

## Chemical composition and biological activity of *Baccharis erioclada* DC. essential oil

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The chemically complex essential oils of *Baccharis* species are associated with several biological activities, such as antimicrobial and antiulcerous properties. However, few studies have investigated *Baccharis erioclada* DC. Therefore, in this study, we aimed to characterize the essential oil of *B. erioclada* and evaluate its antioxidant, antimicrobial, and hemolytic potential. The essential oil was extracted by hydrodistillation using a Clevenger apparatus and analyzed via gas chromatography-mass spectrometry (GC-MS). Phosphomolybdenum complex formation, reducing antioxidant power, and thiobarbituric acid reactive substances (TBARS) methods were used to determine antioxidant potential. To evaluate the essential oil's antimicrobial activity, minimum inhibitory concentrations (MIC) in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* were calculated. Hemolytic activity was determined in sheep red blood cells. Thirty-one compounds were identified via GC-MS analysis, representing 81.60% of the total essential oil. These compounds included: turmerone (27.97%), fokienol (13.47%), ledol (9.78%), and santalol (5.35%). The class of compounds identified was the oxygenated sesquiterpenes (62.52%). Antioxidant activity was confirmed via phosphomolybdenum complex formation and TBARS methods. Moderate antimicrobial activity and low hemolysis rates were displayed at concentrations of 250 and 500 µg/mL.

**Keywords:** Volatile oil. Asteraceae. Vassoura-lageana.

### INTRODUCTION

The genus *Baccharis* (Asteraceae) includes 435 species distributed mainly in South America (Flora do Brasil, 2018). *Baccharis* species produce essential oils (EOs) and are used for pharmaceutical purposes and in the fragrance industry. *Baccharis* species EOs mainly comprise monoterpenoids and sesquiterpenoids, and

several studies have focused on the identification of their constituents and associated biological activities (Budel *et al.*, 2012; Bogo *et al.*, 2016; Campos *et al.*, 2016).

Biological activity assays are of fundamental importance in the screening of plants and their constituents. Toxicological tests complement biological assays (Maciel *et al.*, 2002) and can be conducted commercially under the Administrative Rule 116/1996 of the Health Surveillance Secretariat of the Brazilian Ministry of Health (Brasil, 1996), which regulates chronic and acute toxicity studies for herbal products, or as necessary validation for technological development (Sonaglio *et al.*, 2007). Thus, preliminary biological tests are used to determine the potential biological activities

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and toxicities of such products, and indicate the need for more specific tests. Preliminary tests are excellent tools in studies with medicinal plants, and should be implemented as they contribute to decreasing the use of experimental animals, which has been a concern of ethics committees in animal experimentation (Bednarczuk *et al.*, 2010).

Several biological activities have been reported for the EOs of *Baccharis* species, including anti-inflammatory (Florão *et al.*, 2012), insecticidal (Chaaban *et al.* 2017), antibacterial (Abad; Bermejo, 2007; Negreiros *et al.*, 2016; Pereira *et al.*, 2016), cytotoxic (Búfalo *et al.*, 2010; Pereira *et al.*, 2017), and antiulcerogenic (Massignani *et al.*, 2009).

*Baccharis erioclada* DC., popularly known as “vassoura-lageana”, is a shrub with leaves that are sessile, pinnatinervate, and oblong, with an obtuse apex, attenuated base, and dentate margin above the middle of the leaf blade (Bobek *et al.*, 2015). As observed in other *Baccharis* species, the EO of *B. erioclada* is stored in secretory ducts and glandular trichomes (Bobek *et al.*, 2015; Budel *et al.*, 2018).

Biological activities are exerted by chemical components present in the EO (Barbosa, Filomeno, Teixeira, 2016). However, the composition of the EO may differ as a result of edaphic and environmental factors, volatile oil extraction methods, and storage conditions (Brooker, Kleinig, 2006; Lemos *et al.*, 2012).

Considering the differences in the chemical composition of volatile oils sourced from different locations and the biological activities of *Baccharis* species, the aims of this study were to characterize the EO composition of *B. erioclada* collected in Ponta Grossa, Paraná, Brazil, and to assess its antioxidant, antimicrobial, and hemolytic activities. To the best of our knowledge, there are no previous studies investigating the biological activities of *B. erioclada* EO.

## MATERIAL AND METHODS

### Plant material

The aerial parts (stems, leaves, and flowers) from *B. erioclada* DC. were collected in the region of

Campos Gerais, Ponta Grossa, Paraná, southern Brazil (coordinates: 25° 08' S and 50° 27' W) during the summer of 2013. Plant identification was performed by the botanist Dr Gustavo Heiden (Embrapa - RS), and voucher specimens (ICN 20412) were registered at the herbarium of the State University of Ponta Grossa.

