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, pinnatifid, 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, Teixeiro, 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 °C ± 0.5 °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 × 0.25 mm × 0.25 μm) using splitless injection at 250 °C, and an ion source and interface at 300 °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 °C, the column pressure was 20 psi, starting at 50 °C for 5 min and increasing to 200 °C at a rate of 5 °C/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 |
|-----------------------------|----------------|-----|------|----------------|
| 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         |
| α-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         |
| α-Ylangene                  | S              | 1375| 0.62 | RI, MS         |
| β-Bourbonene                | S              | 1388| 0.79 | RI, MS         |
| (E)-Caryophyllene           | S              | 1419| 1.15 | RI, MS         |
| α-Humulene                  | S              | 1454| 0.29 | RI, MS         |
| γ-Gurjunene                 | S              | 1477| 0.25 | RI, MS         |
| γ-Himachalene               | S              | 1482| 0.55 | RI, MS         |
| α-Vetispirene               | S              | 1490| 0.44 | RI, MS         |
| Viridiflorene               | S              | 1496| 0.74 | RI, MS         |
| α-Muurolene                 | S              | 1500| 0.63 | RI, MS         |
| Epizonarene                 | S              | 1501| 0.18 | RI, MS         |
| δ-Cadinene                  | S              | 1523| 1.56 | RI, MS         |
| α-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         |
| α-Cadinol                  | OS             | 1654| 0.71 | RI, MS         |
| Gymnomitrol                 | OS             | 1660| 2.63 | RI, MS         |
| α-Santalol (7)              | OS             | 1675| 5.35 | RI, MS         |
| Ishwarone                   | OS             | 1681| 1.57 | RI, MS         |
| n-Tetracosane               | AH             | 2400| 0.48 | RI, MS         |
| Total (identified)          |                |     | 81.60|                |

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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 |
|------------|----------------|----|------|----------------|
| (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:

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

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 FeCl₃ 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:

\[ \text{IA\%} = \left[ 1 - \left( \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right) \right] \times 100 \]

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-μm 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 μL, 250-2000 μg/mL) was added to each well containing 170 μL Mueller Hinton broth and 10 μL microorganism suspension, to obtain a final volume of 200 μL in each well. Controls included broth only, broth with bacteria, and broth with 10 μg/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 ≥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 μg/mL and the samples were diluted with 10% methanol and PBS. A solution comprising 200 μL PBS in 200 μL 2% erythrocyte solution was used as a negative control, and the positive control comprised 200 μL distilled water with 200 μL 2% erythrocyte solution. For the solvent control, 20 μL methanol and 180 μL PBS were added to 200 μL 2% erythrocyte solution. This analysis was carried out in Eppendorf tubes containing samples and controls (200 μL) and 200 μL 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): β-pinene (21.44%/1.16%), limonene (15.16%/2.68%), β-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 β-pinene was present in the third sample (34.2%). The principal compounds identified in the EO of B. dracunculifolia DC. and B. uncinella DC., α-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.

| 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        |

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

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 Fe³⁺, with the consequent formation of a colored complex with Fe²⁺. The ferricyanide ion is reduced to ferrocyanide, which, in the presence of the ferric ion (from FeCl₃), forms the Prussian blue complex Fe₄[Fe(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 μg/mL in both *E. coli* and *C. albicans*, and >2000 μg/mL in *P. aeruginosa* and *S. aureus*. In a study by Kurdelas et al. (2012) assessing the EO of *Baccharis darwinii*, MICs were 1000 μg/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).

Ferronatto 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 mg/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± 0.215        |
|              | 75                    | 10.93± 0.0043     |
|              | 100                   | 32.97± 0.0071     |
|              | 250                   | 9.8± 0.004        |
|              | 500                   | 9.26± 0.0035      |
|              | 750                   | 44.68± 0.037      |
| Essential oil| 1000                  | 70.34± 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.

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