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Original Article

In vitro antibacterial activity of the essential oil of Ageratum conyzoides from the Brazilian Amazon

Atividade antibacteriana in vitro do óleo essencial de Ageratum conyzoides da Amazônia Brasileira

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Abstract

Natural products, specifically essential oils (EOs), have exhibited significant biological properties with potential medical applications. In this context, the aim of this study was to analyze the chemical composition and the antibacterial, antioxidant, and cytotoxic activities of the EO of Ageratum conyzoides from the municipality of Santa Rita, Maranhão, in the Brazilian Amazon. The EO was extracted from the fresh aerial parts of A. conyzoides L. using the hydrodistillation method and the oil was analyzed using a GCMS-QP2010 Ultra system. Standard strains of Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, Bacillus cereus and Staphylococcus aureus were used to determine antibacterial activity using the minimum inhibitory concentration (MIC) method and minimum bactericidal concentration (MBC). The antioxidant potential of the EO of A. conyzoides was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging assays. The main constituents found in the oil were precocene I (79.85%) and (E)-caryophyllene (13.55%). The bacteriostatic efficacy of A. conyzoides EO's estimated by MIC and MBC was between 31.25 and 250 µg/mL. Gram-negative bacteria (S. typhimurium and E. coli) were the most resistant. The IC50 values obtained from the DPPH and ABTS assays were 287.94 ± 7.02 µg/mL and 132.23 ± 15.41 µg/mL, respectively. The results show that the essential oil of A. conyzoides has promising antibacterial activity, especially against Gram-positive bacteria.

Keywords: antibacterial, antioxidant activity, pre

cocene I, (E)-caryophyllene.

Resumo

Produtos naturais, especificamente os óleos essenciais (OEs), têm exibido significativas propriedades biológicas, com potenciais aplicações médicas. Neste contexto, o objetivo deste estudo foi analisar a composição química e as atividades antibacteriana, antioxidante e citotóxica do OE de Ageratum conyzoides proveniente do município de Santa Rita, Maranhão, na Amazônia brasileira. O OE foi extraído das partes aéreas frescas de A. conyzoides L. usando o método de hidrodestilação, e o óleo foi analisado utilizando um sistema GCMS-QP2010 Ultra. Cepas padrão de Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, Bacillus cereus e Staphylococcus aureus foram utilizadas para determinar a atividade antibacteriana utilizando os métodos de concentração inibitória mínima (CIM) e concentração bactericida mínima (CBM). O potencial antioxidante do OE de A. conyzoides foi avaliado utilizando ensaios de eliminação de radicais livres 1,1-difenil-2-picrilhidrazil (DPPH) e 2,2-azinobis-(3-etilbenzotiazolina-6-ácido sulfônico) (ABTS). Os principais constituintes encontrados no óleo foram precoceno I (79,85%) e (E)-cariofileno (13,55%). A eficácia bacteriostática do OE de A. conyzoides, estimada por CIM e CBM, variou entre 31,25 e 250 µg/mL. As bactérias Gram-negativas (S. typhimurium e E. coli) foram as mais resistentes. Os valores de IC50 obtidos nos ensaios de DPPH e ABTS foram 287,94 ± 7,02 µg/mL e 132,23 ± 15,41 µg/mL, respectivamente. Os resultados indicam que o óleo essencial de A. conyzoides apresenta atividade antibacteriana promissora, especialmente contra bactérias Gram-positivas.

Palavras-chave: antibacteriano, atividade antioxidante, precoceno I, (E)-cariofileno.

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1. Introduction

Medicinal plants provide antibacterial compounds from their secondary metabolites and can be a good substitute for traditional synthetic or semi-synthetic chemical antibiotics, as well as overcoming multi-resistant bacteria (Voravuthikunchai and Kitpipit, 2005; Othman et al., 2019; Gorlenko et al., 2020). Thus, many antibiotics were initially isolated from natural sources (Rossiter et al., 2017). These plant-derived metabolites not only exhibit effective antibacterial action against resistant strains, but also play an important role in reducing bacterial resistance, offering a promising approach to combating resistance (Foda et al., 2022).

The use of herbal medicines can help reduce dependence on antibiotic therapies and minimize antibiotic resistance. Various plant antimicrobials contain different functional groups, their antibacterial activity is attributed to multiple mechanisms. Therefore, the prospect of developing resistance to plant constituents is relatively lower (Allami et al., 2020).

Essential oils (EOs) are considered to be potential natural antimicrobial agents. Studies have reported their efficacy as having a broad spectrum of antimicrobial activity against various pathogenic microorganisms, which is attributed to their bioactive constituents (Voon et al., 2012). In addition to their antimicrobial efficacy, EOs exhibit a lower potential for adverse reactions compared to synthetic drugs, supporting the development of alternative antimicrobial strategies (Gautam et al., 2014; Soares et al., 2022; Elbestawy et al., 2023).

Ageratum conyzoides Linnaeus (Asteraceae) is a tropical plant very common in West Africa and some parts of Asia and South America. It is a medicinal plant known for its pharmacological and antimicrobial activity. Recent studies have shown that the leaves of A. conyzoides have antibacterial activity against Staphylococcus aureus, Porphyromonas gingivalis, Streptococcus mutans, and Escherichia coli (Sugara et al., 2016; Mentari et al., 2020; Achmad et al., 2020).

Ageratum conyzoides is a plant that produces essential oil with a strong odor in which chemical analysis in different regions has shown a significant variation in its chemical composition. Geographical variations have a major impact on the quantity and/or quality of the chemical constituents of EOs, and the chemical composition of EOs can also vary according to the part of the plant studied (Kouame et al., 2018).

The essential oil of A. conyzoides contains mainly terpinene-4-ol, bornyl acetate, E-caryophyllene, γ-murolene, δ-cadinene, α-murolene, caryophyllene oxide, α-humulene, precocene I and II. The precocenes are the majority compounds in the oil, ranging from 30 to 93% (Castro et al., 2004).

This study aimed to investigate the chemical composition, as well as the antibacterial, antioxidant, and cytotoxic activities, of the EO extracted from the aerial parts of A. conyzoides originating in the municipality of Santa Rita, Maranhão, northeastern Brazil.

