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Essential oil from *Chenopodium ambrosioides* L.: secretory structures, antibacterial and antioxidant activities

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ABSTRACT. The aim of this study was to evaluate the antibacterial and antioxidant activities of essential oil from *Chenopodium ambrosioides* L. and to determine its secretory structures. The essential oil was extracted through the hydrodistillation technique using a modified Clevenger apparatus (2 hours) and chemically characterized by GC/MS and GC-FID. The antioxidant activity was determined by monitoring the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and by the oxidation of the β-carotene/linoleic acid system. The evaluation of antibacterial activity was performed by the agar cavity diffusion technique using the microorganisms *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* Cholerasuis. The characterization of trichomes was accomplished by Scanning Electron Microscopy (SEM) and histochemical tests with Nadi and Sudan IV reagents. The antioxidant activity demonstrated by the β-carotene/acid linoleic test, with IC_{50} = 455.7 μg mL^{-1}. This oil also presented antibacterial activity for both Gram-negative and Gram-positive bacteria. The minimal inhibitory concentration ranged 62.5 to 250 μL mL^{-1}. The presence of terpenes in the glandular trichomes was observed, suggesting that the essential oil is secreted by these structures.

Keywords: natural products, antioxidant assays, antimicrobial, trichomes.

Óleo essencial de *Chenopodium ambrosioides* L.: estruturas secretoras, atividade antibacteriana e antioxidante

RESUMO. Objetivou-se neste trabalho avaliar as atividades antibacteriana e antioxidante do óleo essencial de *Chenopodium ambrosioides* L. e determinar as suas estruturas secretoras. A extração do óleo essencial foi realizada pelo método de hidrodestilação, utilizando o aparelho de Clevenger modificado com duração de 2 horas, sendo, posteriormente, analisado por CG/EM e CG-DIC. A atividade antioxidante foi determinada monitorando-se a redução do radical livre DPPH (2,2-difenil-1-picril-hidrazila) e utilizando o ensaio de oxidação do sistema β-caroteno/ácido linoleico. A avaliação da atividade antibacteriana foi realizada por meio da técnica difusão cavidade em ágar, utilizando os micro-organismos *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* e *Salmonella* Cholerasuis. A caracterização dos tricomas foi realizada por Microscopia Eletrônica de Varredura (MEV) e pelos testes histoquímicos com os reagentes de Nadi e Sudan IV. A atividade antioxidante foi evidenciada pelo teste β-caroteno/ácido linoleico, com CI_{50} 455,7 μg mL^{-1}. O óleo também apresentou atividade antibacteriana tanto para bactérias Gram-negativas como para Gram-positivas. A concentração mínima inibitória do mesmo variou entre 62,5 e 250 μL mL^{-1}. Constatou-se a presença de compostos terpênicos nos tricomas glandulares, inferindo-se que o óleo essencial seja secretado nessas estruturas.

Palavras-chave: produtos naturais, ensaios antioxidantes, antimicrobiano, tricomas.

Introduction

Plant species produce primary metabolites responsible for the synthesis of cellulose, lignin, proteins, lipids and other substances important for the realization of its vital functions. They also produce secondary compounds that are not directly used for nourishment. Essential oils are products of the secondary metabolism of plants and are composed of complex mixtures of volatile substances. They perform certain functions such as self-defense, attraction, protection against water loss and an increase in leaf temperature, among others. Despite advances in the knowledge of the biological and pharmacological potential of essential oils, few studies are described in the literature on the morphology of the cell or organ involved in the production of these plant substances. In the food

industry, in addition to imparting flavor, essential oils have important antioxidant and antibacterial activities that may enhance their use. Thus, to promote food conservation, the use of these oils has been studied, leading to the development of techniques to minimize the negative effects of oxidizing radicals and pathogenic micro-organisms (Andrade et al., 2013; Pereira et al., 2008).

