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Chemistry

Ageratum conyzoides, an invasive species with antioxidant and antifungal potential

Ageratum conyzoides, uma espécie invasiva com potencial antioxidante e antifúngica

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ABSTRACT

This study aimed to evaluate the chemical composition and the antioxidant and antifungal activities of the essential oil (EO) extracted from the aerial parts (leaves and flowers) of *Ageratum conyzoides* (Asteraceae). The EO was extracted by the hydrodistillation process (3h), and the compounds were identified by gas chromatography coupled to mass spectrometry (GC-MS). Antioxidant activity was performed by the β-carotene/linoleic acid co-oxidation system and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging and the iron reduction method (FRAP). The antifungal activity was performed by the broth microdilution (MIC) method using the strains *Rhizopus oryzae* ATCC 7560; *Aspergillus flavus* ATCC 1217; *Aspergillus ochraceus* ATCC 6787 and *Penicillium verrucosum* ATCC 7680. The results indicated a yield of 0.82 mg/kg. Sesquiterpenes hydrocarbons (33.28%) were the major class, and precocene I (48.19%), precocene II (7.38%) and β-caryophyllene (19.66%) were the major constituents. The co-oxidation system of β-carotene/linoleic acid showed 52.18% inhibition of oxidation in the concentration of 1.0 mg/mL. Of the four fungi evaluated, only *Aspergillus ochraceus* showed results, with a MIC of 1250 µL/mL, and the importance of finding activity on this fungus lies in the fact that it is a producer of ochratoxin A, infesting mainly green coffee beans. The results found open up new perspectives in valuing a species considered invasive.

Keywords: Precocenes; β-caryophyllene; *Aspergillus ochraceus*; Mentrast

RESUMO

Este estudo teve como objetivo avaliar a composição química e as atividades antioxidante e antifúngica do óleo essencial (OE) extraído das partes aéreas (folhas e flores) de *Ageratum conyzoides* (Asteraceae). O OE foi extraído pelo processo de hidrodestilação (3h), e os compostos identificados por cromatografia gasosa acoplada à espectrometria de massas (CG-EM). A atividade antioxidante foi avaliada pelo sistema de co-oxidação β-caroteno/ácido linoléico e pelo seqüestro de radicais livres 2,2-difenil-1-picril-hidrazil (DPPH) e pelo método de redução de ferro (FRAP). A atividade antifúngica foi realizada pelo método de microdiluição em caldo (MIC), utilizando as cepas *Rhizopus oryzae* ATCC 7560; *Aspergillus flavus* ATCC 1217; *Aspergillus ochraceus* ATCC 6787 e *Penicillium verrucosum* ATCC 7680. Os resultados indicaram um rendimento de 0,82 mg/kg. Hidrocarbonetos sesquiterpenos (33,28%) foram a classe majoritária e precoceno I (48,19%), precoceno II (7,38%) e β-cariofileno (19,66%) os constituintes majoritários. O sistema de co-oxidação β-caroteno/ácido linoléico apresentou 52,18% de inibição da oxidação na concentração de 1,0 mg/mL. Dos quatro fungos avaliados, apenas *Aspergillus ochraceus* apresentou resultado, com CIM de 1250 µL/mL, e a importância de encontrar atividade sobre esse fungo está no fato de ser produtor de ocratoxina A, infestando principalmente grãos de café verde. Os resultados encontrados abrem novas perspectivas na valorização de uma espécie considerada invasora.

Palavras-chave: Precocenos; β-cariofileno; *Aspergillus ochraceus*; Mentrasto

1 INTRODUCTION

Ageratum conyzoides L. belongs to the Asteraceae family. It is a species native to Central America and the Caribbean and distributed throughout the tropical and subtropical regions (Yadav et al., 2019; Karayat et al., 2024) which justifies the presence of 36 scientific synonyms (Powo, 2023). The common name, "goat weed" or "Billy goat weed", is derived from an Australian male goat due to a close resemblance in odor (Kaur et al., 2023).

In Brazil, it is popularly known as "mentrasto, catinga-de-bode, catinga-de-borrão, erva-de-são-joão, maria-preta, celestina, picão-roxo, erva-de-santa-luzia and camará-opela" (Dores et al., 2014). It is a very widespread weed in all agricultural regions of the country, infesting crops, vegetable gardens and vacant lots (Desai et al., 2024), being able to complete its life cycle in two months, even if its greatest propagation occurs in the summer (Sastry et al., 2019). The inflorescences last two months, have different colors (lilac and white) and undergo self-pollination, which produces about 94,000 seeds per plant (Dhami, 2018), justifying the rapid spread.

In the leaves, flowers and branches of this species are found coumarin, chromene, flavonoids (kaempferol, quercetin, quercetin-3-rhamnopyranoside), caffeic acid, phytol, echinatine (pyrrolizidine alkaloids), sterols (stigmasterol, β-sitosterol, and friedelin) (Satija et al., 2018; Erida et al., 2023). It also contains an essential oil composed of benzofurans (precocene I, precocene II, and ageratochromene dimer), terpenes (α-pinene, β-pinene) and phenylpropanoids (eugenol) possessing diverse herbicidal properties (Erida et al., 2023).