### EO extraction

The EO was extracted from 100 g of dried aerial parts of *B. erioclada* that were ground using a knife mill and subjected to hydrodistillation in a modified Clevenger-type apparatus for 6 h. The EO was stored in a sealed amber jar glass at  $-18\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$  in the dark. EO yield was expressed as the percentage (volume/weight, v/w) of essential oil per 100 g of dried leaves (United States Pharmacopeia (USP), 2002).

### Gas chromatography-mass spectrometry (GC-MS) analysis

*B. erioclada* EO was analyzed via GC-MS using a Shimadzu GC-MS-QP 2010 Plus analyzer (Shimadzu Corp., Kyoto, Japan) equipped with a Rtx-5MS (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ) using splitless injection at 250  $^{\circ}\text{C}$ , and an ion source and interface at 300  $^{\circ}\text{C}$ . The mass range was  $m/z$  40 to  $m/z$  350, and helium was used as the carrier gas. Ramp injection temperature was set at 250  $^{\circ}\text{C}$ , the column pressure was 20 psi, starting at 50  $^{\circ}\text{C}$  for 5 min and increasing to 200  $^{\circ}\text{C}$  at a rate of 5  $^{\circ}\text{C}/\text{min}$ . Identification of EO components was based on the comparison of Kovats retention indices and mass spectra with those reported in the National Institute of Standards and Technology (NIST) library, as well as those described in the literature (Adams, 2007). Analysis was carried out at the Federal University of Paraná and results are listed in Table I.

**TABLE I** - Chemical compounds identified via gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil (EO) of *Baccharis erioclada*

Compounds*	Chemical class	RI	% RA	Identification <sup>c</sup>
Limonene	M	1029	0.17	RI, MS
trans-pinocarveol	OM	1139	1.29	RI, MS
Pinocarvone	OM	1164	0.71	RI, MS
Terpien-4-ol	OM	1177	0.33	RI, MS
$\alpha$ -Terpineol	OM	1188	0.50	RI, MS
Myrtenol	OM	1195	2.89	RI, MS
trans-Carveol	OM	1216	0.73	RI, MS
Carvone	OM	1243	0.97	RI, MS
$\alpha$ -Ylangene	S	1375	0.62	RI, MS
$\beta$ -Bourbonene	S	1388	0.79	RI, MS
(E)-Caryophyllene	S	1419	1.15	RI, MS
$\alpha$ -Humulene	S	1454	0.29	RI, MS
$\gamma$ -Gurjunene	S	1477	0.25	RI, MS
$\gamma$ -Himachalene	S	1482	0.55	RI, MS
$\alpha$ -Vetispirene	S	1490	0.44	RI, MS
Viridiflorene	S	1496	0.74	RI, MS
$\alpha$ -Muurolene	S	1500	0.63	RI, MS
Epizonarene	S	1501	0.18	RI, MS
$\delta$ -Cadinene	S	1523	1.56	RI, MS
$\alpha$ -Calacorene	S	1545	0.81	RI, MS
Palustrol	OS	1568	1.01	RI, MS
Dihydro-ar-turmerone	OS	1595	27.96	RI, MS
Fokienol	OS	1596	13.47	RI, MS
Ledol	OS	1602	9.78	RI, MS
Sesquithuriferol	OS	1605	2.16	RI, MS
1-epi-Cubenol	OS	1628	0.88	RI, MS
$\alpha$ -Cadinol	OS	1654	0.71	RI, MS
Gymnomitrol	OS	1660	2.63	RI, MS
$\alpha$ -Santalol (7)	OS	1675	5.35	RI, MS
Ishwarone	OS	1681	1.57	RI, MS
n-Tetracosane	AH	2400	0.48	RI, MS
<b>Total (identified)</b>			<b>81.60</b>	

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**TABLE I** - Chemical compounds identified via gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil (EO) of *Baccharis erioclada*

Compounds*	Chemical class	RI	% RA	Identification <sup>c</sup>
(M) Monoterpenoid hydrocarbon (1)			0.17%	
(OM) Oxygenated monoterpenoid (7)			7.42%	
(S) Sesquiterpenoid hydrocarbon (12)			8.01%	
(OS) Oxygenated sesquiterpenoid (10)			65.52%	
(AH) Alkane hydrocarbon (1)			0.48%	

\*Name of compounds according to Adams, 2007. RI, retention index, relative to n-alkanes on capillary column; RA, relative area (peak area relative to the peak area), compared with RI reported in the literature; MS, mass spectra, compared with those reported in MS libraries).

