55% SDS solution. The tubes were taken to the water bath at 95 °C for 1 h. After cooling the solution, 1.5 mL of 1-butanol was added to extract the organic phase. The tubes were centrifuged at 3000 rpm for 5 min. The supernatant was then read at 532 nm. 1-butanol was used as white. As negative and positive controls, the same solution was used. The sample was replaced with 0.1 mL of ethanol PA and 0.1 ml of butylated hydroxytoluene (BHT) 0.3% in ethanol PA. The test was performed in triplicate [23]. The Antioxidant Index of the samples in percentage (AI%) was determined by the equation:

$$AI\% = \left[1 - \frac{Abs\ sample - Abs\ blank}{Abs\ control}\right] X\ 100$$

Total antioxidant capacity (Phosphomolybdenum method)

The extract, fractions and standard (ascorbic acid) were diluted in methanol at a concentration of 200 µg/mL as described by Prieto and coauthors [24]. 0.3 mL of each sample was combined with 1 mL of reagent solution (0.1 mol/l of sodium phosphate, 0.03 M of ammonium molybdate and 3 mol/L of sulfuric acid) and completed with ultrapure water until 100 mL. The tubes were incubated at 95 °C for 90 min and cooled to room temperature. The absorbance of the solution was measured at 695 nm. The antioxidant activity (AA%) compared to ascorbic acid was evaluated by the following formula:

$$AA\%\ compared\ to\ ascorbic\ acid = \left[\frac{(Abs\ sample - Abs\ blank)}{(Abs\ control - Abs\ blank)}\right] x\ 100$$

Chromatography analysis

The qualitative and quantitative analyses of the CE were performed on Varian Pro-star SYS-LC-240-E HPLC (Palo Alto, CA, USA) fitted with a DAD detector at 289 nm wavelength, and C18 analytical column (250 x 4.6 mm, 5 µm) maintained at 21 °C.

Chromatographic runs were performed in gradient elution mode. For the mobile phase, an acid phase mixture prepared with distilled water and phosphoric acid pH 3.3 (phase A), methanol (phase B) and acetonitrile:water (90:10) (phase C) was used in the following mixtures: 1-12 min: 70% phase A, 27% phase B and 3% phase C (flow 1.0 mL/min); 12-16 min: 40% phase A, 50% phase B and 10% phase C (flow 1.1 mL/min), 16-25 min: 20% phase A, 50% phase B and 30% phase C (flow 1.2 mL/min), and terminating in 25 min with 5% A phase, 1% B phase and 94% C phase (flow 1.2 mL/min).

About 1 mg of CE was diluted in 2 mL of MeOH. The preparation of the hispidulin calibration curve was done by diluting a 1 mg/mL methanol stock solution of hispidulin in methanol to provide five different concentrations of hispidulin solutions (21, 28, 35, 42 and 49 mg/mL). The solutions were prepared in triplicate. 10 µL of each of the solutions and samples was injected into the chromatographic column. The hispidulin content of the samples was calculated using the equation of the line generated by the analytical curve plotted. The concentration data x area of the peak of the five concentrations of hispidulin were considered.

Quantification of total phenolics and flavonoids

Determination of total phenolic content (TPC)

The Folin-Ciocalteu method was used to evaluate TPC [25]. The CE and fractions were diluted in methanol with concentrations ranging from 80 to 320 µg/mL. The diluted samples were mixed with Folin-Ciocalteu reagent and deionized water. After 10 min, sodium carbonate (10%) was added. The samples were kept at room temperature for 30 min and then measured at 760 nm. TPC were calculated using a calibration curve built with gallic acid (2.5; 5; 7.5; 10; 12.5; 15; 17.5 and 20.0 µg/mL). The results were expressed as gallic acid equivalents (GAE) of a gram of dry plant (mg GAE/g).

Determination of total flavonoids

2 mL of 2% AlCl3 solution was added to the CE diluted in methanol (1000 µg/mL). After 1 h, spectrophotometer readings were performed at 420 nm using quercetin calibration curve at concentrations of 5, 10, 15, 20, 25 and 30 µg/mL as standardized by Chang and coauthors [26]. The total flavonoid contents were determined in quercetin milliequivalent (mEqQ) per gram of CE, based on the equation of the line obtained from the calibration curve constructed: $y = 0.0314 x - 0. 0164$; $R^2 = 0.9996$.