These phytoconstituents have shown diverse pharmacological properties including antimicrobial, anti-inflammatory, analgesic, antioxidant, anticancer, antiprotozoal, antidiabetic, spasmolytic, allelopathy (Yadav et al., 2019), hypoglycemic, analgesic, anti-diarrheal, diuretic, antitussive, antirheumatic (ChabI-Sika et al., 2023), anti-fungal, anti-bacterial, anti-ulcerogenic, anti-malarial, antioxidant, anti-protozoal, anti-Ehrlichia and anti-insecticidal activity (Kouame et al., 2018; Erida et al., 2023).

The pharmacological activities of this species justify its use in folk medicine to treat diseases in central Africa such as pneumonia and burns. The leaves or stems of this plant are utilized against inflammatory stomach or intestine diseases (Quoc & Pham, 2020). Additionally, *A. conyzoides* is used in traditional medicine in Asia, South America and Africa in healing wounds, in rheumatic states, and in fighting fever (Jomba & Kumar, 2023).

The chemical composition of plants is influenced by several external factors, including climate, as some compounds can be accumulated over a given period in response to environmental changes (Hussain et al., 2010). Variability in chemical composition remains a challenge to accurately determine whether these changes will have a positive or negative impact on the chemical constituents of plants (Dobhal et al., 2024). This change may constitute difficulty in the commercialization of essential oils, as they provide color changes, odor and mainly pharmacological changes (Linde et al., 2016), highlighting the antioxidant action, the focus of this research.

There is a lot of interest in discovering natural antioxidants from plants. According to studies on medicinal herbs, the majority of them have substantial antioxidant activity. The medicinal plants include a wide range of natural antioxidants and are used to cure and prevention a variety of diseases such as cancer, diabetes, atherosclerosis, heart disease, nephrotoxicity, hepatotoxicity, cognitive and vision loss (Makhammra et al., 2023).

Antioxidant compounds are essential for maintaining the balance of the organism, acting in the scavenging of free radicals produced in excess during the metabolic process, where they are important mediators of biochemical reactions (Rahaman et al., 2023). In some cases, the production of these molecules increases significantly, which can lead to oxidative stress (Barbosa et al., 2010). Exogenous types of antioxidants such as vitamins, flavonoids, anthocyanins, and some mineral compounds are derived from natural sources but also obtained in synthetic forms, like butylhoxyanisole (BHA), butylhydroxytoluene (BHT), and gallates which are primarily synthetic (Rahaman et al., 2023). The industry has widely used synthetic antioxidants such as butyl hydroxyanisole (BHA) and butyl hydroxytoluene (BHT). However, studies indicate possible toxicity and carcinogenic potential related to these substances (Oyetayo, 2009). In this sense, there is a growing interest in the investigation of antioxidants from natural sources such as extracts and essential oils from plants.

Some species of *Aspergillus* and *Penicillium* are associated with the production of mycotoxins. The main mycotoxin studied in coffee is ochratoxin A (OTA) and its presence has been reported to be associated with the presence of the fungus *A. ochraceus*. Fungi that produce mycotoxins are present in coffee plantations, preparation and storage environments and their relationship with the quality and safety of the final product depends on environmental conditions, crop management and post-harvest processing (Ferreira et al., 2011).

The need for new compounds to be used as therapeutic alternatives, such as those obtained from medicinal plants, can be considered powerful resources in the

development of antioxidant and antimicrobial agents (Rossato Viana et al., 2023). In this context, the objective of the present experiment consisted of the chemical analysis and determination of the antioxidant and antifungal activity of the essential oil of the mixture of flowers and leaves of *Ageratum conyzoides* cultivated in southern Brazil.

2 MATERIALS AND METHODS

2.1. Botanical identification

The culture of *A. conyzoides* is implanted in the Medicinal Garden of Universidade Paranaense – Unipar, located in the city of Umuarama, State of Paraná- Brazil at the coordinates (latitude 23 °C 45' 59" S, longitude 53 °C 19' 30" W and altitude of 442 m).

One specimen was authenticated and deposited in the herbarium of Universidade Educacional Paranaense (HEUP), under number 63. This species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number AD5B6A4.

2.2. Obtaining Plant Material and Essential Oil Extraction

Leaves and flowers were collected in June and July 2018 and 2019, in the morning (7:00-9:00 am). The average temperature in the months of the collection was 18.67 ± 0.71 ºC, where the air humidity remained at 70.69 ± 5.96 (%) and the average rainfall index was 1.46 mm in June and 0.03 mm in July.

The plant material was dried at room temperature. 150g of dry material was subjected to essential oil extraction, using a hydrodistillation process in a modified Clevenger apparatus for 3 hours (Ekundayo et al., 1987); afterwards, the essential oil was removed and stored at 4 °C (Pereira et al., 2016).

The essential oil yield (%) was calculated by the mass (g) of essential oil per mass (g) of dry aerial parts (leaves and flowers). Absolute density was determined in graduated capillaries (5.0 µL), determining the ratio between mass (g) and volume

(mL) of essential oil at 20 ºC. The refractive index was determined using an Abbe refractometer Q767B (model RL3) that was calibrated with distilled water (refractive index 1.3330) at 20 ºC (Farmacopeia Brasileira, 2010).