2. Materials and Methods

2.1. Plant material and oil extraction

The aerial parts of A. conyzoides were collected in March 2022 in the municipality of Santa Rita, state of Maranhão (MA), Northeast region of Brazil (latitude 3°08'37.0"S and longitude 44°19'33.0"W), located in the Brazilian Amazon. Access to the specimen was registered under Nº AA834D0 in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge. The exsiccate containing the aerial organs of the plant species was prepared and identified by Botanist Dr. Eduardo Bezerra Almeida Junior and deposited in the Mar Herbarium of the Federal University of Maranhão under registration number 9099. The essential oil was extracted from the aerial parts (300 g) of fresh A. conyzoides plants by the hydrodistillation method (3 h) using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulfate and its yield was calculated (v/w).

2.2. Oil composition analysis

Oil analysis was performed using a Gas Chromatograph-Mass Spectrometer (GC-MS) QP2010 Ultra system (Shimadzu Corporation, Tokyo, Japan), equipped with GCMS-Solution software containing the Mondello (2011) and Adams (2007) libraries. An Rxi-5ms silica capillary column (30m x 0.25 mm, 0.25 µm film thickness; Restek Corporation, Bellefonte, PA, USA) was used. The analysis conditions were as follows: injector temperature of 250 °C; oven temperature programmed from 60 to 240 °C (3 °C/min); helium as carrier gas, set at a linear speed of 36.5 cm/s (1.0 mL/min); split mode injection for 1.0 µl of sample (oil 6.0 µL: hexane 500 µL); split ratio 1:20; electron impact ionization at 70 eV; ionization source and transfer line temperature of 200 and 250 °C, respectively. The mass spectra were obtained by automatic scanning at intervals of 0.3 s, with mass fragments in the 35-400 m/z range.

The retention index for all the volatile components was calculated using a homologous series of C8-C40 n-alkanes (Sigma-Aldrich, USA), according to the linear equation of Van Den Dool and Kratz (1963). The quantitative data relating to the volatile components was obtained using a GC 2010 series, coupled with an FID Detector, operated under conditions similar to those of the GC-MS system. The components were identified by comparing their retention indices and mass spectra (molecular mass and fragmentation pattern) with those in the GCMS-Solution system libraries.

2.3. Antimicrobial activity

2.3.1. Bacterial strains and culture conditions

Standard strains of Listeria monocytogenes (ATCC 7644), Escherichia coli (ATCC 25922), Salmonella typhimurium (ATCC 14028), Bacillus cereus (ATCC 14579), and Staphylococcus aureus (ATCC 25923), supplied by the Food and Water Quality Control Microbiology Laboratory of the Federal University of Maranhão (PCQA-UFMA), were used. The access was registered under the number A3FAD1C in the National System for the Management of

Genetic Heritage and Associated Traditional Knowledge. They were tested at a cell concentration of 1.5 × 108 CFU/mL following the McFarland scale, recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020).

2.3.2. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The bacterial suspension was standardized by adding a 24-hour culture to a tube containing sterile saline solution until it reached a turbidity equal to the suspension in tube 0.5 of the McFarland scale (approximately 1.5 × 108 CFU/mL), checked by the McFarland Scale Turbidity Meter for reading inoculums at 600 nm and Working Range 0 - 5.0 McFarland; gentamicin (128 to 1 µg/mL) was used as a positive control. The essential oil of A. conyzoides L. was prepared in a stock solution of Mueller-Hinton broth with 0.1% DMSO. In a sterile 96-well microplate, the essential oil of A. conyzoides L. was tested at concentrations of 1000 to 7.8 µg/mL against ATCC bacterial strains. Controls included (i) bacterial growth control, (ii) a commercial antimicrobial control, and (iii) a culture medium control. Immediately after plate assembly, incubation was carried out at 37 °C for 24 hours to allow microorganism growth.

Following the 24-hour incubation, 20 µL of a 3% resazurin solution was added to wells, and the microplate was incubated at 37 °C for an additional 2–4 hours. The final visual reading was then performed. A blue color in the wells was interpreted as the absence of bacterial growth, whereas a pink color as the presence of bacterial growth. The MIC was defined as the lowest concentration of A. conyzoides L. essential oil capable of inhibiting cell growth, i.e. the lowest concentration capable of preventing the color change from blue to pink. Each sample was tested in triplicate.

After determining the MIC, 100 µL were taken for subculture (in triplicate) on Sabouraud dextrose agar plates containing the concentration considered inhibitory and the immediately higher concentrations. After 48 hours of incubation at 37 °C, the minimum bactericidal concentration (MBC) was defined as the lowest concentration that prevented visible growth of the subculture.

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging capacity assay

The radical scavenging activity of A. conyzoides essential oil against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated according to a previously described method by Brand-Williams et al. (1995), with minor modifications. Briefly, 100 µL of the essential oil, prepared at various concentrations (ranging from 1000 to 7.8 µg/mL), dissolved and diluted in ethanol, were mixed with 3.0 mL of a 40 µg/mL ethanolic DPPH solution. The mixture was then kept in the dark at room temperature for 30 minutes, after which its absorbance was measured at 517 nm. Trolox was used as the standard, and the radical scavenging capacity of the essential oil was calculated as µg/mL Trolox equivalents (TE)/g of essential oil.

The antioxidant activity of A. conyzoides essential oil and Trolox was quantified by the concentration values that provided 50% inhibition (IC50) in the DPPH assay. The radical scavenging capacity (%) was obtained using the following formula: (Acontrol − Asample/Acontrol) × 100, where Acontrol is the absorbance of the control and Asample is the absorbance of the sample. After obtaining the inhibition percentage (y) for each concentration (x), the points (x and y) are plotted on a coordinate plane. Subsequently, the equation of the line y = ax + b is determined through linear regression analysis, where a and b are constants, x represents the sample concentration (µg/mL), and y denotes the percentage of inhibition (%). The antioxidant activity is expressed as the 50% Inhibition Concentration (IC50), specifically the sample concentration (x) required to reduce 50% of radicals (y = 50).