Statistical analysis

All calculations were performed in Microsoft Excel 2010 and SISVAR 5.3 software. The data were analyzed using the analysis of variance (ANOVA) software, and the Duncan test was used to compare the means of the activity indexes. The differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Identification of hispidulin

It was possible to identify the compound isolated from the ethyl acetate fraction of the aerial parts of *B. erioclada* species by analyzing the data obtained from the 1H spectrum and two-dimensional analyzes of HSQC and HMBC (Table 1), spectral data and their comparison with literature data, suggesting that it is the flavone hispidulin (Figure 1).

Table 1. $^{13}C\{1H\}$ NMR spectroscopic data of hispidulin.

Position	Experimental		Literature	
	^{1}H δ (mult., *J* in Hz)[a]	^{13}C (δ)[a,b]	^{1}H δ (mult., *J* in Hz)[a]	^{13}C (δ)[a,b]
2		166.4		164.7
3	6.59 (1H, *s*)	103.5	6.53	102.2
4		184.2		182.7
5		-		153.1
6		133.7		131.2
7		158.9		156.9
8	6.56 (1H, *s*)	95.3	6.54	94.0
9		154.7		152.3
10		105.8		104.5
1’		123.4		121.7
2’/6’	7.84 (1H, *d*, 8,8)	130.0	7.79	127.9
3’/5’	6.92 (1H, *d*, 8,8)	117.5	6.93	115.6
4’		162.8		160.8
6-O$\underline{C}H_3$	3,88 (3H, *s*)	61,4	3.9	59.9

$^{13}C\{1H\}$ NMR data was acquired using an NMR 400 MHz (CD3OD) following Njeh and coauthors [27].

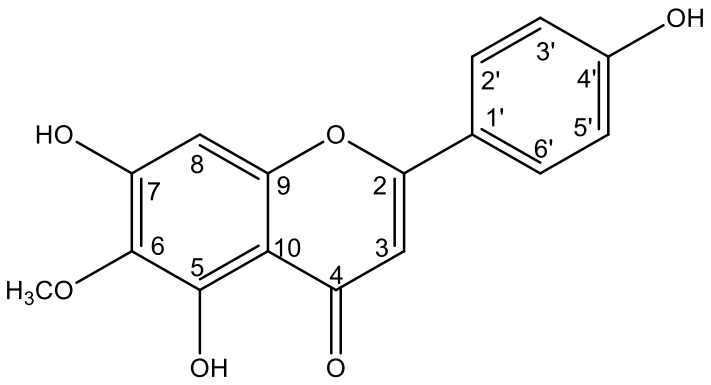

Figure 1. Chemical structure of Hispidulin.

Hispidulin is commonly found in many plants such as *Grindelia argentina* Deble&A.S.Oliveira, *Fridericia chica* (Bonpl.) L.G. Lohmann (synonym *Arrabidaeachica*), *Saussurea involucrata* (Kar. &Kir.) Sch.Bip., *Crossostephium chinense* (L.) Makino, *Artemisia* spp., *Salvia* spp. [28], as well as in *Baccharis* species, *B. crispa*[29,30] and B. *linearifolia* (Lam.) Pers. (synonym *B. pseudotenuifolia*) [31].

There are two duplets at 7.94 and 6.92 ppm with displacement and coupling constant characteristic of the presence of para-disubstituted ring in the molecule (Figure 1 and Table 1). In addition, the simplet signals in 6.59 and 6.56 ppm suggest the presence of some electron-withdrawing group attached to the same carbon (i.e., hydroxyl and carbonyl grouping). It is worth mentioning the presence of a simplet with integral referring to 3 atoms of hydrogen and chemical displacement in 3.88 ppm characteristic of methoxyl groups.

Hispidulin has widely been reported to have various biological activities. It has shown to exhibit dose-dependent inhibitory effects on lipid peroxidation [32], pro-oxidant activity by stimulating the deoxyribose degradation in a system containing Fe^{+3} EDTA in the presence of hydrogen peroxide (H_2O_2), and *in vivo* antioxidant activity promoting inhibition of bromobenzene-induced hepatotoxicity by neutralizing the effects of glutathione depletion similar to that observed with N-acetylcysteine [33].

Antioxidant assays *in vitro*

Due to the complexity of the chemical composition of extracts and fractions from medicinal plants, the use of more than one method to evaluate the antioxidant potential of plant extracts is recommended [34]. Table 2 shows the values obtained for all the antioxidant tests performed.

Table 2. Antioxidant activity of the crude extract and fractions by different methods.