2.3. Chemical Characterization

The chemical identification of the essential oil was performed by GC-MS (Agilent 7890B-5977A MSD). The capillary column was HP-5MS IU 5% (30 m x 0.25 mm x 0.25 µm), with initial temperature of 80 °C and heating to 185 °C (2 °C min) maintained for 1 minute, followed by heating to 275 °C (9 °C min) maintained for 2 minutes and final heating to 300 °C (25 °C min) maintained for 1 min. Helium was used as carrier gas at a linear velocity of 1 mL/min. The injector temperature was 280 °C; the injection volume was 1 µL; the injection took place in Split mode (2:1). The transfer line was maintained at 280 °C, the ionization source and quadrupole at 230 °C and 150 °C, respectively. The EM detection system was used in "scan" mode, at a mass/charge ratio (m/z) of 40-600, with a "solvent delay" of 3 min. Compounds were identified by comparing mass spectra found in NIST 11.0 libraries and comparing retention indices (RI) obtained by a standard homologous series (C7-C28) (Adams, 2017).

2.4 Antioxidant activity

2.4.1 Determination of antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\cdot}$) scavenging assay

DPPH$^{\cdot}$ assays were performed according to Rufino et al. (2007). A 10 µL aliquot of essential oil from *A. conyzoides* leaves and flowers at different concentrations (1,00; 0,75; 0,50; 0,25; 0,125; 0,062; 0,03; 0,01 mg/mL) was added to 290 µL of methanolic DPPH$^{\cdot}$ solution (60 µM). The negative control was 10 µL of methanolic DPPH$^{\cdot}$ solution (60 µM). The mixtures were kept in the dark at room temperature for 30 min. The reduction in absorbance was measured at 515 nm by using a Spectra Max Plus 384

microplate reader. The total antioxidant capacity of essential oil was calculated by using a standard solution of quercetin (60 μM) as a 100% reference. The concentration required to scavenge 50% of free radicals (IC_{50}) was determined from absorbance versus sample concentration curves.

2.4.2 Determination of antioxidant activity by the β-carotene/linoleic acid co-oxidation assay

The ability of essential oil from *A. conyzoides* leaves and flowers to inhibit β-carotene/linoleic acid co-oxidation was assessed according to Rufino et al. (2006). To a beaker were added 20 μL of linoleic acid, 265 μL of Tween 40, 25 μL of β-carotene solution (20 mg/mL), and 0.5 mL of chloroform. The solvent was removed using a dryer. Then, the emulsion was dissolved in 20 mL of hydrogen peroxide. The antioxidant activity was determined by adding 280 μL of emulsion and 20 μL of essential oil at different concentrations (1.00, 0.75, 0.50, and 0.25 mg/mL), incubating the samples for 120 min, and measuring the absorbance at 470 nm. A Trolox solution was a used as control. The results are expressed as a percentage of oxidation inhibition, as given by Eqs. (1), (2), and (3).

$$A_{red} = A_i - A_f \quad (1)$$

$$O = [(A_{redsample} \times 100]/(A_{redsystem}) \quad (2)$$

$$I = 100 - (O) \quad (3)$$

Where A_{red} is the reduction in absorbance, A_i the initial absorbance, A_f the final absorbance, O the oxidation percentage, and I the inhibition percentage.

2.4.3 Determination of antioxidant activity by the ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the procedures described by Benzie and Strain (1996) and modified by Rufino et al. (2006). Briefly, the FRAP reagent was prepared by mixing 25 mL of acetate buffer (0.3 M), 2.5 mL of an aqueous solution of 2,4,6-Tris (2-pyridyl)-*s*-triazine (TPTZ, 10 mM), and 2.5 mL of an aqueous solution of ferric chloride (20 mM). Then, 10 µL of essential oil from *A. conyzoides* leaves and flowers at different concentrations (1.00, 0.75, 0.50, and 0.25 mg/mL) and 290 µL of FRAP reagent were added to the wells of a 96-well microplate. The microplate was placed on a Spectra Max Plus 384 reader, homogenized by vigorous shaking, and kept at 37 °C for 30 min. The change in absorbance was read at 595 nm. Antioxidant activity was calculated against a standard curve of ferrous sulfate (1000 µM).

2.5. Microbiological tests and fungal used

For this test, four fungal strains were used: *Rhizopus oryzae* ATCC 7560; *Aspergillus flavus* ATCC 1217; *Aspergillus ochraceus* ATCC 6787 and *Penicillium verrucosum* ATCC 7680.

2.5.1 Fungal Suspension

For each fungal sample, a standardized suspension was made from a 24-hour culture in Müeller Hinton broth (MHB) (DIFCO®). In a tube containing sterile saline (NaCl 0.85%), the fungal suspension was added drop by drop until a turbidity identical to that compared to the 0.5 tube on the McFarland scale ($BaSO_4$ suspension corresponding to 1.0 x 10^8 CFU/mL). Subsequently, a 1:10 dilution was performed in an eppendorf tube containing CMH, in order to obtain a bacterial suspension of 10^7 CFU/mL, whose inoculum was used in the assay to determine the (MIC) (NCCLS, 2002).

2.5.2 Sensitivity tests to determine the Minimum Inhibitory Concentration (MIC)

The antifungal activity of the essential oil of *A. conyzoides* was evaluated by the broth microdilution method (MIC), determining the minimum inhibitory concentration (MIC) of the essential oil for the strains. This methodology was performed according to the M27-A2 broth microdilution reference protocols (NCCLS, 2002).

The essential oil of *A. conyzoides* (40 mg/mL) was diluted in a 2% aqueous solution of polysorbate 80. In a 96-well plate, 95 µL of Müeller Hinton broth was added to each well; 100 µL of essential oil (dissolved in 2% polysorbate) and 5 µL of inoculum, resulting in a final volume of 200 µL in each well. Microplates were incubated at 37 °C for 24 h. As a positive control, Levofloxacin was used. MIC was defined as the lowest concentration that resulted in the inhibition of microbial growth. 2,3,5-triphenyltetrazolium chloride was used as a developer (Beloti et al., 1999).