The species *Chenopodium ambrosioides* L. is a plant of the Chenopodiaceas family, popularly known in Brazil as 'erva-de-santa-maria', 'mentruz', 'mastruço' and its use is widespread throughout the country (Di Stasi & Hiruma-Lima, 1989). Studies report that the essential oil obtained from this species has antihelmintic activity (particularly against *Ascaris lumbricoides*), antifungal activity (Prasad, Shukla, Kumar, & Dubey, 2010), activity against Leishmanial (Monzote, García, Montalvo, Linares & Scull, 2009; Monzote, Nance, García, Scull & Setzer, 2011) and acaricide activity (Chiasson, Bostanian, & Vincent, 2004). In the present study, the essential oil from *Chenopodium ambrosioides* was extracted to determine the secretory structures and to evaluate the biological potential as an antioxidant and antibacterial agent.

Material and methods

The *C. ambrosioides* leaves were collected in the Garden of Medicinal Plants in Lavras, Minas Gerais State. The taxonomic identification of the species was performed in the ESAL Herbarium by Professor Mariana Mansanares, and a voucher specimen was incorporated into the collection of the Herbarium under registration number 26769.

The essential oil was extracted from the leaves by hydrodistillation for two hours using a modified Clevenger apparatus coupled to a 5 L round bottom flask. The hydrolact was separated by centrifugation in a horizontal crosshead centrifuge at 1100 *g* for 5 min. The essential oil was removed with the aid of a Pasteur pipette and transferred to a glass vial, which was wrapped with aluminum foil and stored under refrigeration (Agência Nacional de Vigilância Sanitária [ANVISA], 2010).

Chemical analysis of the essential oil

Identification of constituents of the essential oils

The GC-MS analyses were performed on a Perkin Elmer Autosystem XL gas chromatograph equipped with a fused silica column (30 m x 0.25 mm ID, DB-1 film thickness, 0.25 mM; J & W Scientific Inc.) coupled to a Perkin Elmer TurboMass mass spectrometer (software version 4.1). The oven temperature was programmed from 45 to 175°C at a rate of 3°C min^{-1}, and, subsequently, 15°C min^{-1} to 300°C, where the temperature was held constant for 10 min. The transfer line temperature was 280°C, the temperature of the ionization chamber was 220°C, and the carrier gas was helium at a linear velocity of 30 cm s^{-1}. The split ratio was 1:40.

The identities of the compounds were determined by comparison of their retention indices with those of the C_9-C_{21} n-alkanes and by comparing the mass spectra with those of standard commercial and reference compounds present in existing oils in the laboratory, as well as by comparison with a mass spectral library developed at the laboratory of the *Centro de Biotecnologia Vegetal, Faculdade de Ciências, Universidade de Lisboa* (Mendes et al., 2011).

Quantification of constituents of essential oils

The essential oils were analyzed by gas-liquid chromatography on a Perkin Elmer 8700 gas chromatograph equipped with two flame ionization detectors, a system for processing data and an autoinjector. Two columns of different polarity were installed with the following characteristics: DB-1 methyl silicone immobilized phase in a fused silica column (30 m x 0.25 mm ID, film thickness 0.25 mm; J & W Scientific Inc.); DB-17HT phenylmethylsilicone stationary phase (30 m x 0.25 mm id; film thickness 0.25 mm). The oven temperature was programmed from 45 to 175°C at a rate of 3°C $min.^{-1}$, and, subsequently, 15°C $min.^{-1}$ to 300°C, where the temperature was maintained for 10 min. The temperature of the injector and detector ports were 290 and 280°C respectively. The carrier gas was hydrogen, adjusted to a linear velocity of 30 cm s^{-1}. The split ratio was 1:50. The percentage composition of the oils was determined by integration of peak areas without using correction factors. The values given represent the average of two injections (Mendes et al., 2011).

Morphological and histochemical analyses

Fresh leaves from the fourth node of the *C. ambrosioides* L. plant were washed under running water and cut into 0.5 x 0.5 cm squares. The materials were immersed in modified Karnovisky solution (2.5% glutaraldehyde and 2.5% formaldehyde in 0.05 M sodium cacodylate buffer with 0.001M $CaCl_2$) (Karnovsky, 1965), pH 7.2, for a period of 24 hours. The materials were washed three times in 0.05 M cacodylate buffer during 10 minutes for each wash. They were then transferred

on a solution of 1.0% osmium tetroxide (OsO_4) for a period of one hour.