2.4.2. ABTS radical scavenging activity assay

The radical cation scavenging capacity of 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by the essential oil of A. conyzoides was evaluated according to Re et al. (1999). The ABTS radical cation (3.0 mL), kept in the dark, was mixed with a 30 µL aliquot of various concentrations of the essential oil (1000 to 7.8 µg/mL) dissolved and diluted in ethanol, homogenized using a vortex mixer. After 6 minutes, the absorbance of the reaction mixture was measured with a spectrophotometer at a wavelength of 734 nm. Samples were prepared in triplicate. Trolox was used as a standard, and the results were calculated as µg/mL Trolox equivalents (TE)/g of essential oil. The antioxidant activity of A. conyzoides essential oil and Trolox by the ABTS assay was also reported as IC50, as described for the DPPH assay.

2.5. Cytotoxicity assay

2.5.1. Cell culture

Assays were performed using RAW 264.7 murine macrophages cell line (ATCC TIB-71TM), cultured in DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco, Gaithersburg, MD, USA), penicillin (100 U/mL) and streptomycin (100 µg/mL) (Sigma-Aldrich, St Louis, MO, USA) at 37 °C with 5% CO2 in 75 cm2 cell culture flasks.

2.5.2. MTT assay

The cytotoxicity of the A. conyzoides EO was evaluated as described by Teles et al. (2021). Different concentrations of EO (1000 to 15.62 µg/mL) or gentamicin (64 µg/mL) were prepared by serial dilutions (1:2), and 100 µL per well of each concentration was added to 96-well plates containing RAW 264.7 cells (5 × 105 cells/mL). The experimental design included three control groups: a blank control (wells with culture medium without cells), an untreated control (cells and DMSO 1%), and a reference drug control (gentamicin). Following 24 hours of incubation, the cell viability was determined by reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The resulting formazan was measured by recording absorbance changes at 570 nm using a spectrophotometer (Riss et al., 2013).

2.6 Statistical analysis

Statistical analyses were carried out using GraphPad Prism 7.0 software (GraphPad Software, La Jolla California USA). Student's t-test and analysis of variance (ANOVA) were used to compare the rate of inhibition of the microorganism and the treatment time analyzed. Differences with values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Chemical characterization of the essential oil of A. conyzoides L.

The essential oil of A. conyzoides was obtained by hydrodistillation with a yield of 0.7% (v/w). As presented in Table 1, ten constituents were identified and quantified by GC-MS, representing 98.45% of the total composition of the EO. The main constituents were precocene I (79.85%) and (E)-caryophyllene (13.55%).

3.2. Antimicrobial potential of the essential oil of A. conyzoides L.

The antimicrobial activity of the essential oil of A. conyzoides was evaluated using the microdilution method, tested against five pathogenic microorganisms. The estimated minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranged from 31.25 to 250 µg/mL (as shown in Table 2).

Table 1. Compounds found in the essential oil of Ageratum conyzoides L.

Yield (%)			**0.7**
Constituents (%)	**RI_C**	**RI_L**	
Isobornyl formate	1236	1235[a]	0.2
Bornyl acetate	1290	1287[a]	1.25
β-Cubebene	1395	1392[b]	0.3
(E)-Caryophyllene	1432	1424[b]	**13.55**
α-Humulene	1455	1452[a]	0.3
Precocene I	1473	1464[b]	**79.85**
γ-Amorphene	1496	1495[a]	1.3
α-Muurolene	1505	1500[a]	0.5
β-Sesquiphellandrene	1530	1523[b]	1.0
(E)-Nerolidol	1564	1561[a]	0.2
Monoterpene hydrocarbons			-
Oxygenated monoterpenes			1.75
Sesquiterpene hydrocarbons			16.65
Oxygenated sesquiterpenes			0.2
Chromenes			79.85
Total (%)			98.45

RIC: Retention index calculated, RIL: Retention Index according to literature sources. [a]Retention index reported by Adams (2007); [b]Retention index reported by Mondello (2011).

3.3. Antioxidant activity

The antioxidant potential of A. conyzoides essential oil was evaluated using DPPH and ABTS free radical scavenging assays. The antioxidant activity results, measured by IC50 values for A. conyzoides EO and Trolox, are presented in Table 3. In the DPPH and ABTS assays, the IC50 values for A. conyzoides EO were determined to be 287.94 ± 7.02 µg/mL and 132.23 ± 15.41 µg/mL, respectively. In comparison, Trolox, used as the antioxidant standard, exhibited IC50 values of 10.14 ± 0.0003 µg/mL (DPPH) and 4.055 ± 0.0004 µg/mL (ABTS), indicating that the studied EO demonstrated a significantly lower capacity to reduce free radical concentration. In the DPPH and ABTS assays, the Trolox equivalent antioxidant capacity (TEAC) of A. conyzoides EO was 196.11 ± 16.41 µg trolox/g and 1087 ± 4.94 µg trolox/g, respectively (as shown in Table 3).

3.4. Cytotoxicity

We assessed the viability of RAW 264.7 cells exposed to different concentrations of the tested treatment. No toxicity was observed at the highest concentration (1000 µg/mL) when compared with the negative control group, as demonstrated by the absence of significant differences in cell viability observed in Figure 1.

4. Discussion

The main constituents observed were precocene I (79.85%) and (E)-caryophyllene (13.55%), results consistent with the findings reported by Furtado et al. (2005), Martins et al. (2005), and Rosário et al. (2023). However, the chemical composition of the essential oil is quite variable, exhibiting both quantitative and qualitative differences. This variability arises because the plant's physiological processes fluctuate throughout the

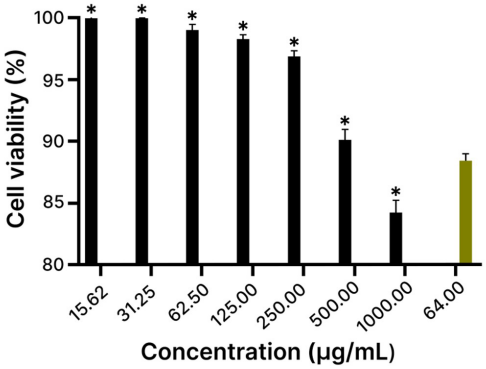

Figure 1. Cytotoxic effect of Ageratum conyzoides L. essential oil (15.62 to 1000 µg/mL, black bars) and gentamicin (64 µg/mL, green bar) on murine macrophages RAW 264.7 cell line for 24 hours, evaluated by cell viability. Each row represents mean and standard deviation of three independent assays. Where *P > 0.05 demonstrated that there was no statistically significant difference in relation to the control group according to the unpaired Student's t-test with a 95% confidence index.