2.6 Statistical analysis

All experiments were performed in triplicate. Data were subjected to analysis of variance (ANOVA) and differences between means were determined by Tukey's test at the 5% significance level using Minitab version 17 software.

3 RESULTS

The essential oil of *A. conyzoides* showed a light yellow color and a strong floral odor. The yield of essential oil from aerial parts (leaves and flowers) was 0.82 ± 0.14% (dry basis), density 0.95 ± 0.04 g/mL, and refractive index 1.5355.

The essential oil from aerial part presented 54 compounds, predominantly sesquiterpene hydrocarbons (33.28%) and chromenes (55.57%) (Table 1). The major compounds were precocene I (48.19%), precocene II (7.38%) and β –caryophyllene (19.66%) (Table 1 and Figure 1). Figure 1 represents the mass spectra of the major compounds precocene I, precocene II and β-caryophyllene found in the essential oil of *A. conyzoides*, thus indicating a higher concentration of Precocene in the EO of *A. conyzoides*.

Table 1 – Chemical composition of *Ageratum conyzoides* aerial parts (leaves and flowers) essential oil

(Continued)

Ret Time	Compounds	Relative area %	RI calc	RI Lit.	MF	MM	Identification Methods
3.672	ni	0.36	858				a,b,c
5.188	α-pinene	0.16	938	939	$C_{10}H_{16}$	136	a,b,c
5.552	Camphene	0.57	953	952	$C_{10}H_{16}$	136	a,b,c
6.258	β-pinene	0.07	980	980	$C_{10}H_{16}$	136	a,b,c
6.613	β-myrcene	0.1	992	992	$C_{10}H_{16}$	136	a,b,c
6.912	α-terpinene	0.47	1002	1003	$C_{10}H_{16}$	136	a,b,c
7.762	D-limonene	0.2	1031	1030	$C_{10}H_{16}$	136	a,b,c
9.791	Terpinolene	0.03	1089	1088	$C_{10}H_{16}$	136	a,b,c
10.222	Linalool	0.05	1099	1100	$C_{10}H_{18}O$	154	a,b,c
10.834	ni	0.07	1117				a,b,c
12.774	Borneol	0.03	1167	1166	$C_{10}H_{18}O$	154	a,b,c
15.281	Endo fenchyl acetate	1.58	1228	1222	$C_{12}H_{20}O_2$	196	a,b,c
17.762	ni	0.71	1287				a,b,c
19.392	α-longipinene	0.1	1327	1330	$C_{15}H_{24}$	204	a,b,c
20.297	α-cubebene	0.63	1349	1349	$C_{15}H_{24}$	204	a,b,c
21.382	α-copaene	0.56	1374	1376	$C_{15}H_{24}$	204	a,b,c
21.739	β-cuvebene	3.95	1382	1388	$C_{15}H_{24}$	204	a,b,c
22.061	β-caryophyllene	19.66	1390	1399	$C_{15}H_{24}$	204	a,b,c
24.015	α-bergamotene	0.34	1439	1433	$C_{15}H_{24}$	204	a,b,c
24.388	γ-elemene	0.06	1457	1458	$C_{15}H_{24}$	204	a,b,c
24.753	Humulene	2.1	1457	1456	$C_{15}H_{24}$	204	a,b,c
25.799	Precocene I	48.19	1482	1471	$C_{12}H_{14}O_2$	190	a,b,c
27.508	α-farnesene	3.73	1526	1515	$C_{15}H_{24}$	204	a,b,c
27.876	β –sesquiphellandrene	0.15	1536	1523	$C_{15}H_{24}$	204	a,b,c
28.166	δ-cadinene	1.89	1544	1541	$C_{15}H_{24}$	204	a,b,c
28.345	Trans-cadina- 1, 4- diene	0.11	1548	1548	$C_{15}H_{24}$	204	a,b,c
28.634	Trans-sesquisabinene hydrate	0.2	1556	1565	$C_{15}H_{26}O$	222	a,b,c
28.936	Nerolidol	0.74	1564	1565	$C_{15}H_{26}O$	222	a,b,c
29.125	ni	0.06	1568				a,b,c
29.692	Germacrene D-4-ol	0.4	1583	1579	$C_{15}H_{26}O$	220	a,b,c
30.037	Caryophyllene oxide	1.06	1591	1589	$C_{15}H_{24}O$	220	a,b,c
30.172	Widdrol	0.19	1594	1592	$C_{15}H_{26}O$	222	a,b,c
30.603	Humulene epoxide II	0.16	1606	1605	$C_{15}H_{24}O$	222	a,b,c
30.840	Patchoulane	0.17	1613	1618	$C_{15}H_{26}O$	222	a,b,c
31.107	ni	0.47	1620				a,b,c
31.494	α-cadinol	0.47	1631	1632	$C_{15}H_{26}O$	220	a,b,c
32.009	Precocene II	7.38	1645	1656	$C_{13}H_{16}O_3$	220	a,b,c
32.266	ni	0.09	1652				a,b,c
32.452	Acorenone	0.48	1657	1655	$C_{15}H_{24}O$	222	a,b,c

Table 1 – Chemical composition of *Ageratum conyzoides* aerial parts (leaves and flowers) essential oil

(Conclusion)