## EO antioxidant activity

### Formation of the phosphomolybdenum complex method

The EO and standards (ascorbic acid and rutin) were diluted in methanol to a concentration of 200 µg/mL, and the method used was previously described by Prieto *et al.* (1999). The EO (300 µL) was diluted in 1 mL reagent solution (0.03 M ammonium molybdate, 0.1 M sodium phosphate, and 3 M sulfuric acid) and made up to 100 mL with distilled water. The tube was sealed and transferred to a water bath at 95 °C for 90 min. It was then cooled to room temperature (25 ± 30 °C), and absorbance was measured at 695 nm. The AA% relative to ascorbic acid was calculated using the following formula:

$$\text{AA\% compared to ascorbic acid} = \frac{[(A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}}) - (A_{\text{blank}})] \times 100}{1}$$

Where  $A_{\text{sample}}$  is the absorbance of the test compound,  $A_{\text{blank}}$  is the absorbance of the blank, and  $A_{\text{control}}$  is the absorbance of ascorbic acid.

### Reducing antioxidant power method

The reducing antioxidant power assay, also known as the Prussian blue assay, was performed in 25-mL test tubes containing 200 µg/mL *B. erioclada* EO. Potassium phosphate buffer (pH 7.0, 0.2 M) and 1.0% potassium

ferricyanide were added. The mixture was incubated at 45 °C for 20 min, before the addition of 1% trichloroacetic acid. Approximately 2.5 mL was transferred to 5-mL test tubes, and 1.5 mL distilled water, 1.0 mL ethanol, and 0.5 mL FeCl<sub>3</sub> were added adjust the concentration to 1.0% (w/v). Absorbance was then measured at 700 nm using a spectrophotometer (Yen, Chen, 1995; Morais *et al.*, 2006).

### Thiobarbituric acid reactive substances (TBARS) method

Antioxidant activity assessment was performed according to the method described by Morais *et al.* (2006). The following were added to a test tube: 0.1 mL of a 0.3% sample solution in ethanol, 0.4 mL water, 0.5 mL 5% (w/v) egg yolk solution previously prepared in 0.55% sodium dodecyl sulfate (SDS), 50 µL 0.035% 2,2'-azo-bis-2-amidinopropane chloride (ABAP), 1.5 mL 20% acetic acid (pH 3.5), and 1.5 mL 0.4% thiobarbituric acid (TBA) also prepared in 0.55% SDS solution. The tubes were kept in a water bath at 95 °C for 1 h. After cooling the solution, 1.5 mL 1-butanol was added to extract the organic phase, and the tubes were centrifuged at 3000 rpm for 5 min. Absorbance of the supernatant was measured at 532 nm in triplicate using a spectrophotometer, and 1-butanol was used as a blank. The same solution was used as a positive control and the sample was replaced with 0.1 mL of 0.3% butylated hydroxytoluene (BHT) in ethanol. The same solution was used as a negative control and the sample

was replaced with 0.1 mL ethanol. The antioxidant index (IA) of the samples was calculated as a percentage (IA%) according to the following equation:

$$IA\% = [1 - (A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}}] \times 100$$

Where,  $A_{\text{sample}}$  = sample absorbance,  $A_{\text{blank}}$  = blank absorbance;  $A_{\text{control}}$  = control absorbance

### Antibacterial activity

All assays were performed in triplicate using the following strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), and the yeast fungus *Candida albicans* (ATCC 10231). The EO was prepared in 0.5% polysorbate 80 and filtered through a 0.22- $\mu\text{m}$  Millipore membrane (Merck Millipore, Burlington, MA, USA) to guarantee its sterility. Inoculation was performed by diluting three to four colonies of the isolated strains in saline solution to obtain a turbidity equivalent to 0.5 on the McFarland scale. The minimum inhibitory concentration (MIC) was determined via broth microdilution method (Lima, Luna, Santos, 2006; Santos *et al.*, 2007).

Tests were performed in a sterile, 6-well, “sensitive microtiter”, enzyme-linked immunosorbent assay (ELISA) plate containing Mueller Hinton broth. EO (20  $\mu\text{L}$ , 250-2000  $\mu\text{g}/\text{mL}$ ) was added to each well containing 170  $\mu\text{L}$  Mueller Hinton broth and 10  $\mu\text{L}$  microorganism suspension, to obtain a final volume of 200  $\mu\text{L}$  in each well. Controls included broth only, broth with bacteria, and broth with 10  $\text{mg}/\text{mL}$  chlorhexidine (Merthiolate®).

Plates were incubated at 35 °C for 24 h. Absorbance was measured at a wavelength of 650 nm using an ELISA plate reader. Results were expressed as MIC, representing the lowest concentration of the extract capable of preventing  $\geq 90\%$  microbial growth.

### In vitro hemolytic activity

This method was carried out following the protocol reported by Banerjee *et al.* (2008) with some modifications. Lamb blood was purchased from Newprov® and was lightly homogenized before

transferring 5 mL to a test tube for centrifugation for 5 min at 3000 rpm. The supernatant was discarded, and the remaining solution was washed with ice-cold phosphate-buffered saline (PBS). This process was repeated until the supernatant was completely clear. The erythrocyte pellet was diluted with PBS to obtain a 2% dilution. For the hemolysis test, the EO was used at concentrations of 100-1000  $\mu\text{g}/\text{mL}$  and the samples were diluted with 10% methanol and PBS. A solution comprising 200  $\mu\text{L}$  PBS in 200  $\mu\text{L}$  2% erythrocyte solution was used as a negative control, and the positive control comprised 200  $\mu\text{L}$  distilled water with 200  $\mu\text{L}$  2% erythrocyte solution. For the solvent control, 20  $\mu\text{L}$  methanol and 180  $\mu\text{L}$  PBS were added to 200  $\mu\text{L}$  2% erythrocyte solution.