Sample	DPPH (IC50 µg/mL)	Reduction power (%)[a]	TBARS (%)[b]	Total antioxidant capacity (%)
CE	127.000 ± 0.165[b]	61.710 ± 0.013[b]	56.070 ± 0.18[a]	51.140 ± 3.408[a]
FHex	276.000 ±0.874[d]	49.370 ± 0.014[d]	28.970 ± 0.019[b]	24.970 ± 1.162[b]
FCl	301.800 ±0.546[c]	53.410 ± 0.026[c]	29.440 ± 0.050[b]	15.030 ± 0.916[c]
FEa	247.870 ±0.472[c]	57.420 ± 0.003[c]	7.850 ± 0.085[c]	23.470 ± 0.191[b]
FMe	276.980 ±0.367[d]	62.520 ± 0.005[b]	31.240 ± 0.062[b]	8.000 ± 0.478[d]
BHT	-	-	50.550 ± 0.009[a]	-
Rutin	7.540 ±0.0400[a]	-	-	-
Ascorbic acid	6.000 ± 0.002[a]	100[a]	-	100.000 ± 0.184

Data expressed as mean ± standard deviation. [a] Data compared to control. *Statistical difference ($p<0.05$) determined by one-way ANOVA Duncan test.

Based on the results obtained in the different methods, it is possible to determine that the CE showed higher antioxidant capacity since it presented lower values of IC_{50} or greater reductive capacity compared to the fractions. It is also worth noting that in the TBARS and total antioxidant capacity assays, the values obtained for the CE were higher than those obtained for the positive control. The best response obtained for the CE can be justified by the presence of a high number of total phenols or even by the synergism of the compounds present in the fractions.

The DPPH reduction assay determines, by colorimetry, the concentration of substances capable of donating electrons in order to stabilize the radical. It is described as an easy, accurate and reproducible method to determine the antioxidant activity of plant extracts and pure substances [35]. It can be used to screen natural products, making it a critical preliminary test for determining the antioxidant potential of a pure extract, fraction or substance. Several chemical substances have shown a close correlation between DPPH and antioxidant-sequestering activities determined in biological and non-biological models [36].

Tapia and coauthors [37] found that the aqueous extracts of *B. grisebachii* Hieron. inhibited the formation of free radicals in different *in vitro* systems in concentrations of 50-100 µg/ml. Dias and coauthors [38] reported the antioxidant activity of *B. crispa* in powdered drug preparations, lyophilized CE, crude lyophilized resin extract and chloroform, ethyl acetate, absolute ethanol and ethanol 50% extracts, presented EC_{50} values that ranged from 22.74 to 18.19 µg/ml. In an earlier study, Simões-Pires and coauthors [39] observed significant antioxidant activity of aqueous extracts of *B. crispa* and *B. usterii*Heering against DPPH radical, suggesting that the action is due to the presence of caffeine-like acids.

In the study performed by Akaike and coauthors [40], the isolated compounds of *B. gaudichaudiana*, such as quercetin-3-OH-rhamnoside, eupafoline, rutin and 3,5-dicyclohexylacetyl were evaluated against DPPH radical, which showed moderate activity when compared to Trolox standards (1.0 mM) or quercetin (1.9 mM). Leaf extracts of *B. dracunculifolia* showed significant antioxidant activity with IC_{50} of 5 µg/ml [41]. These results confirmed the antioxidant activity previously reported for *B. dracunculifolia* extracts [42].

Another method used was TBARS, which uses thiobarbituric acid reactive substances. This method evaluates the protection of lipoperoxidation promoted by antioxidant substances present in plant matrices with the aid of a rich source of lipids [43]. Secondary metabolites, such as flavonoids, coumarins, phenylpropanoids and terpenoids, have been reported as lipid peroxidation inhibitors [44].

Mongelli and coauthors [45] verified the antioxidant activity of the aqueous extract obtained from the aerial parts of *B. coridifolia* DC. in comparison with the thiobarbituric acid reactive test (TBARS), which has an IC_{50} value of 556 µg/ml. Studying another species, Tapia and coauthors [37] observed the antioxidative effect of *B. grisebachii* extracts, attributed to isolated phenolic compounds (IC_{50} = 10 µg/ml). Both studies suggested that the presence of these secondary metabolites justify the widespread use of these species in the treatment of gastric disorders. Previous research has reported that the formation of reactive oxygen species (ROS), such as superoxide anion and hydroxyl radicals, could exert harmful effects on the cell membrane and tissues since many gastrointestinal problems are linked to oxidative stress [46].