Table 2. MIC and MBC values of Ageratum conyzoides L. essential oil on different bacterial cultures after 24 hours of treatment.

Samples Analyzed	MIC/MBC (μg/mL)				
	E. coli	***S. typhimurium***	***S. aureus***	***L. monocytogenes***	***B. cereus***
EO of *A. conyzoides*	250/250	250/250	31.25/31.25	31.25/31.25	62.50/125
Gentamicin	16.0	16.0	16.0	32.0	64.0

year, leading to changes in the concentration of active components across different seasons. External factors such as temperature, rainfall, wind, soil, latitude, altitude and season significantly affect the production and yield of these compounds (Neves et al., 2021).

It is likely that the compounds found in A. conyzoides L. act in different ways against microorganisms, meaning that their antimicrobial activity is not related to a single mechanism of action but rather involves multiple targets in the bacterial cell (Tariq et al., 2019). The most widely accepted idea is that essential oils develop their action against bacteria through interaction with the cell membrane, including electron transport, ion gradients, protein translocation, phosphorylation and other enzyme-dependent reactions (Andrade-Ochoa et al., 2021).

The yield obtained in this study differs from those reported in the literature for the essential oils of the species A. conyzoides L. Patil et al. (2010), Liu and Liu (2014), and Lima et al. (2014) found values of 0.18%, 0.11% and 0.46% (w/w), respectively. These differences may be related to the methodology used in the extraction and the collection site. Castro et al. (2004) when evaluating A. conyzoides essential oil extracted from five different locations, obtained contents ranging from 0.48% to 0.70%. Other authors such as Vieira et al. (2012), Kumar et al. (2016), and Gutiérrez et al. (2021) found yields of 0.46%, 0.1% and 0.26% respectively. This is mainly due to the different climate, sunlight, relative humidity and soil type.

In the present study, A. conyzoides L. essential oil inhibited the growth of all bacteria tested, especially Gram-positive bacteria (S. aureus, L. monocytogenes and B. cereus), as evidenced by MIC values of 31.25 to 62.50 μg/mL. This corroborates findings from previous research conducted in diverse geographic regions with this plant species. For instance, Kouame et al. (2018) observed similar antibacterial activity in plants collected in western Ivory Coast, while Quoc (2020) reported analogous results in Vietnam.

Gram-negative bacteria, such as S. typhimurium and E. coli, exhibited the highest resistance to the tested essential oil. This observation is consistent with the findings of previous studies, which have attributed the higher resistance of Gram-negative cells to the greater structural complexity of their cell envelope (Swamy et al., 2016; Silva et al., 2021). This complexity is characterized by a relatively thin peptidoglycan layer underlying an outer membrane composed of glycolipids, mainly lipopolysaccharides (Silhavy et al., 2010), which exhibit a hydrophilic nature that selectively limits the diffusion of hydrophobic compounds from EOs (Tariq et al., 2019). Implying a differential sensitivity profile between Gram-positive and Gram-negative bacteria.

Table 3. Antioxidant activity of Ageratum conyzoides L. essential oil. Mean values of triplicates ± standard deviation. Means followed by different letters in the same column differ statistically (P < 0.05) by the Unpaired t-test.

Samples	IC_{50} (μg/mL)	
	DPPH	**ABTS**
Essential oil	287.94^{a} ± 7.02	132.23^{a} ± 15.41
Trolox	10.14^{b} ± 0.0003	4.055^{b} ± 0.0004
Sample	**Antioxidant activity (μg trolox/g)**	
Essential oil	196.11 ± 16.41	1087 ± 4.94

Mean values of triplicates ± standard deviation. Means followed by different letters in the same column differ statistically (P < 0.05) by the Unpaired t-test.

Kurade et al. (2010) had already reported that Ageratum houstonianum, which also contains precocene I as one of its major compounds, has demonstrated proven antibacterial activity. Studies have also been conducted to confirm the antibacterial activity of caryophyllene (the second major compound of EO A. conyzoides) against various bacterial strains (Dahham et al., 2015; Dickson et al., 2023).

As reported by Dahham et al. (2015), β-caryophyllene exhibited antibacterial activity against six bacterial strains, including S. aureus (MTCC 7405) and B. cereus (MTCC 1307). In a murine model of urinary tract infection (UTI) by the uropathogenic strain of E. coli CFT073 (ATCC 700928), antibacterial effects of caryophyllene were observed, with a significant reduction in bacterial load in the urine and bladder tissue of treated animals (Dickson et al., 2023).

Perigo et al. (2016) investigated the correlation between the chemical composition of essential oils from plants of different species of the genus Piper and their biological activity, demonstrating that the presence of high concentrations of trans-caryophyllene was linked to the inhibition of bacterial growth of E. coli (ATCC 8739) and S. aureus (ATCC 6538).

Van de Vel et al. (2019) pointed out that several factors can interfere with the different MIC values of plant essential oils, such as the technique applied, the strain of microorganism used, factors related to the plant, such as planting location, time of collection and whether the oil was obtained from fresh or dried plants, and the quantity tested. Essential oils from plant species can often have a more effective antimicrobial action against pathogens due to synergism between the bioactive constituents.

Following a therapeutic approach, the use of essential oils from plants, synergistically associated with antibiotic therapy, can be very promising for the treatment of bacterial

diseases, and can contribute to reducing bacterial resistance caused by the drugs of choice and reducing treatment costs (Majeed et al., 2023; Apinundecha et al., 2023).

Due to the increased rate of antibiotic-resistant enteric bacteria, enteritis treatment can be carried out in conjunction with herbal medicines (Gillings and Stokes, 2012). Ostrosky et al. (2008) have reported on the importance of developing and producing pharmaceutical and cosmetic products from plant extracts with antimicrobial action. Thus, research with plants leads to a promising and effective way of discovering new medicines, and it is necessary to elucidate the active components present in them, as well as their mechanisms of action.