Subsequently, the materials were washed three times with distilled water, once each with aqueous acetone in concentrations of 25, 50, 75, and 90% and three times with 100% acetone (10 minutes for each treatment). After dehydration, the acetone was replaced by CO_2 in the Balzers CPD 030 critical point apparatus, and the drying was complete (Robards, 1978). The specimens were mounted on stubs on a sheet of aluminum foil with carbon tape, covered with gold in a Balzers SCD 050 evaporator and observed in a Scanning Electron Microscope.

The plant material was collected for histochemical analysis and stored in 70% ethanol solution. The material was subjected to cross sections with the aid of a model LPC table microtome, and the observations were performed in an Olympus model CX31 photonic microscope coupled to a Moticam 2500 digital camera.

Cross sections of the material were transferred to 70% ethanol solution for detection of lipid compounds and stained with Sudan IV. The sections were washed in 70% ethanol and mounted between slide and coverslip with 50% glycerol (Gerlach, 1984). The slides were immediately photographed. A red stain indicated a positive reaction for lipids.

Sections were transferred to freshly prepared NADI reagent for a period of one hour at room temperature in the dark for detection of terpenoid compounds. The material was washed in 0.1 M sodium phosphate buffer, pH 7.2, for two minutes (David & Carde, 1964). Subsequently, the sections were mounted on semi-permanent slides with glicerinized water and observed under a microscope. The terpene compounds stained blue and resins stained dark red. The NADI (Figure 1) reagent consisted of a mixture of naphthol and α-dimethyl-ρ-phenylenediamine hydrochloride, which forms the blue indophenol dye upon oxidation (Figueiredo, Barroso, Pedro, & Pedroso, 2007).

Antioxidant activity

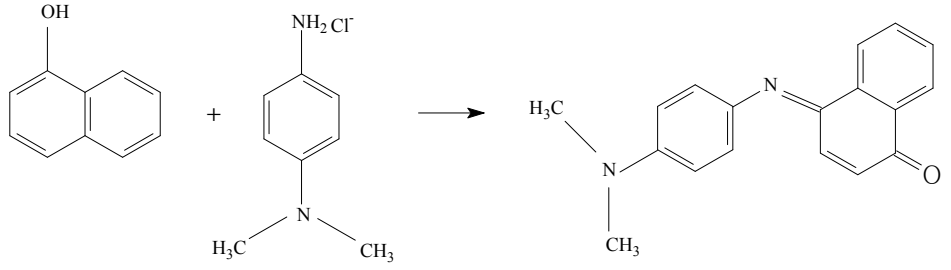
β-Carotene–linoleic acid assay

Evaluating the oxidation of the β-carotene/linoleic acid system, a solution of β-carotene in chloroform was prepared. It was added 60 µL of linoleic acid, 600 mg Tween 20 and 1.5 mL of chloroform, and the chloroform was evaporated on a rotary vacuum evaporator (Rotavapor R Bücher 114). Subsequently, 150 mL of distilled water, previously saturated with oxygen, was added to the mixture (emulsion A). The emulsion A (2.8 mL) was added to test tubes, and 200 μL of the essential oil, at concentrations of 25, 50, 100, 150, 200, 250, 300 e 500 μg mL^{-1} in methanol, was added. In parallel, two control solutions were prepared: a blank contained emulsion A without the addition of β-carotene and the other contained 2.8 mL of the emulsion A and 0.2 mL of methanol (Wang, Wu, Zu & Fu, 2008; Lopes-Lutz, Alviano, Alviano & Kolodziejczyk, 2008).