This analysis was carried out in Eppendorf tubes containing samples and controls (200  $\mu\text{L}$ ) and 200  $\mu\text{L}$  2% erythrocyte solution. The tubes were manually homogenized via gentle shaking and incubated for 3 h at 37 °C. Subsequently, they were centrifuged at 3000 rpm for 5 min. The supernatant was transferred to a 96-well ELISA plate, and absorbance was measured at 540 nm. Duncan’s test (Duncan, 1955) was used to compare the means of the activity indices (IA%). Differences were considered statistically significant if  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Yield and chemical composition of the EO

In the present study, the chemical composition of the EOs and their biological activities were investigated. The EO from the aerial parts of *B. erioclada* had a light-yellow color, characteristic aroma, and lower density than water. The yield was 0.4%, relative to the weight of the dry material. The yield of EOs from *Baccharis* species is not high, and ranged from 0.17% for *B. megapotamica* Spreng. and *B. anomala* DC. (Budel *et al.*, 2012), to 0.5% for *B. articulata* (Lam.) Pers., and 0.3% for *B. oxyodonta* DC. (Agostini *et al.*, 2005).

The GC-MS analysis of EOs led to the identification of 31 different compounds (Table I), representing 81.60% of the total EO components of the aerial parts of *B. erioclada*. The principal class of compounds represented was the sesquiterpenoids, comprising oxygenated

sesquiterpenoids (62.52%) and sesquiterpenoid hydrocarbons (8.01%). These are also the principal compounds of the EOs of several *Baccharis* species (Lago *et al.*, 2008; Budel *et al.*, 2012; Bogo *et al.*, 2016; Campos *et al.*, 2016; Pereira *et al.*, 2016). However, Agostini *et al.* (2005) observed a predominance of monoterpenoids in the EO of *Baccharis uncinella*.

In the present study, turmerone (27.97%), fokienol (13.47%), ledol (9.78%), and santalol (5.35%) were the principal compounds identified in the EO of *B. erioclada*. However, a different chemical composition was identified for specimens of this species collected in Campos do Jordão, São Paulo (female/male):  $\beta$ -pinene (21.44%/1.16%), limonene (15.16%/2.68%),  $\beta$ -caryophyllene (4.21%/10.70%), and spathulenol (6.61%/12.57%; Ferracini *et al.*, 1995). Although the chemical composition is related to seasonal conditions and environmental influences (Heinzmann, Spitzer, Simões, 2017), the link between variations in the composition of the EO and different chemotypes of *B. erioclada* should be investigated.

In this context, a different chemical composition was identified in three samples of *B. milleflora* (Pereira *et al.*, 2016). Spathulenol was present in two samples (16.2% and 25.3%), and  $\beta$ -pinene was present in the third sample (34.2%). The principal compounds identified in the EO of *B. dracunculifolia* DC. and *B. uncinella* DC.,  $\alpha$ -pinene and E-nerolidol, were present at levels between 18.76-27.45% and 12.96-14.02%, respectively (Fabiane *et al.*, 2008). However, Boix *et al.* (2010) identified verbenone (10.1%), myrcene (10.2%), 1,8-cineol (10.4%), and camphor (25.2%) as the principal compounds of *B. dracunculifolia*. These differences in chemical composition reinforce the

importance of characterizing essential oils via GC-MS to establish a correlation between chemical composition and biological activities.

Furthermore, the EO of *B. erioclada* contains four major compounds in higher concentration than others, which are present only in trace amounts, and these compounds are fundamental for the pharmacological actions of other compounds (Bakkali *et al.*, 2008; Galindo *et al.*, 2010). The principal compound identified in the EO of *B. erioclada* was turmerone, which has shown antifungal activity against *Aspergillus flavus* (Ferreira *et al.*, 2013) and larvicidal activity against the malaria vector *Anopheles gambiae* (Ajaiyeoba *et al.*, 2008).

### EO antioxidant activity

It is important to investigate the antioxidant potential from EOs, as these compounds possess the ability to stabilize free radicals and other reactive oxygen species, which, when present in the organism, may lead to several cellular changes related to various diseases, including heart disease, cancer, diabetes, and Alzheimer's disease (Miguel, 2010; Li, Wang, Luo, 2012).