Ret Time	Compounds	Relative area %	RI calc	RI Lit.	MF	MM	Identification Methods
33.142	α –bisabolol	0.61	1676	1681	$C_{15}H_{26}O$	222	a,b,c
33.489	Cis-farnesol	0.15	1685	1697	$C_{15}H_{26}O$	222	a,b,c
33.630	ni	0.06	1689				a,b,c
33.807	Farnesol	0.23	1693	1695	$C_{15}H_{26}O$	222	a,b,c
34.564	Eudesm-7(11)-en-4α-ol	0.24	1715	1700	$C_{15}H_{26}O$	222	a,b,c
37.030	ni	0.81	1785				a,b,c
39.917	Kaur-16-ene	0.02	2071	2061	$C_{20}H_{32}$	272	a,b,c
44.849	ni	0.13	2164				a,b,c
	Total identified	97.23					
	Monoterpene hydrocarbons	1.60					
	Oxygenated monoterpenes	0.08					
	hydrocarbon sesquiterpenes	33.28					
	oxygenated sesquiterpenes	5.10					
	hydrocarbon diterpenes	0.02					
	Chromens	55.57					
	not identified	2.76					
	Other compounds	1.58					

[a]Compounds listed according to HP-5MS elution order; [b]retention rate (RR) calculated using C_7 to C_{26} *n*-alkanes in capillary column (HP-5MS); [c]Identification based on comparison of mass spectra from Wiley 275 libraries; Relative Area (%): Percentage of the area (%) that the compound occupies within the chromatogram; RI: Retention Index, MF: Molecular Formula and MM: Molecular mass of chemical compounds from *Ageratum conyzoides*; n.i = Not identified

The results of the antioxidant activity of the essential oil of *A. conyzoides* are detailed in Tables 2 and 3 and Figure 2. The results of antifungal activity indicated that of the four fungi used, only *Aspergillus ochraceus* presents a result, with a MIC of 1250 µL/mL; the other fungi did not show a viable result.

Figure 1 – Mass spectra obtained by GC-MS from the Precocene I (m/z = 190), Precocene II (m/z = 220) and caryophyllene (m/z = 204) found in *Ageratum conyzoides* aerial parts (leaves and flowers) essential oil

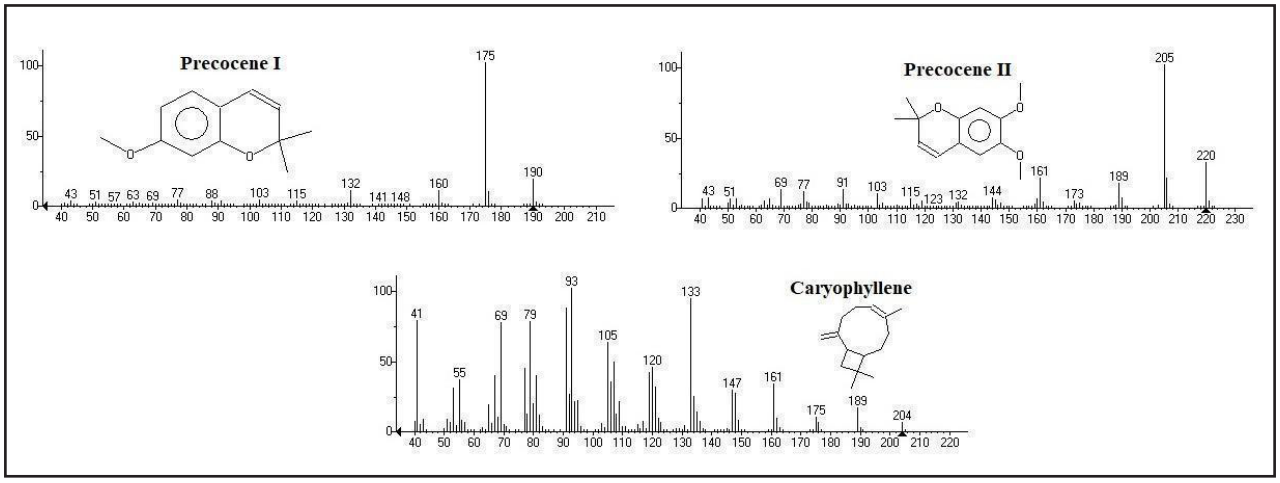


Table 2 – Antioxidant activity of *Ageratum conyzoides* aerial parts (leaves and flowers) essential oil by DPPH• and FRAP methods

Antioxidant activity	**DPPH•** IC_{50} **(mg/mL)**	**FRAP** **(μM Fe^{2+}/mg de amostra)**
Essential oil	5.01 ± 0.99[b]	0.46 ± 0.05[b]
Quercetin	0.03 ± 0.001[a]	–
Trolox	–	9.18 ± 0.83[a]