Studies by Oliveira and coauthors [47,48] have shown that crude extracts (ethanolic and aqueous) and fractions (ethyl acetate, n-butanol and aqueous residue) of *B. crispa*, *B.usterii* and *B. spicata* (Lam.) Baill. presented antioxidant activity since they inhibited lipid peroxidation, thus decreasing the production of MDA, a TBA reagent. The study also showed that the more polar fractions presented higher antioxidant activity. The most active fraction in *B. spicata* and *B. crispa* was n-butanol, while for *B. usterii* both the aqueous fraction and n-butanol presented similar activity.

Vieira and coauthors [49] compared the antioxidant properties of aqueous and ethanolic extracts obtained from *B. articulata*, *B. crispa*, *B. spicata* and *B. usterii*. The aqueous extracts were more efficient than

the ethanolic extracts in lipid peroxidation of microsomes induced by the ascorbyl radical assay. However, comparing the aqueous and ethanolic extracts, significant differences were observed only between the extracts of *B. articulata* and *B. crispa* in protecting liposomes against ascorbyl radical.

The reduction power test is based on the reduction of the ferricyanide to ferrocyanide ion, which in the presence of the ferric ion (from $FeCl_3$) forms Prussian blue dye. It is worth mentioning the reductive power value presented by EB was 51.13% inhibition when compared to vitamin C. No trials were found in the literature with other species of *Baccharis* using this technique. However, the results of the evaluation of *Calendula officinalis* L. (Asteraceae), a commercial reducer, indicated low reductive capacity of the ethanolic extracts ranging from 4.38 to 9.06% [50].

The total antioxidant capacity of the extracts and fractions is measured by the hydrophilic and lipophilic substances present in them. It can be evaluated by the phosphomolybdenum complex reduction method, which is based on spectrophotometric determination of the reduction of molybdenum VI to molybdenum V with the formation of Mo^{+5} phosphate [24]. The results obtained indicate that the reduction of molybdenum VI to molybdenum V may inhibit the action of xanthine oxidase since molybdenum is used as a cofactor of this enzyme [51]. Xanthine oxidase is responsible for producing superoxide radicals during the biotransformation of xanthine and hypoxanthine to uric acid.

In the xanthine oxidase inhibition test performed by Borgo and coauthors [52], the *in vitro* antioxidant potential of three extracts obtained by different drying methods was verified, in which the concentration of 100 µg/ml reduced about 80% of the dihydroxybenzoic acid (DHBA) in relation to the negative control. It is suggested that this effect observed in the extracts may be due to the presence of the flavonoid quercetin and/or to the synergistic action of other phenolic compounds.

Chromatography analysis

Chromatographic profile

After observing the best antioxidant results of the CE *in vitro*, a qualitative evaluation was performed using the high-performance liquid chromatography (HPLC) technique. The chromatographic profile of the CE was analyzed at wavelength 289 nm (Figure 2). The chromatogram showed peaks with different retention times indicating the presence of substances with different degrees of polarity, with the largest area corresponding to hispidulin (Figure 2).