The safe therapeutic application of A. conyzoides EO, as with any other compound, requires an evaluation of its cytotoxicity to achieve a balance between antibacterial activity and cell viability after exposure to the essential oils. The present analysis of cytotoxic activity, assessed using the RAW 264.7 cell line in an MTT assay, demonstrates that A. conyzoides EO, at concentrations ranging from 15.62 to 1000 µg/mL, did not result in a significant reduction in the viability of RAW 264.7 cells, suggesting a favorable safety profile.

The antioxidant activity of essential oils has been extensively studied due to their ability to neutralize free radicals and reduce oxidative stress, a key factor in the development of various chronic diseases, including heart disease, cancer, and age-related disorders (Huang et al., 2005; Mishra et al., 2012; Sharopov et al., 2015). Additionally, essential oils exhibit antimicrobial properties. Their hydrophobic nature enables interaction with cellular membranes, leading to structural damage, leakage of essential compounds, and disruption of energy production, ultimately compromising cell viability (El-Sherbiny and Elbestawy, 2022).

In the present evaluation of the in vitro antioxidant activity of A. conyzoides essential oil using DPPH and ABTS radical scavenging assays, a relatively low capacity to reduce DPPH (IC50: 287.94 ± 7.02 µg/mL; TEAC: 196.11 ± 16.41 µg trolox/g) and ABTS (IC50: 132.23 ± 15.41 µg/mL; TEAC: 1087 ± 4.94 µg trolox/g) free radical concentration was observed. These results are consistent with previous studies, such as those by Patil et al. (2010), which reported that the antioxidant activity of A. conyzoides EO in DPPH radical reduction assays, measured by an IC50 of 570.00 ± 6.00 mg/mL, was substantially lower than that of methanol extract, which had an IC50 of 22.50 ± 3.18 mg/mL, while ascorbic acid, used as a standard antioxidant, had an IC50 of 3.70 ± 1.00 µg/mL. The authors attribute the higher antioxidant activity of A. conyzoides methanol extract to its higher flavonoid content. These observations are consistent with those of Bayala et al. (2014), who found that A. conyzoides EO showed limited DPPH radical reduction capacity (32.37 ± 4.25%) and ABTS (0.53 ± 0.02 mmol TE/g) in vitro, whereas quercetin (1 mg/mL), used as a reference antioxidant, showed a DPPH radical reduction of 73.13 ± 5.25% and an ABTS radical reduction capacity of 8.96 ± 0.07 mmol TE/g.

The literature indicates that among the constituents of EOs, particularly the class of terpenes and their oxygenated derivatives (terpenoids), are often found in greater abundance and possess significant antioxidant potential, with terpenoid and phenolic components being notable examples (Bakkali et al., 2008; Jugreet and Mahomoodally, 2020). Previous studies have shown that β-caryophyllene (a sesquiterpene) exhibits free radical scavenging activity, as demonstrated by the DPPH and Ferric Reducing Antioxidant Power (FRAP) assays (Dar et al., 2011; Dahham et al., 2015). However, linking the antioxidant activity of an EO to a limited number of active compounds presents significant challenges. This is due to the underlying mechanisms of this activity, which emerge from the complex interaction between the chemical composition of the EO, featuring different profiles of bioactive compounds, and the presence of synergistic or antagonistic components affecting antioxidant activity. Additionally, the structural characteristics of the bioactive compounds and the experimental conditions also play crucial roles (Sanchez-Moreno, 2002; Viuda-Martos et al., 2010).

5. Conclusion

The results show that the essential oil of A. conyzoides has promising antibacterial activity, especially against gram-positive bacteria. It could emerge as a promising alternative for controlling antimicrobial-resistant bacteria. The essential oil also demonstrated a low antioxidant capacity. Additionally, it was found that the essential oil exhibited no cytotoxicity against the macrophage cell line, indicating a favorable safety profile. However, in vivo investigations are needed to determine the true potential.

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Data Availability Statement

The entire data set that supports the results of this study was published in the article itself.

References

ACHMAD, H., ADAM, A.M., AZIZAH, A., SUKMANA, B.I., KHERA, S.N. and RAMADHANY, Y.F., 2020. A review of bandotan leaf extract (Ageratum conyzoides L.) in inhibition test to the growth of bacteria (Porphyromonas gingivalis) case of periodontitis disease. Systematic Reviews in Pharmacy, vol. 11, no. 4, pp. 390-395. http://doi.org/10.31838/srp.2020.4.58.

ADAMS, R.P., 2007. Identifcation of essential oil components by gas chromatography/mass spectrometry. 4th ed. Carol Stream, IL: Allured Publishing Corporation, 809 p.

ALLAMI, R.H., MOUHAMAD, R.S., ABDULATEEF, S.A. and ABEDALELAH AL-KHAFAJI, K., 2020. Antimicrobial activity of herbal mixture extract combination on microorganisms isolated from urinary tract infection. Bionatura, vol. 5, no. 4, pp. 1346-1351. http://doi.org/10.21931/RB/2020.05.04.11.

ANDRADE-OCHOA, S., CHACÓN-VARGAS, K.F., SÁNCHEZ-TORRES, L.E., RIVERA-CHAVIRA, B.E., NOGUEDA-TORRES, B. and NEVÁREZ-MOORILLÓN, G.V., 2021. Differential antimicrobial effect of essential oils and their main components: insights based on the cell membrane and external structure. Membranes, vol. 11, no. 6, pp. 405. http://doi.org/10.3390/membranes11060405. PMid:34071618.

APINUNDECHA, C., TEETHAISONG, Y., SUKNASANG, S., AYAMUANG, I. and EUMKEB, G., 2023. Synergistic Interaction between Boesenbergia rotunda (L.) Mansf. essential oil and cloxacillin on methicillin-resistant Staphylococcus aureus (MRSA) Inhibition. Evidence-based Complementary and Alternative Medicine, vol. 2023, no. 1, pp. 3453273. http://doi.org/10.1155/2023/3453273.

BAKKALI, F., AVERBECK, S., AVERBECK, D. and IDAOMAR, M., 2008. Biological effects of essential oils: a review. Food and Chemical Toxicology, vol. 46, no. 2, pp. 446-475. http://doi.org/10.1016/j.fct.2007.09.106. PMid:17996351.