The analyses were performed with three replications, and after incubation of the tubes at 50°C for 60 minutes for the oxidation reaction, the absorbance readings were performed on a Shimatzu UV-160 1PC spectrophotometer at a wavelength of 470 nm. The percentage of inhibition (percentage of antioxidant activity AA%) was calculated by the following formula:

$$AA\% = 100 \times (DC - DA) / DC$$

where AA is the antioxidant activity; DC is the degree of degradation of the control [ln (a / b) / 60]; DA is the degree of degradation in the presence of the sample [ln (a / b) / 60]; a is the absorbance at time 0; b is the final absorbance after 60 minutes of incubation. For comparison, the synthetic butylated hydroxytoluene (BHT) antioxidant was used at the same concentrations as those of the essential oil.



α- naphthol dimethyl-ρ-phenylenediamine hydrochloride blue indophenol dye

Figure 1. Formation reaction NADI reagent.
Source: Figueiredo et al. (2007).

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

The stock solution of DPPH in methanol (40 mg L^{-1}) was prepared. The essential oil was diluted in methanol to concentrations of 25, 50, 100, 150, 200, 250, 300 and 500 μg mL^{-1}; 2.7 mL of the solution of DPPH-stock solution was added to a test tube, followed by the addition of 0.3 mL of the dilutions of the essential oil. The blank contained 2.7 mL of methanol and 0.3 mL of the most concentrated dilution of the essential oil. The control contained 2.7 mL of the DPPH-stock solution and 0.3 mL of methanol. The analyses were performed in triplicate, and after 60 minutes (tubes wrapped to protect from the light), the absorbance readings were performed at 515 nm. The percent antioxidant activity was calculated according to the following equation:

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

where A_{sample} is the absorbance of the solution containing all of the reagents and $A_{control}$ is the absorbance of the control (solution containing all the reagents except the essential oil (Sousa et al., 2007). The synthetic antioxidant BHT was also used for comparison.

Data evaluation and statistical analysis

The IC_{50} values (concentration that presents 50% inhibition of oxidation) of the essential oil and the antioxidant compound used as reference (BHT) were calculated by regression analysis of the concentration of the essential oil with its antioxidant activity. This analysis was performed only for the essential oil or the compound that presented greater than 50% inhibition of oxidation in each method. A completely randomized design (CRD) was used. Data were subjected to analysis of variance and means were compared by the Tukey test at 5% probability using the statistical program Sisvar (Ferreira, 2011).

Antibacterial activity

The antibacterial activity of the essential oil was evaluated using the agar cavity diffusion test. Pure strains of *Staphylococcus aureus* ATCC 13565, *Listeria monocytogenes* ATCC 19117, *Escherichia coli* ATCC 11229, and *Salmonella* Cholerasuis ATCC 6539 were used.

Bacteria were subcultured in BHI broth and incubated at 37°C for 24 hours. Subsequently, aliquots of the medium were transferred to a tube containing 5 mL of tryptic soy broth (TSB). The tubes were incubated at 37°C until the turbidity reached that of a standard McFarland 0.5 solution, resulting in a suspension containing 10^8 CFU mL^{-1}. The turbidity readings were obtained on a Shimadzu UV-160 PC 1 spectrophotometer at a wavelength of 625 nm (National Committee for Clinical Laboratory Standards [NCCLS], 2003).

The inoculum concentration, obtained by the McFarland scale of 0.5 (10^8 CFU mL^{-1}), was diluted to 10^6 UFC mL^{-1} and transferred to TSA culture medium for the *Listeria monocytogenes* species and to Mueller-Hinton agar for the other species. The agar in which the bacterial culture was inoculated was deposited on a layer of agar to which sterile glass beads 4 mm in diameter were added to form wells in the culture medium. These wells were filled with 10 µL of the controls and the essential oil diluted in dimethylsulfoxide (DMSO) at concentrations of 0; 3.90; 7.81; 15.62; 31.25; 62.5; 125; 250 and 500 µL mL^{-1} (Pereira et al, 2008).

Three replicates were tested for each treatment, a relative control in which 10 μL of DMSO was applied, and, for comparison, a solution of the antibiotic Chloramphenicol (CL). Subsequently, the plates were incubated in BOD at 37°C for 24 hours, and diametrically opposite measurements of the inhibition halos were measured. From the diameters, the susceptibility profile of bacteria in different concentrations of essential oil could be evaluated. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of essential oil for which the presence of an inhibitory halo was observed (Andrade, Cardoso, Batista, Mallet, & Machado, 2012).