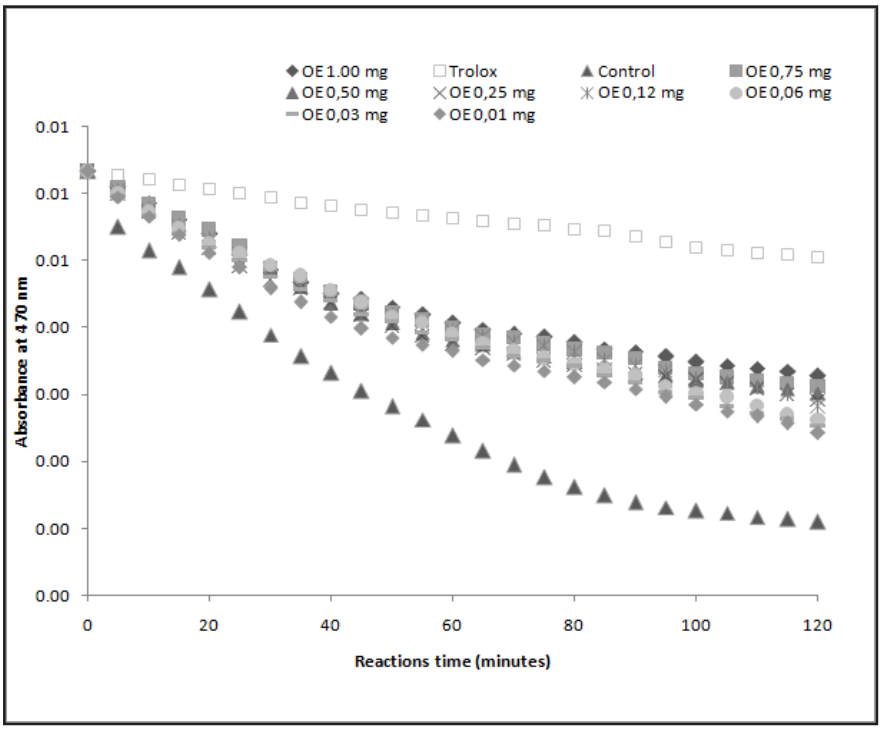
Values are the mean ± standard deviation (n = 3). The statistical analysis used was analysis of variance (ANOVA), and differences between means determined by Tukey's test (p ≤ 0.05). Values in the same column with different letters show significant difference (p ≤ 0,05). IC50: required amount of the sample to reduce 50% of the free radical DPPH (2,2 diphenyl-1-picrylhydrazyl); FRAP: iron-reducing antioxidant power. Positive control: quercetin (for DPPH) and trolox (for FRAP).

Table 3 – Percent inhibition of oxidation of *Ageratum conyzoides* aerial parts (leaves and flowers) essential oil by the β-carotene/linoleic acid co-oxidation system (BCLA). Results are expressed as β-carotene color protection (%) during 120 minutes of oxidative reaction

Samples (mg/mL)	Protective effect (%)
Trolox	79.85[a] ± 6.27
1.00	52.18[b] ± 7.81
0.75	50.97[b] ± 6.29
0.50	47.38[b] ± 7.16
0.25	46.13[b] ± 7.72
0.125	45.23[b] ± 5.89
0.625	44.63[b] ± 4.70
0.03	43.91[b] ± 6.64
0.01	40.24[b] ± 7.69
Negative control	0.00[c] ± 0.00

Values are the mean ± standard deviation (n = 3). Data were subjected to analysis of variance (ANOVA), and differences between means were assessed by Tukey's test ($p \leq 0.05$). Values in the same column followed by different letters are significantly different ($p \leq 0.05$).

Figure 2 - Absorbance at 470 nm over time (120 minutes) of β-carotene co-oxidation reaction / linoleic acid in *Ageratum conyzoides* aerial parts (leaves and flowers) essential oil at concentrations of 1.00; 0.75; 0.50; 0.25; 0.12; 0.06; 0.03 and 0.01 mg/mL



4 DISCUSSION

The collection of *A. conyzoides* in June and July (which corresponds to the winter season in the southern hemisphere) occurred when the plant suffered interference in the rainfall regime, since, according to climatological data, the average rainfall index was 1.46 mm in June and 0.03 mm in July, indicating a period with low rainfall where the crop is installed. *A. conyzoides* requires low irrigation however, it is observed that plants in more humid places, as in the vicinity of treetops and mountain regions, they are more developed, thus indicating their preference for more humid places (Ming, 1999).

The average temperature in the months of the collection was 18.67 ± 0.71ºC, where the air humidity remained at 70.69 ± 5.96 (%). *A. conyzoides* is considered an annual species, invasive in pastures, wastelands and cultivated areas, as it adapts to both wet and dry areas (Castro et al., 2016). For fresh parts of the plant essential oil yields of 0.11% are reported for forming aerial parts (Liu & Liu, 2014), 0.22% and 0.19% for flowers and stems (Kouame et al., 2018), 0.16 to 0.26% for flowers (Dung et al., 1989) and 1.60% for flowers (Kasali et al., 2002). For dry parts, the essential oil yield was: 2,8% for inflorescences, 1.0% for flowers, 0.6% for roots and 0.5% for stems (Zoghbi et al., 2007). The yield of the essential oil of *A. conyzoide*s obtained in our study (0.82% of dry plants) is within the variability of results described in the literature for this plant.

One of the challenges for using essential oils in food preservation is the high cost, which can be up to six times higher than chemical fungicides (Kouassi et al., 2012). Burt (2004) reports that the increased demand for essential oil for uses mainly in the chemical, cosmetic and food industries may lead to bioengineering of its synthesis in plants. According to the European Pharmacopoeia, for the development of applications with essential oils, a minimum of 2 mL/kg (0.2%) of plants is required (Nemeth & Bernath, 2008). *A. conyzoides* has a high essential oil extraction yield of up to 2.8% of dry plants (Zoghbi et al., 2007), and in our results, the essential oil of *A. conyzoides* presented a yield of 0.82%, indicating that it is a species with high potential for the development of applications due to its high yield of essential oil.