BAYALA, B., BASSOLE, I.H.N., GNOULA, C., NEBIE, R., YONLI, A., MOREL, L., FIGUEREDO, G., NIKIEMA, J.B., LOBACCARO, J.M.A. and SIMPORE, J., 2014. Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from Burkina Faso. PLoS One, vol. 9, no. 3, pp. e92122. http://doi.org/10.1371/journal.pone.0092122. PMid:24662935.

BRAND-WILLIAMS, W., CUVELIER, M.E. and BERSET, C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft + Technologie, vol. 28, no. 1, pp. 25-30. http://doi.org/10.1016/S0023-6438(95)80008-5.

CASTRO, H.G., OLIVEIRA, L.O., BARBOSA, L.C.D.A., FERREIRA, F.A., SILVA, D.J.H.D., MOSQUIM, P.R. and NASCIMENTO, E.A., 2004. Teor e composição do óleo essencial de cinco acessos de mentrasto. Quimica Nova, vol. 27, no. 1, pp. 55-57. http://doi.org/10.1590/S0100-40422004000100011.

CLINICAL AND LABORATORY STANDARDS INSTITUTE – CLSI, 2020. Performance standards for antimicrobial susceptibility testing. 30th ed. Wayne: Clinical and Laboratory Standards Institute. CLSI supplement M100.

DAHHAM, S., TABANA, Y., IQBAL, M., AHAMED, M., EZZAT, M., MAJID, A. and MAJID, A., 2015. The Anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna. Molecules, vol. 20, no. 7, pp. 11808-11829. http://doi.org/10.3390/molecules200711808. PMid:26132906.

DAR, M.Y., SHAH, W.A., RATHER, M.A., QURISHI, Y., HAMID, A. and QURISHI, M.A., 2011. Chemical composition, in vitro cytotoxic and antioxidant activities of the essential oil and major constituents of Cymbopogon jawarancusa (Kashmir). Food Chemistry, vol. 129, no. 4, pp. 1606-1611. http://doi.org/10.1016/j.foodchem.2011.06.016.

DICKSON, K., SCOTT, C., WHITE, H., ZHOU, J., KELLY, M. and LEHMANN, C., 2023. Antibacterial and analgesic properties of beta-caryophyllene in a murine urinary tract infection model. Molecules, vol. 28, no. 10, pp. 4144. http://doi.org/10.3390/molecules28104144. PMid:37241885.

ELBESTAWY, M.K.M., EL-SHERBINY, G.M. and MOGHANNEM, S.A., 2023. Antibacterial, antibiofilm and anti-inflammatory activities of eugenol clove essential oil against resistant helicobacter pylori. Molecules, vol. 28, no. 6, pp. 2448. http://doi.org/10.3390/molecules28062448. PMid:36985419.

EL-SHERBINY, G.M. and ELBESTAWY, M.K.M., 2022. A review: plant essential oils active against Helicobacter pylori. Journal of Essential Oil Research, vol. 34, no. 3, pp. 203-215. http://doi.org/10.1080/10412905.2022.2025464.

FODA, A.M., KALABA, M.H., EL-SHERBINY, G.M., MOGHANNEM, S.A. and EL-FAKHARANY, E.M., 2022. Antibacterial activity of essential oils for combating colistin-resistant bacteria. Expert Review of Anti-Infective Therapy, vol. 20, no. 10, pp. 1351-1364. http://doi.org/10.1080/14787210.2022.2101997. PMid:35839089.

FURTADO, R.F., LIMA, M.G.A., ANDRADE NETO, M., BEZERRA, J.N.S. and SILVA, M.G., 2005. Atividade larvicida de óleos essenciais contra Aedes aegypti L. (Diptera: culicidae). Neotropical Entomology, vol. 34, no. 5, pp. 843-847. http://doi.org/10.1590/S1519-566X2005000500018.

GAUTAM, N., MANTHA, A.K., and MITTAL, S., 2014. Essential oils and their constituents as anticancer agents: a mechanistic view. BioMed Research International, vol. 2014, no. 1, pp. 154106. http://doi.org/10.1155/2014/154106.

GILLINGS, M.R. and STOKES, H.W., 2012. Are humans increasing bacterial evolvability? Trends in Ecology & Evolution, vol. 27, no. 6, pp. 346-352. http://doi.org/10.1016/j.tree.2012.02.006. PMid:22459247.

GORLENKO, C.L., BUDANOVA, E.V., ZAMYATNIN, A.A. and IKRYANNIKOVA, L.N., 2020. Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics? Antibiotics, vol. 9, no. 4, pp. 170. http://doi.org/10.3390/antibiotics9040170. PMid:32290036.

GUTIÉRREZ, I.E.M., SOUZA, L.M.C., MAGALHÃES, A.O., PERALTA, E.D., DE OLIVEIRA, L.M., LUCCHESE, A.M. and DA SILVA, L.T.S., 2021. Characterization the herbal drug and antioxidant activity of Ageratum conyzoides L. Brazilian Journal of Development, vol. 7, no. 8, pp. 78766-78781. http://doi.org/10.34117/bjdv7n8-209.

HUANG, D., OU, B. and PRIOR, R.L., 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, vol. 53, no. 6, pp. 1841-1856. http://doi.org/10.1021/jf030723c. PMid:15769103.

JUGREET, B.S. and MAHOMOODALLY, M.F., 2020. Pharmacological properties of essential oil constituents and their mechanisms of action. In: M.K. SWAMY, ed. Plant-derived bioactives. Singapore: Springer, pp. 387-415. http://doi.org/10.1007/978-981-15-2361-8_18.

KOUAME, B.K.F.P., TOURE, D., KABLAN, L., BEDI, G., TEA, I., ROBINS, R., CHALCHAT, J.C. and TONZIBO, F., 2018. Chemical constituents and antibacterial activity of essential oils from flowers and stems of Ageratum conyzoides from Ivory Coast. Records of Natural Products, vol. 12, no. 2, pp. 160-168. http://doi.org/10.25135/rnp.22.17.06.040.