The effects of the antioxidant activity of the EO from the aerial parts of *B. erioclada* on reduction of the phosphomolybdenum complex, lipid peroxidation (TBARS), and reducing power (Prussian blue) were evaluated (Table II). However, few studies regarding the antioxidant activity of the EO of *Baccharis* species are available, in comparison to those involving extracts and fractions, thus highlighting the need to carry out these investigations.

**TABLE II** - Antioxidant activity of *Baccharis erioclada* determined via different methods

Sample	Phosphomolybdenum complex assay (%)	TBARS (%)	Reducing power (%)*
Essential oil	50.02 ± 1.31	20.26 ± 0.14	-
BHT	-	56.07 ± 0.17	88.57 ± 0.002
Rutin	36.15 ± 1.21	-	107.01 ± 0.03
Ascorbic acid	100 ± 0.18	-	90.03 ± 0.08

TBARS, thiobarbituric acid reactive substances; BHT, butylated hydroxytoluene. \*Total antioxidant activity is considered to be 100% in relation to the antioxidant potential of ascorbic acid and rutin.

Different techniques are used to determine the antioxidant activity of substances. Among these techniques, the phosphomolybdenum method is preferable as it provides information regarding total antioxidant capacity. It is based on the reduction of molybdenum (VI) to molybdenum (V) in the presence of certain substances with antioxidant properties, leading to the formation of a green complex comprising phosphate/molybdenum (V) (Prieto, Pineda, Aguilar, 1999). The EO of *B. erioclada* displayed 50.02% antioxidant activity, and was superior to that of rutin which was used as a standard. Pereira *et al.* (2017) evaluated *B. milleflora* EO samples during different seasons throughout the year using the phosphomolybdenum method and showed that samples collected in autumn and winter exhibited 79.81% and 79.1% antioxidant activity.

The antioxidant capacity of a compound may also be evaluated by its ability to inhibit lipid peroxidation by quantifying the formation of malondialdehyde, which then reacts with thiobarbituric acid to generate a derivative that can be measured spectrophotometrically (Morais, 2006). The sample displayed 20.26% inhibition of lipid peroxidation, which is lower than that exhibited by BHT (56.07%). In the test performed on *B. milleflora* EO, an antioxidant activity of 29.06% was observed for the sample collected in winter. This activity was superior to that shown by the standard BHT (26.42%). The sample collected in autumn showed an antioxidant IA close to that of BHT (25.91%; Pereira *et al.*, 2016).

Evaluation of the reducing power is based on the ability of phenolic compounds to reduce  $\text{Fe}^{3+}$ , with the consequent formation of a colored complex with  $\text{Fe}^{2+}$ . The ferricyanide ion is reduced to ferrocyanide, which, in the presence of the ferric ion (from  $\text{FeCl}_3$ ), forms the Prussian blue complex  $\text{Fe}_4[\text{Fe}(\text{Cn})_6]_3$  (Yen, Chen, 1995; Jayanthi, Lalitha, 2011). The EO did not demonstrate any antioxidant activity at the concentration tested and no trials involving the use of this technique on other *Baccharis* species were reported in the literature. However, ethanolic extracts of *Calendula officinalis* L, which is also part of the Asteraceae family, exhibited low reductive capacity in relation to routine commercial measurements varying from 4.38 to 9.06% (Santos *et al.*, 2015).

Considering the results from the different assays performed, we concluded that the EO of *B. erioclada* shows antioxidant activity at the tested concentrations. Because EOs are complex mixtures, antioxidant capacity may result from the presence of antioxidant compounds or synergism between these compounds. Antioxidant compounds exert beneficial effects because of their ability to prevent oxidative damage, thus preventing the progression of various diseases.

### Antimicrobial activity

A large number of studies on the antimicrobial activity of *Baccharis* species have been performed (Kurdelas *et al.*, 2012; Campos *et al.*, 2016). The EO of *B. erioclada* contains constituents that may be considered potent antimicrobial agents. The MICs of the EO were 1000  $\mu\text{g}/\text{mL}$  in both *E. coli* and *C. albicans*, and >2000  $\mu\text{g}/\text{mL}$  in *P. aeruginosa* and *S. aureus*. In a study by Kurdelas *et al.* (2012) assessing the EO of *Baccharis darwinii*, MICs were 1000  $\mu\text{g}/\text{mL}$  in *E. coli*, *Yersinia enterocolitica*, and *Salmonella enterica*.

The EO of *B. uncinella* was inactive against all bacteria tested, and that of *Baccharis semiserrata* DC. showed moderate activity against *S. aureus* (Vannini *et al.*, 2012).

Ferronato *et al.* (2007) demonstrated that the EOs from *B. uncinella* and *B. dracunculifolia* were active against *S. aureus*, *E. coli*, and *P. aeruginosa*. Zapata *et al.* (2010) showed that the EO of *Baccharis latifolia* (Ruiz & Pav.) Pers. was active against *Aspergillus fumigatus* (MIC = 157.4  $\text{mg}/\text{mL}$ ). In a study by Parreira *et al.* (2010), the EO from *B. dracunculifolia* showed no activity against yeasts belonging to the genus *Candida*.