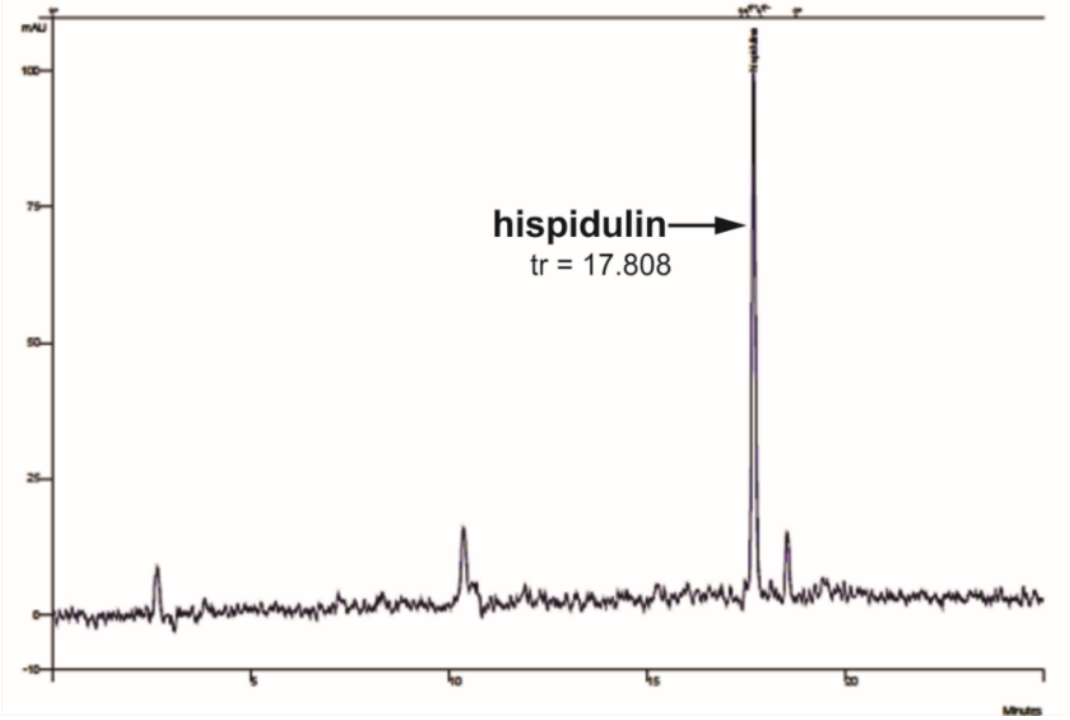

Figure 2. HPLC chromatogram of *Baccharis erioclada* crude extract.

HPLC has become a dominant analytical tool for the separation and determination of polyphenols with different detection systems, such as the diode array detector (DAD) and mass or tandem mass spectrometry. DAD is the most used in the literature on flavonoids in which HPLC is used as an analytical technique since

it allows the real-time scanning of the entire UV/VIS spectrum and provides more information about complex mixtures such as crude vegetable extracts [53–55].

Khoddami and coauthors [56], in a review article on techniques for the analysis of phenolic compounds derived from plants, highlight the HPLC method as the first choice for separation and quantification of these compounds. The authors state that it also provides the necessary conditions for researching several plant species, classes of compounds and vegetal derivatives, citing more than 30 different samples.

Quantification of Hispidulin

For the preparation of herbal products, standardized extracts are crucial to guarantee end products with adequate quality requirements. Several analytical techniques can be used to quantify the main constituents present in these preparations. HPLC is one of the most widely used techniques in routine analysis [57].

The calibration curve constructed with different concentrations of hispidulin presented a high coefficient of determination, with R^2 = 0.9944. By correlating the area value of the hispidulin chromatographic peak present in the CE sample at a retention time of 17.5 min with the equation of the line obtained in the calibration curve (y = 205878x + 488056), it was possible to determine the content of this flavonoid in the CE, obtaining a value of 27,2296 g/100 g of dried extract of *B. erioclada*.

Quantification of total phenolics and flavonoids

Considering the CE of *B. erioclada* presented a promising antioxidant capacity, the chromatogram indicated the presence of several phenolic compounds. The total phenolic compounds and flavonoids in the CE were quantified as 160.66 mg/g and 47.98 mg/g, respectively.

Excellent results were obtained in relation to the concentration of total polyphenols and total flavonoids in the study species. According to Chew and coauthors [58], concentrations of phenols above 50 mg EAG/g are considered high and between 50-30 mg EAG/g medium-high. Therefore, the EB of *B. erioclada* presented a high concentration of phenols. Moreover, secondary metabolites such as phenolic acids, coumarins, flavonoids, simple phenols, lignins, lignans, hydrolysable tannins and condensates have shown positive activity in the Folin Ciocalteu analysis [59].

Verdi and coauthors [5] stated that polyphenols and flavonoids are common in *Baccharis*. Therefore, the results obtained for the CE of the aerial parts corroborate with data described in the literature for other species in the genus. It is known that many compounds present in some plant species have antioxidant and photoprotective activity, which may intensify the final protection promoted by the product or neutralize the free radicals produced in the skin after exposure to the sun [60].

Flavonoids naturally neutralize reactive oxygen species, which cause cell degeneration. For this reason, the phytochemical studies of natural extracts are of great importance since one of the tendencies of the pharmaceutical market is to develop products with natural assets to prevent cutaneous aging [61,62].

CONCLUSION

The results obtained from this study further reinforce the view that *Baccharis* extracts are promising sources of antioxidant compounds. Further studies are needed to examine the potential use of *B. erioclada*, taking advantage of the possible synergy between the molecules present in the extracts, conferring protection against the most dangerous oxidative species. Such properties may prevent pathologies induced by oxidative stress, including inflammatory disorders, various gastrointestinal tract diseases and neurodegenerative diseases.

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Conflicts of Interest: The authors declare no conflict of interest.

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