Results and Discussion

Chemical composition of the essential oil from *C. ambrosioides*

The percentages and retention indices of chemical constituents of the essential oil from *C. ambrosioides* are presented in Table 1. Monoterpenes hydrocarbons (62.8%) were the most abundant in the oil, while oxygenated monoterpenes were found in concentrations of 13.2%. The principal constituents found were α-terpinene, *p*-cymene and *trans*-ascaridole. The remaining components identified were found in percentages ≤0.5% (Santiago et al., 2014).

According to the literature, the differences at the chemical compositions of the essential oils in the same botanical species are probably due to the method of extraction, the region of origin of the plants and different climatic conditions (Gobbo-Neto & Lopes, 2007). Therefore, variations in the

percentage of analyzed compounds can be observed. The constituent ρ-cymene showed 21.8% of the essential oil. Chekem et al. (2010) studying the compounds of *C. ambrosioides* in vegetable material collect in Cameroon found 23.4% of ρ-cymene. Different results were also found by Singh, Batish, Kohli, Mittal, & Yadav (2008), in their study were found 25.77% ρ-cymene in the oils extracted of vegetable materials from India.

Table 1. Composition of the essential oil from *C. ambrosioides* L.

Compound	RI	Percent Composition (%)
Benzaldehyde	927	v
α-Pinene	930	v
n-Octanal	973	v
β-Myrcene	975	v
α-Terpinene	1002	40.73
ρ-Cymene	1003	21.81
β-Phellandrene	1005	v
Limonene	1009	0.24
trans-β-Ocimene	1027	v
γ-Terpinene	1035	v
n-Octanol	1045	v
Dimethyl styrene	1059	v
n-Nonanal	1073	v
cis-Piperitone epoxide	1211	0.34
trans-Piperitone epoxide	1258	0.35
trans-Ascaridole	-	12.49
Total identified		75.95%

* $RI_{cal.}$ = Calculated retention index, N. area= Normalization of the area, v= vestigial

In the present study, *trans*-ascaridole appeared at a concentration of 12.5% in the oil. Borges et al. (2012) found ascaridole (17.1%) in plants collected in northeastern Brazil. On the other hand, Degenhardt et al. (2016) studying the essential oil of *C. ambrosioides* from central-west region, Brazil, found higher concentrations of ascaridole, with percentages of 49.77%.

The results obtained for the chemical composition of the aerial parts from *C. ambrosioides* corroborate with the results found by Owolabi et al. (2009), which identified and quantified the compounds α-terpinene (63.1%), ρ-cymene (26.4%) and ascaridole (3.9%). According to Johnson e Croteau (1984), ascaridole is a sensitive heat component, which has a biosynthetic pathway related to the formation of α-terpinene. This fact can be associated with the high concentration of this component in the oil (40.7%) and low percentage of *trans*-ascaridole.

Glandular and non-glandular trichomes

The presence of vesicular glandular trichomes and non-glandular tector trichomes was observed in the surfaces of the leaf images obtained by SEM (Figure 2A and B). The structures were restricted to the abaxial side and were located in depressions located on the leaf lamina. The non-glandular tector trichomes (Figure 2B) have a tapered structure and are present in both abaxial and adaxial surfaces. However, the distribution of these trichomes proved variable because the adaxial trichomes are present in greater numbers than those seen on the abaxial surface. They usually form a dense cover and may serve as a mechanical barrier against various external factors such as herbivores and pathogens. They also protect the foliar surface from ultraviolet radiation, extreme heat and excessive water loss (Valkama, Salminen, Koricheva, & Pihlaja, 2003).