### In vitro hemolytic activity

The use of plants by the general population, and the interest of industries and research institutes have shown a remarkable increase in recent years. Toxicological screening of plant species is therefore necessary. *In vitro* toxicology studies are useful in screening plants that have toxic effects, reduce costs, provide rapid responses, and contribute to replacement, reduction, and refinement. *In vitro* and/or

alternative tests allow the preliminary identification of plants with potential toxic effects and the reduction of experimental animals (Bednarczuk *et al.* 2010).

The hemolytic activity of the *B. erioclada* EO was evaluated in sheep erythrocytes. The concentrations studied ranged from 1000 to 75 µg/mL, no direct or

inversely proportional relationship was observed between the increase in concentrations and hemolytic activity. This observation may be explained by the synergism of the compounds present in the oil, which may be more or less active, depending on the concentration. The hemolytic potential of the EO is listed in Table III.

**TABLE III** - Evaluation of the hemolytic activity of the essential oil (EO) of *Baccharis erioclada*

Sample	Concentration (µg/mL)	Hemolysis (%)
Control	-	100 <sup>a</sup> ± 0.215
	75	10.93 <sup>e</sup> ± 0.0043
Essential oil	100	32.97 <sup>d</sup> ± 0.0071
	250	9.8 <sup>ef</sup> ± 0.004
	500	9.26 <sup>f</sup> ± 0.0035
	750	44.68 <sup>c</sup> ± 0.037
	1000	70.34 <sup>c</sup> ± 0.003

Note: same letters do not differ statistically.

*In vitro* hemolytic activity may be considered to be a good toxicity screening test for extracts and plant fractions, as by evaluating the mechanical stability of the sheep erythrocyte membrane, we can characterize the damage that a compound may cause (hemolysis) and correlate the toxicity of extracts or fractions with potential therapeutic activity (Zohra, Fawzia, 2014).

## CONCLUSION

The yield of the EO of *B. erioclada* obtained was 0.4%, and was composed of 31 compounds. The oxygenated sesquiterpenes were the main class of components, and turmerone, fokienol, ledol, and santalol were the principal compounds identified. The phosphomolybdenum method revealed that the antioxidant activity of the EO of *B. erioclada* was higher than that of the standard rutin, and a reducing antioxidant power assay further showed the EO's excellent activity. Moderate antimicrobial activity and hemolytic potential were also

observed. This study contributes to the enrichment of the database concerning the specie *B. erioclada* EO and your biological activities and antioxidant properties.

## ACKNOWLEDGEMENTS

The authors would like to thank CAPES for financial support and the Department of Chemistry of the Federal University of Paraná, Brazil, for their assistance in GC-MS analysis.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

Abad MJ, Bermejo P. *Baccharis* (Compositae): a review update. *Caldasia*. 2007;7:76-96.