Regarding the color of the oil, the reports in the literature are also varied from reddish orange (Kasali et al., 2002; Ekundayo et al., 1988) to light yellow (Liu & Liu, 2014; Kouame et al., 2018). Differences in yield and characteristics of the essential oil obtained from *A. conyzoides* may be related to sample processing before extraction. In our study, dried flowers and leaves were used; while Liu and Liu (2014) used fresh aerial parts, and Dung et al. (1989) and Kasali et al. (2002) used fresh leaves. The use of different parts of *A. conyzoides* results in different EO yields as demonstrated by Zoghbi et al. (2007). Sample preparation and other biotic and abiotic factors can also affect the yield and chemical composition of essential oils (Bettiol, 2009).

One of the important factors in the chemical composition of essential oils are the genetic characteristics that generate the chemotypes and these directly influence the biological activities of the essential oil. Chart 1 shows 3 chemotypes identified in the essential oil of *A. conyzoides*, which may suggest that the essential oil identified in our experiment belongs to chemotype I.

For Kasali et al. (2002), the species *A. conyzoides* has insecticidal, healing, bactericidal, fungicidal and anti-gonadotropic properties. The insecticidal action occurs in chemotypes that have precocene as the majority, because according to Binder et al. (1991), precocenes have caused a direct impact on the insects, and according to Kafi-Farashah et al. (2018), act as anti-juvenile hormones and cause genotoxicity. Ekundayo et al. (1988), also demonstrated the juvenileizing hormonal action of precocene I and II in insects, the most common effect being precocious metamorphosis, producing sterile or dying adults.

Chart 1 – Main chemotypes of *Ageratum conyzoides* essential oil and biological activities (Continued)

Chemotypes	Regions	Temperature and Rainfall	Part of the plant
I	Umuarama northwest region of Paraná - Brazil	20.7 °C and 1512 mm.	Dried leaves and flowers
	Northwest Karnataka, India	25°C.	Fresh leaves
			Fresh flowers
			Fresh stem
			Fresh root
	Belém and Santarém Novo, state of Pará- Brazil	25.9 °C and 2150 mm.	Leaves
			Twigs
			Roots
			Inflorescences
	Ivory Coast-Africa	Semi-humid tropical	Fresh flowers
			Fresh twigs
	Ribeirão Pires, State of São Paulo – Brazil	17.2°C and 2159 mm.	Fresh leaves
	Badagry Lagos Nigeria	33 ºC and 100mm	Fresh mature leaves hydrodistillation 4 hours
	Kumaun Himalaya	20.9ºC and 389.52mm	Fresh flowering aerial part
II	Minas Gerais - Brasil	21°C and 1341 mm.	Dry aerial part - Access Piranga-MG
	Minas Gerais - Brasil	25°C	Dry aerial part –Viçosa-MG
			Dry aerial part-Mariana MG
III	Nova Santa Rita, Rio Grande do Sul –Brasil	19.5°C and 1419 mm.	Fresh aerial part
	Hanoi, Vietnam	23.8°C and 1684 mm.	Fresh leaves
	Pakistan	Tropical to temperate	Fresh flowers
			Fresh leaves

Chart 1 – Main chemotypes of *Ageratum conyzoides* essential oil and biological activities (Conclusion)

Chemotypes	Majority (%)			EO Yield (%)	Biological Activities/ References
	Precocene I	Precocene II	β- caryophyllene		
I	48.19	7.38	19.66	0.82%	Antioxidant Our results
	72.30	3.10	12.10	0.15%	Test not performed Joshi, (2014)
	66.50	10.50	10.20	0.17%	
	50.30	0.30	14.60	0.5%	
	79.30	0.40	6.00	0.08%	
	69.60	-	14.40	1.0%	Test not performed Zoghbi et al. (2007)
	71.60	-	12.80	0.5%	
	67.40	-	15.30	0.6%	
	55.50	-	19.40	2.8%	
	58.80	-	15.20	0.22%	Antimicrobial moderate against gram positive bacteria
	76.50	-	8.10	0.19%	Kouame et al. (2018)
	79.11	10.39	-	0.11 a 0.19%	Antifungal Esper et al. (2015)
	63.08	5.04	-	1.6 %	Kasali et al. (2002)
	16.7	42.5	20.7	0.30%	Padalia et al. (2010)
II	76.5	-	-	0.48%	Test not performed Castro et al. (2014)
	15.63	76.71	-	0.49%	
	10.46	70.77	-	0.49%	
III	28.24	28.55	-	0.13%	Test not performed Barros et al. (2015)
	29.00	31.10	-	0.16 to 0.26%	Dung et al. (1989)
	30.30	34.90	14.40	0.5%	Antifungal namaticide Riaz et al. (1995)

The antioxidant capacity values vary depending on the methodology used, and vegetables that contain a higher concentration of phenolic compounds do not always display a greater activity to counteract free radical effects (Viana et al., 2023). In this sense, it could be observed in the present experiment that of the three methods used to evaluate the antioxidant potential of *A. conyzoide*s EO, the best results were obtained by the β-carotene/linoleic acid co-oxidation system (Table 3) indicating a moderate activity (52.18%) of oxidation inhibition at the concentration of 1.0 mg/mL and (40.24%) at the concentration of lowest concentration tested (0.01 mg/mL), with no significant difference between the highest and lowest concentration tested (Table 3) whose difference was 100 times. This protective action found in the essential oil of *A. conyzoides* is positive and may suggest the use of this oil in products that are susceptible to oxidative effects during storage, mainly in products susceptible to lipid peroxidation. The β-carotene/linoleic acid co-oxidation system determines the activity of a sample or compound in protecting a lipid substrate such as essential oils from oxidation, evaluating the level of inhibition of free radicals generated during linoleic acid peroxidation (Duarte-Almeida et al., 2006).