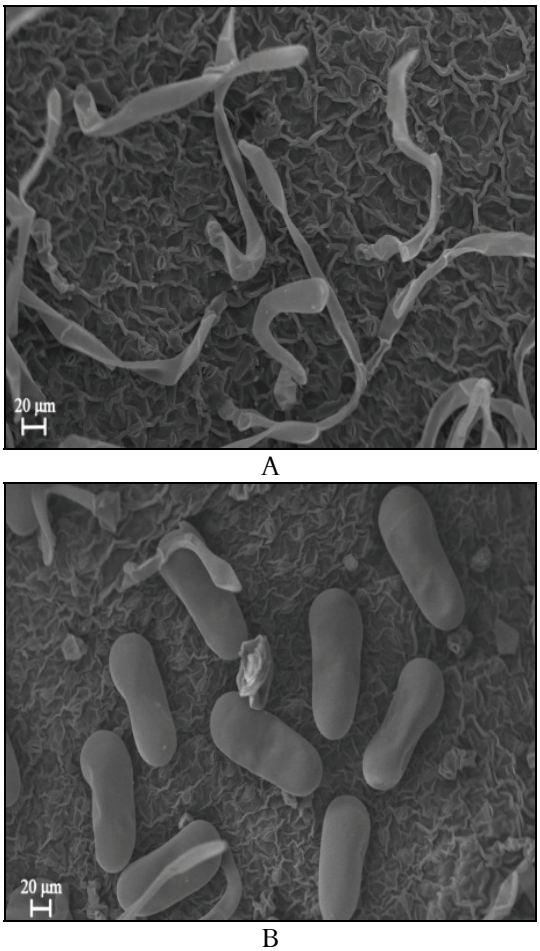


Figure 2. Scanning electron micrographs of adaxial and abaxial leaf surface of *Chenopodium ambrosioides* L. A- Glandular and non-glandular tector trichomes present on abaxial surface. B- Tector trichomes present on the adaxial surface.
Source: Author.

Costa and Tavares (2006) studied the leaf anatomy of *Chenopodium ambrosioides* and established the presence of non-glandular tector trichomes and vesicular glandular trichomes, as was observed in the present study. The non-glandular tector trichomes are multicellular and uniserated. The basal cell is rounded, and the terminal cell is elongated in the shape of a sickle and occurs on both sides. The vesicular glandular trichomes are present only on

the abaxial surface. They are composed of an elliptical basal cell, two or more flat intermediate cells in the periclinal direction and a glandular head.

Histochemical analysis of cross sections of leaf laminae revealed the presence of lipids within the glandular trichomes by means of positive reactions with the Sudan IV (red color) and Nadi (blue color) reagents (Figure 3). The presence of terpenes was observed, suggesting that the cellular secretory glandular trichomes are responsible for storing substances present in the essential oil of *C. ambrosioides*.

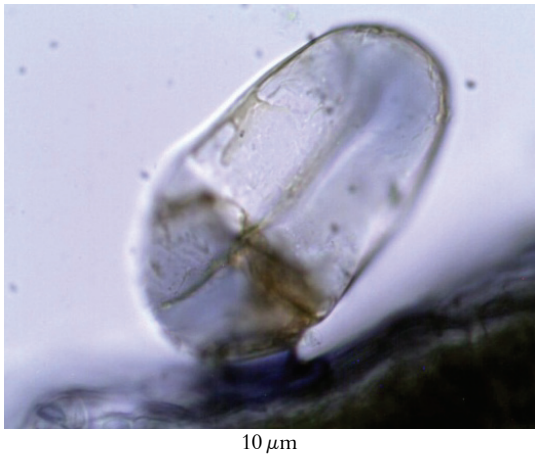


Figure 3. Histochemical characterization of this substance in the vesicular trichomes (cross section of leaf lamina from *Chenopodium ambrosioides* L.): glandular trichome with a positive reaction (blue color) with the Nadi reagent.
Source: Author.

Antioxidant activity

The antioxidant activities of the essential oil and the BHT standard corresponding to the concentrations evaluated with the β-carotene/linoleic acid system are presented in Table 2. The highest antioxidant activity was observed at an essential oil concentration of 500 μg mL^{-1}. The lowest antioxidant potentials for the essential oil and the standard were observed at the concentration of 25 μg mL^{-1}.

Table 2. Percentage of antioxidant activity (%AA) of the essential oil from *Chenopodium ambrosioides* L. and the BHT standard determined by the β-carotene/linoleic acid method.