- Adams RP. Identification of essential oils components by gas chromatography mass spectroscopy. 4.ed. Illinois: Allured Publishing Corporation. Carol Stream. 2007.
- Agostini F, Santos ACA, Rossato M, Pansera MR, Zattera F, Wasum R, et al. Estudo do óleo essencial de algumas espécies do gênero *Baccharis* (Asteraceae) do sul do Brasil. *Rev Bras Farmacogn*. 2005;15(3):215–220.
- Ajaiyeoba EO, Sama W, Essien EE, Olayemi JO, Ekundayo O, Walker TM, et al. Larvicidal activity of turmerone-rich essential oils of *Curcuma longa* leaf and rhizome from Nigeria on *Anopheles gambiae*. *Pharm Biol*. 2008;46(4):279-282.
- Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oil: a review. *Food Chem Toxicol*. 2008;46(2):446-75.
- Banerjee A, Kunwar A, Mishra B, Priyadarsini KI. Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of red blood cells. *Chem-Biol Interact*. 2008;174(2):134-139.
- Barbosa LCA, Filomeno CA, Teixeira RR. Chemical variability and biological activities of *Eucalyptus* spp. volatile oils. *Molecules*. 2016;21(12):1671.
- Bednarczuk VO, Verdum MCS, Miguel MD, Miguel OG. Testes in vitro e in vivo utilizados na triagem toxicológica de produtos naturais. *Visão acadêmica*. 2010;11(2):43-50.
- Bobek VB, Almeida VP, Pereira CB, Heiden G, Duarte MR, Budel JM, et al. Comparative pharmacobotanical analysis of *Baccharis caprariifolia* DC. and *B. erioclada* DC. from Campos Gerais, Paraná, Southern Brazil. *Lat Am J Pharm*. 2015;34(7):1396-402.
- Bogo CA, Andrade MH, Paula JP, Farago PV, Doll-Boscardin PM, Budel JM. Comparative analysis of essential oils of *Baccharis* L.: a review. *Rev Stricto Sensu*. 2016;1(2):1-11.
- Boix YF, Victório CP, Lage CLS, Kuster RM. Volatile compounds from *Rosmarinus officinalis* L. and *Baccharis dracunculifolia* DC. growing in southeast coast of Brazil. *Quim Nova*. 2010;33(2):255-257.
- BRASIL. Ministério da Saúde. Secretaria de Vigilância Sanitária. Portaria n. 116, de 8.8.1996. Diário Oficial da República Federativa do Brasil, 12 ago 1996.
- Brooker MIH, Kleinig DA. Field guide to Eucalyptus (3rd ed.), Vol. 1. Melbourne: Blooming; 2006.
- Budel JM, Duarte MR, Doll-Boscardin PM, Farago PV, Matzenbacher NI, Sartoratto A, et al. Composition of essential oils and secretory structures of *Baccharis anomala*, *B. megapotamica* and *B. ochracea*. *J Essent Oil Res*. 2012;24(1):19-24.
- Budel JM, Raman V, Monteiro LM, Almeida VP de, Bobek VB, Heiden G, et al. Foliar anatomy and microscopy of six Brazilian species of *Baccharis* (Asteraceae). *Microsc Res Tech*. 2018;81(8):832-842.
- Búfalo MC, Candeias JM, Sousa JP, Bastos JK, Sforcin JM. In vitro cytotoxic activity of *Baccharis dracunculifolia* and propolis against HEp-2 cells. *Nat Prod Res*. 2010;24(18):1710-1718.
- Campos FR, Bressan J, Jasinski VCG, Zuccolotto T, Silva LE, Cerqueira, LB. *Baccharis* (Asteraceae): Chemical constituents and biological activities. *Chem Biodivers*. 2016;13(1):1-17.
- Chaaban A, Martins CEN, Bretanha LC, Micke GA, Carrer AR, Ferreira L, et al. Insecticide activity of *Baccharis dracunculifolia* essential oil against *Cochliomyia macellaria* (Diptera: Calliphoridae). *Nat Prod Res*. 2017;32(24):2954-2958.
- Duncan DB. Multiple range and multiple F tests. *Biometrics*. 1955;11:1-42.
- Fabiane KC, Ferronato R, Santos AC, Onofre SB. Physicochemical characteristics of the essential oils of *Baccharis dracunculifolia* and *Baccharis uncinella* DC. (Asteraceae). *Rev Bras Farmacogn*. 2008;18(2):197-203.
- Ferracini VL, Paraiba LC, Leitão-Filho HF, Silva AG, Nascimento LR, Marsaioli AJ. Essential oils of seven Brazilian *Baccharis* species. *J Essen Oil Res*. 1995;7(4):355-367.
- Ferreira FD, Kimmelmeier C, Arrotéia CC, da Costa CL, Mallmann CA, Janeiro V, et al. Inhibitory effect of the essential oil of *Curcuma longa* L. and curcumin on aflatoxin production by *Aspergillus flavus* Link. *Food Chem*. 2013;136(2):789-793.
- Ferronato R, Marchesan ED, Pezenti E, Bednarski F, Onofre SB. Atividade antimicrobiana de óleos essenciais produzidos por *Baccharis dracunculifolia* DC. e *Baccharis uncinella* DC. (Asteraceae). *Rev Bras Farmacogn*. 2007;17(2):224-230.
- Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro. [Cited 2018 June 6]. Available from: <http://floradobrasil.jbrj.gov.br>.
- Florão FA, Budel JM, Duarte MR, Marcondes A, Rodrigues RAF, Rodrigues MVN, et al. Essential oil from *Baccharis* species (Asteraceae) have anti-inflammatory effects for human cells. *J Essent Oil Res*. 2012;24(6):561-570.
- Galindo LA, Pultrini AM, Costa M. Biological effects of *Ocimum gratissimum* L. are due to synergic action among multiple compounds present in essential oil. *J Nat Med*. 2010;64(4):436-441.
- Heinzmann BM, Spitzer V, Simões CMO. Óleos essenciais. In: Simões CMO, Schenkel EP, Mello JCP, Mentz LA,