As for the moderate action found, we followed the parameters established by Rufino et al. (2010) who consider high antioxidant activity above 70% and moderate between 40 and 70% of oxidation inhibition.

Low antioxidant activities were observed by the DPPH (IC_{50}= 5.01 mg/ mL) and FRAP (0.46 μM Fe^{2+}/mg sample) methods (Table 2). These results are related to the methods employed since the DPPH method is routinely used for hydroorganic extracts containing hydrophilic and lipophilic compounds, whose mechanism of action consists of the ability of antioxidant compounds to sequester or donate hydrogen. The FRAP method is indicated for hydrophilic compounds, which analyzes the ability of the sample to donate electrons. Another point that must be considered is that the DPPH and FRAP methods correlate and are related to the presence of phenolic compounds (Pérez-Jiménez et al., 2008; Rufino et al., 2010) and this correlation was observed in the results of the oil essential *A. conyzoides*.

For chromenes, precocone I and II there are no reports of antioxidant activity, since these compounds are recognized as potent natural insecticides (Edwin & Kester, 2018), being base compounds to produce synthetic bioactive analogues (Ekundayo et al., 1988).

The second most abundant compound in the essential oil of *A. conyzoides* was β-caryophyllene (19.66 %) (Table 1) and for this compound, there are conflicting results in the literature, because according to Ruberto and Baratta (2000), β-caryophyllene showed low antioxidant efficacy (only 8.9% protection against 93.5% for α-tocopherol, both at 1.0 mg/mL) using the non-polar method of thiobarbituric acid reactive species. Calleja et al. (2012) tested the antioxidant activity of β-caryophyllene and the positive control α-tocopherol using different methods. For the DPPH radical, the IC_{50} values were 132 and 1.8 mg/mL, respectively. For the inhibition of lipoxygenase activity, β-caryophyllene inhibited antioxidant activity higher than α-tocopherol (27.9% inhibition compared to 12.9% for α-tocopherol).

Patil et al. (2010) evaluated the antioxidant potential of the essential oil *A. conyzoides*, whose chemical composition indicated the presence of 26.22% of β-caryophyllene and 52.18% of Precocene I. The results showed better results for the non-polar methods, with the β-carotene/linoleic acid co-oxidation system method with a maximum inhibition of lipid peroxidation of the essential oil IC_{50} of 0.015 ± 0.005 mg/mL, while butyl hydroxy anisole (BHA), a standard antioxidant, had an IC_{50} of 0.023 ± 0.011 mg/mL. Lower antioxidant potential was verified for the more polar methods, DPPH (IC_{50} 0.57 mg/mL) and FRAP (3.21 mg/mL). The high concentration of β-caryophyllene found by these authors in the essential oil suggests that β-caryophyllene may have influenced the antioxidant response found.

β-caryophyllene is a naturally occurring volatile bicyclic sesquiterpene present in numerous herbs, spices and foods (Basha & Sankaranarayanan, 2016). Due to its weak aromatic taste, β-caryophyllene is commercially used as a food additive and in cosmetics. The Flavoring and Extract Manufacturers Association has granted

β-caryophyllene "generally recognized as safe" status, and it has been approved by the U.S. Food and Drug Administration for food use due to its low toxicity (Calleja et al., 2012). Sivakumar and Bautista - Banos (2014), recommend the use of essential oils in food preservation and Burt (2004), recommends concentrations from 0.1 to 6%. The antioxidant concentrations of the essential oil of *A. conyzoides*, reported in our study for antioxidant action, are from 1.00 to 0.01 mg/mL, that is, from 0.1 to 0.001%. Thus, the values found in our study are within the concentration range of essential oils applied in food preservation (Sivakumar & Bautista-Banos, 2014), making the essential oil of our study an alternative for the development of applications in food preservation. Jiang and Xiong (2016) conducted a review on the use of natural antioxidants for meat conservation, for these authors the production of meat and meat products using chemical components potentially impacting health is a major challenge for meat and food scientists.

The antifungal potential of *A. conyzoides* EO on the fungus *A. ochraceus* indicates the potential that essential oil of this species in controlling ochratoxin A, an mycotoxin which mainly attacks green coffee beans during the drying process, which is one of the routes of infestation by ochratoxigenic *Aspergillus* species and consequently of contamination by ochratoxin A (Ferreira et al., 2011). The importance of finding activity in this fungus lies in the fact that it is a food contaminant, and *A. conyzoides* EO can act as a natural preservative.

5 CONCLUSION

The essential oil of *Ageratum conyzoides* presented high concentrations of hydrocarbon sesquiterpenes (33.28%), with the majority being β-caryophyllene (19.66%), and Chromens (55.57%), with emphasis on Precocene I (48.19%) and Precocene II (7.38%). The essential oil provided a protective effect of 52.18 to 44.63% at concentrations ranging from 1.00 to 0.01 mg/mL through the β-carotene/linoleic

acid co-oxidation system, suggesting that the essential oil has promising potential to increase the shelf life of pharmaceutical, cosmetic and food products. The action against the fungus *Aspergillus ochraceus*, suggests the fungicidal action of the essential oil, controlling ochratoxin A, a mycotoxin that contaminates food, indicating that this oil can also act as a natural preservative.

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