Concentration (μg mL^{-1})	%AA *C. ambrosioides* L.	%AA BHT
25	6.02 fB	64.33 dA
50	11.16 eB	73.12 cA
100	20.74 dB	77.75 bA
150	20.76 dB	77.49 bA
200	32.95 cB	80.28 abA
250	37.69 bB	81.11 aA
300	40.17 bB	80.74 aA
500	51.23 aB	83.17 aA

Means followed by the same lowercase letter in the same column and the same capital letter in the same row do not differ by the Tukey test at 5% probability.

The antioxidant activities of the essential oil and the BHT standard observed in the test with the stable DPPH free radical are shown in Table 3. The highest percentages of capture of the DPPH radical were obtained with essential oil concentrations of 300 and 500 μg mL^{-1}, with no significant difference among the other concentrations. The highest activity for BHT was observed at 200 μg mL^{-1}. The synthetic antioxidant had a higher antioxidant potential than that of the essential oil. The IC_{50} observed for the antioxidant activity of the essential oil from *C. ambrosioides* L. in the β-carotene/linoleic acid test was 455.7 μg mL^{-1}. The IC_{50} observed for the antioxidant activity of the the synthetic antioxidant BHT was less than 25 μg mL^{-1}. It was not possible to calculate the IC_{50} for the essential oil in the DPPH method.

Table 3. Percentage of antioxidant activity (%AA) of the essential oil and the BHT standard determined by the scavenging of the DPPH radical.

Concentration (μg mL^{-1})	%AA *C. ambrosioides* L.	%AA BHT
25	3.37 bB	27.94 eA
50	4.06 bB	51.42 dA
100	4.59 dB	59.32 cA
150	4.71 dB	86.81 bA
200	4.73 cB	90.18 abA
250	4.98 bB	91.08 aA
300	11.94 aB	91.59 aA
500	15.79 aB	91.92 aA

Means followed by the same lowercase letter in the same column and the same capital letter in the same rows do not differ by the Tukey test at 5% probability.

Because of the complex composition of essential oils, it is often difficult to establish a relationship between the structures of the compounds and their antioxidant activities. Some reactions between the products present in the assay medium can occur, and the occurrence of synergism, additivity and antagonism between the minor components is possible. Other factors may also influence the antioxidant test, such as concentration, temperature, light, type of substrate and physical state of the system (Tomaino et al., 2005; Viuda-Martos, Navajas, Zapata, Fernández-López, & Pérez-Alvarez, 2010).

In general, according to the results, it can be said that the essential oil (rich in terpenes) showed better values for the antioxidant activity in the β-carotene/linoleic acid system oxidation assay. This technique can be especially useful for investigations of lipophilic antioxidants (Andrade et al., 2013).

Miranda, Cardoso, Batista, Rodrigues, & Figueiredo (2016) evaluated the antioxidant properties of essential oils of fresh leaves from *Coniza bonariensis*, *Parthenium hysterophorus*, *Tithonia diversifolia*, *Ambrosia polystachya*, *Hedychium coronarium* and *Baccharis dracunculifolia*, extracted by

hydrodistillation. It was observed essential oils from *C. bonariensis*, *B. dracunculifolia* and *A. polystachya* are rich in monoterpenes hydrocarbons compounds, with percentage of 86.6, 58.2 and 32.7%. Using β-carotene/linoleic acid methodology, these oils showed IC_{50} greater than the highest concentration evaluated, but, none of the essential oils assessed by DPPH radical sequestration presented significant activity.

The high values for the antioxidant activities presented by BHT and the low values presented by the essential oil can be explained by the fact that the reduction of DPPH occurs via transfer of a hydrogen atom or a mechanism of electron transfer from the antioxidant compound. In general, the constituents of the essential oil did not resemble the phenolic structure of BHT, which can donate the hydrogen atom of the hydroxyl group to form a phenol radical that can be stabilized by resonance with the double bonds of the aromatic ring (Hidalgo et al., 2009). Besides, terpenes show low solubility in the reaction medium of the assay, because this test utilizes methanol or ethanol as solvent. Thus, the fact that the essential oils studied did not show significant antioxidant activity can be explained; the oil is composed almost entirely of monoterpenes, especially monoterpenes hydrocarbons.