- Petrovick PR, editors. *Farmacognosia: do produto natural ao medicamento*. Porto Alegre: Artmed; 2017.
- Jayanthi P, Lalitha P. Reducing power of the solvent extracts of *Eichhorniacrassipes* (mart.) Solms. *Int J Pharm Pharm Sci*. 2011;3(3):126-128.
- Kurdelas RR, Kurdelasa RR, Sandra López S, Lima B, Feresin GE, Zygadloc J, et al. Chemical composition, anti-insect and antimicrobial activity of *Baccharis darwinii* essential oil from Argentina, Patagonia. *Ind Crops Prod*. 2012;40:261-267.
- Lago JHG, Romoff P, Fávero AO, Soares MG, Baraldi PT, Corrêa AG, et al. Composição química dos óleos essenciais das folhas de seis espécies do gênero *Baccharis* de “Campos de Altitude” da mata atlântica paulista. *Quím Nova*. 2008;31(4):727-730.
- Lemos DRH, Melo EC, Rocha RP, Barbosa LCA, Pinheiro AL. Influence of drying air temperature on the chemical composition of the volatile oil of melaleuca. *Eng Agric*. 2012;20(1):5-11.
- Li X, Wang W, Luo M. Solvent-free microwave extraction of essential oil from *Dryopteris fragrans* and evaluation of antioxidant activity. *Food Chem*. 2012;133(2):427-444.
- Lima MRF, Luna JS, Santos AF, Andrade, MCC, San't Ana, AEG, Genet, JP. Anti-bacterial activity of some Brazilian medicinal plants. *J Ethnopharmacol*. 2006;105:137-14.
- Maciel MAM, Pinto AC, Veiga JRVV, Grynberg NF, Echvarria A. Plantas medicinais: A necessidade de estudos multidisciplinares. *Quím Nova*. 2002;25(3):429-438.
- Massignani JJ, Lemos M, Maistro EL, Schaphauser HP, Jorge RF, Sousa JP, et al. Antiulcerogenic activity of the essential oil of *Baccharis dracunculifolia* on different experimental models in rats. *Phytother Res*. 2009;23:1355-60.
- Miguel, M. G. Antioxidant and anti-inflammatory activities of essential oils: a short review. *Molecules*. 2010;15(12):9252-9287.
- Morais SM, Catunda Júnior FEA, Silva ARA, Martins Neto JS. Atividade antioxidante de óleos essenciais de espécies de *Croton* do nordeste do Brasil. *Quím Nova*. 2006;29(5):907-10.
- Negreiros MO, Pawlowski A, Zini CA, Soares GL, Motta AS, Frazzon AP. Antimicrobial and antibiofilm activity of *Baccharis psiadioides* essential oil against antibiotic-resistant *Enterococcus faecalis* strains. *Pharm Biol*. 2016;54(12):3272-3279.
- Parreira NA, Magalhães LG, Morais, DR, Caixeta, SC, Sousa JP, Bastos JK, et al. Antiprotozoal, schistosomicidal, and antimicrobial activities of the essential oil from the leaves of *Baccharis dracunculifolia*. *Chem Biodivers*. 2010;7(4):993-1001.
- Pereira CB, Farago PV, Borsato DM, Folquitto DG, Maia BHLNS, Esmerino LA, et al. Chemical composition and biological activities of *Baccharis milleflora* essential oil. *Lat. Am J Pharm*. 2016;35(10):2225-2233.
- Pereira CB, Kanunfre CC, Farago PV, Borsato DM, Budel JM, de Noronha Sales Maia BHL, et al. Cytotoxic mechanism of *Baccharis milleflora* (Less.) DC. essential oil. *Toxicol In Vitro*. 2017;42:214-221.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999;269:337-341.
- Santos LMO, Oliveira LA, Tibulo EPS, Lima CP. Análise de amostras de flores de Calêndula (*Calendula officinalis* L., Asteraceae) comercializadas na grande Curitiba. *Rev Ciênc Farm Básica Apl*. 2015;36:251-258.
- Santos SC, Ferreira FS, Rossi-Alva JC, Fernandez LG. Atividade antimicrobiana in vitro do extrato de *Abarema cochliocarpos* (Gomes) Barneby & Grimes. *Rev Bras Farmacogn*. 2007;17(2):215-219.
- Sonaglio D, González Ortega G, Petrovick PR, Bassani VL. *Farmacognosia – da planta ao medicamento*. Capítulo 13: Desenvolvimento Tecnológico e Produção de Fitoterápicos. 6. ed. Editora da UFRGS. Editora da UFSC. 2007.
- The United States Pharmacopeia. 25th ed. Rockville: United States Pharmacopeial Convention; 2002.
- Vannini AB, Santos TG, Fleming LRP, Purnhagen LA, Lourenço ETB, Butzke M, et al. Chemical characterization and antimicrobial evaluation of the essential oils from *Baccharis uncinella* DC. and *Baccharis semiserrata* DC. (Asteraceae). *J Essent Oil Res*. 2012;24(6):547-554.
- Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem*. 1995;43(1):27-32.
- Zapata B, Duran C, Stashenko E, Betancur-Galvis L, Mesa-Arango AC. Actividad antimicrobica y citotóxica de aceites esenciales de plantas de la familia Asteraceae. *Rev Iberoam Micol*. 2010;27(2):101-103.
- Zohra M, Fawzia A. Hemolytic activity of different herbal extracts used in Algeria. *Int J Pharma Sci Res*. 2014;5(8):495-500.

Received for publication on 30<sup>th</sup> January 2019Accepted for publication on 12<sup>th</sup> June 2019