KUMAR, K.G.A., TAYADE, A.B., KUMAR, R., GUPTA, S., SHARMA, A.K., NAGAR, G., TEWARI, S.S., KUMAR, B., RAWAT, A.K.S., SRIVASTAVA, S., KUMAR, S. and GHOSH, S., 2016. Chemo-profiling and bioassay of phytoextracts from Ageratum conyzoides for acaricidal properties against Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) infesting cattle and buffaloes in India. Ticks and Tick-Borne Diseases, vol. 7, no. 2, pp. 342-349. http://doi.org/10.1016/j.ttbdis.2015.12.005. PMid:26723275.

KURADE, N.P., JAITAK, V., KAUL, V.K. and SHARMA, O.P., 2010. Chemical composition and antibacterial activity of essential oils of Lantana camara, Ageratum houstonianum and Eupatorium adenophorum. Pharmaceutical Biology, vol. 48, no. 5, pp. 539-544. http://doi.org/10.3109/13880200903193336. PMid:20645797.

LIMA, R.K., CARDOSO, M., MORAES, J.C., CARVALHO, S.M., MELO, B.A. and VIEIRA, S.S., 2014. Composição química e toxicidade de óleos essenciais para o pulgão-verde Schizaphis graminum (Rondani, 1852). Arquivos do Instituto Biológico, vol. 81, no. 1, pp. 22-29. http://doi.org/10.1590/S1808-16572014000100005.

LIU, X.C. and LIU, Z.L., 2014. Evaluation of larvicidal activity of the essential oil of Ageratum conyzoides L. aerial parts and its major constituents against Aedes albopictus. Journal of Entomology and Zoology Studies, vol. 2, no. 4, pp. 345-350.

MAJEED, A., GULERIA, S., SHARMA, N., SALARIA, K.H., AIMAN, F., SINGH, B. and GUPTA, V.K., 2023. Antioxidant capacity and combinatorial antimicrobial effects of Nardostachys jatamansi essential oil with conventional antibiotics against some drug resistant bacteria. Current Research in Biotechnology, vol. 5, pp. 100118. http://doi.org/10.1016/j.crbiot.2022.100118.

MARTINS, A.P., SALGUEIRO, L.R., GONÇALVES, M.J., VILA, R., CAÑIGUERAL, S., TOMI, F. and CASANOVA, J., 2005. Essential oil composition and antimicrobial activity of Ageratum conyzoides from S. Tomé and Príncipe. The Journal of Essential Oil Research, vol. 17, no. 3, pp. 239-242. http://doi.org/10.1080/10412905.2005.9698888.

MENTARI, I.A., WIRNAWATI, W., and PUTRI, M.R., 2020. Karakterisasi simplisia dan ekstrak daun bandotan (Ageratum conyzoides L) sebagai kandidat obat karies gigi. Jurnal Ilmiah Ibnu Sina (JIIS): Ilmu Farmasi dan Kesehatan, vol. 5, no. 1, pp. 1-9. http://doi.org/10.36387/jiis.v5i1.346.

MISHRA, K., OJHA, H. and CHAUDHURY, N.K., 2012. Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. Food Chemistry, vol. 130, no. 4, pp. 1036-1043. http://doi.org/10.1016/j.foodchem.2011.07.127.

MONDELLO, L., 2011. Flavors and fragrances of natural and synthetic compounds. Italy: Messina University, Wiley.

NEVES, D.S.C., SANTANA, G.N. and KREPSKY, P.B., 2021. Variação intraespecífica na composição e teor do óleo essencial de Lippia thymoides. Revista Fitos, vol. 15, no. 2, pp. 192-203. http://doi.org/10.32712/2446-4775.2021.1062.

OSTROSKY, E.A., MIZUMOTO, M.K., LIMA, M.E.L., KANEKO, T.M., NISHIKAWA, S.O. and FREITAS, B.R., 2008. Métodos para avaliação da atividade antimicrobiana e determinação da Concentração Mínima Inibitória (CMI) de plantas medicinais. Revista Brasileira de Farmacognosia, vol. 18, no. 2, pp. 301-307. http://doi.org/10.1590/S0102-695X2008000200026.

OTHMAN, L., SLEIMAN, A. and ABDEL-MASSIH, R.M., 2019. Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. Frontiers in Microbiology, vol. 10, pp. 911. http://doi.org/10.3389/fmicb.2019.00911. PMid:31156565.

PATIL, R.P., NIMBALKAR, M.S., JADHAV, U.U., DAWKAR, V.V. and GOVINDWAR, S.P., 2010. Antiaflatoxigenic and antioxidant activity of an essential oil from Ageratum conyzoides L. Journal of the Science of Food and Agriculture, vol. 90, no. 4, pp. 608-614. http://doi.org/10.1002/jsfa.3857. PMid:20355088.

PERIGO, C.V., TORRES, R.B., BERNACCI, L.C., GUIMARÃES, E.F., HABER, L.L., FACANALI, R., VIEIRA, M.A.R., QUECINI, V. and MARQUES, M.O.M., 2016. The chemical composition and antibacterial activity of eleven Piper species from distinct rainforest areas in Southeastern Brazil. Industrial Crops and Products, vol. 94, pp. 528-539. http://doi.org/10.1016/j.indcrop.2016.09.028.

QUOC, L.P.T., 2020. Physicochemical properties and antibacterial activity of essential oil of Ageratum conyzoides L. leaves. ACS. Agriculturae Conspectus Scientificus, vol. 85, no. 2, pp. 139-144.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. and RICE-EVANS, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, vol. 26, no. 9-10, pp. 1231-1237. http://doi.org/10.1016/S0891-5849(98)00315-3. PMid:10381194.

RISS, T.L., MORAVEC, R.A., NILES, A.L., DUELLMAN, S., BENINK, H.A., WORZELLA, T.J. and MINOR, L., 2013. Cell viability assays. In: S. MARKOSSIAN, A. GROSSMAN, H. BASKIR, M. ARKIN, D. AULD, C. AUSTIN, J. BAELL, K. BRIMACOMBE, T. D. Y. CHUNG, N. P. COUSSENS, J. L. DAHLIN, V. DEVANARAYAN, T. L. FOLEY, M. GLICKSMAN, K. GORSHKOV, S. GROTEGUT, M. D. HALL, S. HOARE, J. INGLESE, P. W. IVERSEN, M. LAL-NAG, Z. LI, J. R. MANRO, J. MCGEE, A. NORVIL, M. PEARSON, T. RISS, P. SARADJIAN, G. S. SITTAMPALAM, M. A. TARSELLI, O. J. TRASK, J. R. WEIDNER, M. J. WILDEY, K. WILSON, M. XIA and X. XU, eds. Assay guidance manual. Bethesda: Eli Lilly & Company and the National Center for Advancing Translational Sciences, pp. 403-427.