Colorado, Restrepo, and Delgado (2012) evaluated the chemical composition and antioxidant activity of the essential oil from *C. ambrosioides* from Colombia. The main constituents found were α-terpinene, *ρ*-cymene, *trans*-4-carene and ascaridole. Among others, estragole, thymol and carvacrol were present in small amounts. The essential oil presented antioxidant activity with 84.89% inhibition of the oxidation of the DPPH radical. This activity was attributed, among other things, to the presence of phenolic compounds such as estragole, carvacrol and thymol in the essential oil that they studied.

Antibacterial activity

The minimum inhibitory concentrations (MICs) of the essential oil from *C. ambrosioides* that inhibited the growth of the bacteria under study are listed in Table 4. The essential oil presented antibacterial activity at the concentrations evaluated, inhibiting the growth of Gram-positive and Gram-negative bacteria. The MIC for the bacterium *Listeria monocytogenes* (Gram-positive) was higher than those observed with the other bacteria. This fact differs from most studies, which report that Gram-positive bacteria are more sensitive to essential oils than the Gram-negative bacteria. In most cases, Gram-negative bacteria are less sensitive that Gram-positive bacteria, because cell wall of Gram-negative is rich in polysaccharides, with inhibits the penetration of antimicrobial substances (Burt, 2004).

Table 4. Minimum inhibitory concentrations of the essential oil from *Chenopodium ambrosioides* L. against the microrganisms *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* Cholerasuis.

Bacteria	Gram	MIC (μL mL^{-1})	DMSO	CF
S. aureus ATCC 13565	+	62.5	NI	TI
L. monocytogenes ATCC 19117	+	250	NI	TI
E. coli ATCC 11229	-	125	NI	TI
S. Cholerasuis ATCC 6539	-	125	NI	TI

NI= no inhibition; DMSO (dimethyllsulfoxide); CF (chloranphenicol), TI= total inhibition.

According to Valeriano, Piccoli, Cardoso, and Alves (2012), the moderate antibacterial activity determined by the agar diffusion method can be attributed to the low affinity between less polar components of the essential oil and the polar substrate (agar). Moreover, although the essential oils present difficulty in diffusing uniformly throughout the culture medium, its volatility contributes to the formation of inhibition zones, making this technique a valid method for the determination of antimicrobial activity (Lambert, Skandamis, Coote, & Nychas, 2001; Inouye, Uchida, Maruyama, Yamaguchi, & Abe, 2006).

Owolabi et al. (2009) evaluated the antibacterial activity of the essential oil from *C. ambrosioides* species grown in Nigeria. These species presented no antibacterial activity against Gram-positive bacteria such as *Bacillus cereus* and *Staphylococcus aureu*s or against Gram-negative bacteria such as *Escherichia coli*. It is noteworthy that a difference in the constitution or in the concentration of the constituents of the essential oil that they studied can directly influence the antimicrobial activity. It should be emphasized that, in addition to the classes to which the constituents belong, other factors must be taken into account in assigning the antibacterial activity, such as isomerism and the synergism between the components.

According to Bakkali, Averbeck, Averbeck, and Idaomar (2008) compounds hydrophobic, as such as monoterpenes hydrocarbons, will probably prefer the aqueous phase to move toward the membrane structures. The accumulation of lipophilic compounds into lipid bilayers may enhance their availability to the cell but may also cause toxicity problems.

Conclusion

Chenopodium ambrosioides L. possessed terpene compounds in glandular trichomes, suggesting that the essential oil is secreted by such structures. The antioxidant activity was best demonstrated by the β-carotene/linoleic acid test (IC_{50} = 455.7 mg L^{-1}). The essential oil presented antibacterial activity against both Gram-negative (*E. coli* and *S.* Cholerasuis) and Gram-positive bacteria (*S. aureus* and *L. monocytogenes*).

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