ROSÁRIO, C.J.R.M., LIMA, A.S., MENDONÇA, C.J.S., SOARES, I.S., JÚNIOR, E.B.A., GOMES, M.N., COSTA-JUNIOR, L.M., MAIA, J.G.S. and ROCHA, C.Q., 2023. Essential oil Ageratum conyzoides chemotypes and anti-tick activities. Veterinary Parasitology, vol. 319, pp. 109942. http://doi.org/10.1016/j.vetpar.2023.109942. PMid:37178553.

ROSSITER, S.E., FLETCHER, M.H. and WUEST, W.M., 2017. Natural products as platforms to overcome antibiotic resistance. Chemical Reviews, vol. 117, no. 19, pp. 12415-12474. http://doi.org/10.1021/acs.chemrev.7b00283. PMid:28953368.

SANCHEZ-MORENO, C., 2002. Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Science & Technology International, vol. 8, no. 3, pp. 121-137. http://doi.org/10.1177/1082013202008003770.

SHAROPOV, F.S., WINK, M. and SETZER, W.N., 2015. Radical scavenging and antioxidant activities of essential oil components: an experimental and computational investigation. Natural Product Communications, vol. 10, no. 1, pp. 153-156. http://doi.org/10.1177/1934578X1501000135. PMid:25920239.

SILHAVY, T.J., KAHNE, D. and WALKER, S., 2010. The bacterial cell envelope. Cold Spring Harbor Perspectives in Biology, vol. 2, no. 5, pp. a000414. http://doi.org/10.1101/cshperspect.a000414. PMid:20452953.

SILVA, B.D., BERNARDES, P.C., PINHEIRO, P.F., FANTUZZI, E. and ROBERTO, C.D., 2021. Chemical composition, extraction sources and action mechanisms of essential oils: natural preservative and limitations of use in meat products. Meat Science, vol. 176, pp. 108463. http://doi.org/10.1016/j.meatsci.2021.108463. PMid:33640647.

SOARES, G.A.B., BHATTACHARYA, T., CHAKRABARTI, T., TAGDE, P. and CAVALU, S., 2022. Exploring pharmacological mechanisms of essential oils on the central nervous system. Plants, vol. 11, no. 1, pp. 21. http://doi.org/10.3390/plants11010021. PMid:35009027.

SUGARA, T.H., IRAWADI, T.T., SUPRAPTO, I.H. and HANAFI, M., 2016. Anti bacteria activity of ethyl acetate fraction bandotan leaf (Agerantum conyzoides L). Journal Ilmiah Ibnu Sina, vol. 1, no. 1, pp. 88-96. http://doi.org/10.36387/jiis.v1i1.34.

SWAMY, M.K., AKHTAR, M.S. and SINNIAH, U.R., 2016. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence-Based Complementary and Alternative Medicine, vol. 2016, no. 1, pp. 1-21. http://doi.org/10.1155/2016/3012462. PMid:28090211.

TARIQ, S., WANI, S., RASOOL, W., SHAFI, K., BHAT, M.A., PRABHAKAR, A., SHALLA, A.H. and RATHER, M.A., 2019. A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. Microbial Pathogenesis, vol. 134, pp. 103580. http://doi.org/10.1016/j.micpath.2019.103580. PMid:31195112.

TELES, A.M., SILVA-SILVA, J.V., FERNANDES, J.M.P., ABREU-SILVA, A.L., CALABRESE, K. S., MENDES FILHO, N.E., MOUCHREK, A.N., and ALMEIDA-SOUZA, F., 2021. GC-MS characterization of antibacterial, antioxidant, and antitrypanosomal activity of Syzygium aromaticum essential oil and eugenol. Evidence-Based

Complementary and Alternative Medicine, vol. 2021, no. 1, pp. 6663255. http://doi.org/10.1155/2021/6663255.

VAN DE VEL, E., SAMPERS, I. and RAES, K., 2019. A review on influencing factors on the minimum inhibitory concentration of essential oils. Critical Reviews in Food Science and Nutrition, vol. 59, no. 3, pp. 357-378. http://doi.org/10.1080/10408398.2017.1371112. PMid:28853911.

VAN DEN DOOL, H. and KRATZ, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas: liquid partition chromatography. Journal of Chromatography. A, vol. 11, pp. 463-471. http://doi.org/10.1016/S0021-9673(01)80947-X. PMid:14062605.

VIEIRA, S.S., CARDOSO, M.G., SOUSA, P.E., GUIMARÃES, L.G.L., ANDRADE, M.A. and ANDRADE, J., 2012. Composição química e atividade fungitóxica do óleo essencial de Ageratum conyzoides L. (Mentrasto). Magistra, vol. 24, no. 1, pp. 55-62.

VIUDA-MARTOS, M., RUIZ NAVAJAS, Y., SÁNCHEZ ZAPATA, E., FERNÁNDEZ-LÓPEZ, J. and PÉREZ-ÁLVAREZ, J.A., 2010. Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour and Fragrance Journal, vol. 25, no. 1, pp. 13-19. http://doi.org/10.1002/ffj.1951.

VOON, H.C., BHAT, R. and RUSUL, G., 2012. Flower extracts and their essential oils as potential antimicrobial agents for food uses and pharmaceutical applications. Comprehensive Reviews in Food Science and Food Safety, vol. 11, no. 1, pp. 34-55. http://doi.org/10.1111/j.1541-4337.2011.00169.x.

VORAVUTHIKUNCHAI, S.P. and KITPIPIT, L., 2005. Activity of medicinal plant extracts against hospital isolates of methicillin-resistant Staphylococcus aureus. Clinical Microbiology and Infection, vol. 11, no. 6, pp. 510-512. http://doi.org/10.1111/j.1469-0691.2005.01104.x. PMid:15882206.