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Chemical Composition and Antimicrobial Activity of the Essential Oils of *Ageratum fastigiatum* (Asteraceae)

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Abstract: Essential oils from different organs of *Ageratum fastigiatum* (Gardn.) R. M. King et H. Rob., obtained by hydrodistillation were analyzed through Gas Chromatography and Gas Chromatography/Mass Spectrometry. The essential oils were tested for antimicrobial activity by the agar-diffusion method and minimal inhibitory concentration (MIC). The main compounds found were β-caryophyllene, germacrene D and 1,10-di-epi-cubenol. The samples were active against *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus faecalis*, *Salmonella typhosa*, *Escherichia coli*, but not *Pseudomonas aeruginosa* and fungi. The MIC ranged from 5.0 to 18.0 mg/mL.

Keywords: *Ageratum fastigiatum*; essential oil composition; *β*-caryophyllene; germacrene-D; 1,10-di-epi-cubenol; antimicrobial activity.

1. Introduction

Essential oils are important constituents of some higher plants comprising monoterpenes, sesquiterpenes, arylpropanoids, and other derivatives. The antimicrobial properties of essential oils have been recognized long ago and they have been scientifically established [1]. The family

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Asteraceae includes about 25000 cosmopolitan species, many of which are rich in essential oil producers with biological activity [2]. The genus *Ageratum* belongs to the Eupatoriae tribe [3] and many of its species, such as *A. conyzoides*, have been chemically studied [2,4,5].

Ageratum fastigiatum (Gardn.) R. M. King et H. Rob. is a plant found in Minas Gerais, Southestern Brazil [6,7]. Phytochemical studies have identified diterpenes, triterpenes, and derivatives from *A. fastigiatum* [8,9]. This species presented antinociceptive and antimicrobial activities and the leaves have been used in folk medicine to treat inflammation, infections and as analgesic [10].

Up to the present there is no study of the chemical constituents and antimicrobial activity of the *A. fastigiatum* oils. In this paper, the composition of the essential oils from leaves, branches, and roots of *A. fastigiatum* and their antimicrobial tests will be described.

2. Materials and Methods

2.1. Plant Material

Plant material from species *A. fastigiatum* was collected in March 2005 in the county of São João Del-Rei, state of Minas Gerais, Brazil. The species was identified by Dr. Roberto Lourenço Esteves, and a voucher specimen (nº 10329) has been deposited in the Herbarium of the State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil.

2.2 Isolation of the Essential Oils

Fresh leaves, branches and roots of *A. fastigiatum* (200 g) were separated, triturated and hydrodistilled in a Clevenger-type apparatus [11]. After 2 h of distillation, the essential oils were removed from the surface of the water. The oils were dried over anhydrous sodium sulphate. The samples were sealed and kept in dark glass vials in the refrigerator for further analysis

2.3 Gas chromatography analysis (GC/FID)

Capillary gas chromatography was performed using a Hewlett-Packard 6890 gas chromatograph; fused silica capillary column HP-5 (5% diphenyl and 95% dimethylpolysyloxane, 60 m x 0.25 mm, 0.25 μm film thickness); helium as carrier gas (1 mL/min); and temperature programming from 70 to 290°C (2°C/min); injector temperature 270°C and detector temperature 300°C.

2.4 Gas chromatography/mass spectrometry analysis (GC/MS)

The GC/MS analysis of the oils were performed on a Hewlett Packard series 6890 gas chromatograph coupled to MS HP5972 mass spectrometer under the following analytical conditions: ZB-5MS column (30 m x 0.25 mm x 0.25 μm film thickness); helium (1 mL/min); programmed temperature 60º-240ºC (3ºC/min); injector temperature (260ºC) and interface (200ºC); ionization energy, 70 eV; scan range, 30-300 amu; scan time, 1 s. Compound identification was based on the comparison of retention indices (determined relatively to the retention times of a n-alkanes series), mass spectra and the NIST spectrometer data bank, as well as comparison with literature data [12].

2.5 Microbial strains

The essential oils of *A. fastigiatum* were tested against a panel of microorganisms including *Staphylococcus aureus* (ATCC 6538), *Streptococcus mutans* (ATCC 25175), *Streptococcus faecalis* (ATCC 29212), *Salmonella typhosa* (ATCC 10708), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 15442), *Candida albicans* (ATCC10231) and *C. tropicalis* (ATCC 13803). These microorganisms, belonging to the American Type Culture Collection (ATCC), were supplied by the Instituto Nacional de Controle e Qualidade em Saúde of the Fundação Oswaldo Cruz, Rio de Janeiro, Rio de Janeiro, Brazil.

2.6 Screening for antimicrobial activity

The antimicrobial activity was tested through agar diffusion method [13]. Tryptone soy agar was used as the standard test medium for bacteria and Sabouraud agar for yeast. Fresh cultures were prepared and used to inoculate 50 mL of tryptone soy broth that was incubated at 35 ºC for 18 h. Organisms were washed twice with phosphate buffer saline (PBS) and resuspended. Overnight broth cultures were prepared, adjusted in peptone-physiological salt solution (1 g peptone and 8.5 g/L NaCl) to yield approximately 10^6 bacteria/mL and 10^5 conidia/mL. The agar plates were prepared in 90 mm Petri dishes with 22 mL of agar medium giving a final depth of 3 mm. Cylinders (diameter 5.5 mm) were placed on the inoculated agar surfaces and filled with 100 µL of diluted oil in hexane. Each 100 µL had 10, 25 or 50 mg of essential oil. Hexane (100 µL) was used as negative control. All plates were aerobically incubated at 35 ºC for 18-24 h (bacteria) and at 22 ºC for 48 h (yeast). The antimicrobial activity was estimated by measuring the radius of the inhibition zone (mm). Each test was performed in duplicate and the results were shown as means. Chloramphenicol (30 µg) and nystatin (100 units) were used as positive controls.

2.7 Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined through the dilution method [14]. Bacteria were grown in nutrient broth (brain heart infusion liquid medium) for 6 h. After this, 20 µL of 10^6 cells/mL were inoculated in tubes with nutrient broth supplemented with eight different concentrations (25, 50, 100, 200, 400, 500, 600, and 800 µL) of the oils (30 mg/mL). After 24 h at 37 ºC, the MIC of each sample was measured through optical density in the spectrophotometer (620 nm), through the comparison of the sample readout with the non-inoculated nutrient broth [15]. All determinations were performed in duplicate.

3. Results and Discussion

The fresh parts of *A. fastigiatum* were found to contain 0.4, 0.2, and 0.2% of essential oil for leaves, branches and roots, respectively. Twenty compounds were identified in the essential oil from leaves, thirteen from branches, and fifteen from roots, representing 100.0, 98.7, and 100.0% of each total oil respectively (Table 1). The compounds identified in the essential oils from *A. fastigiatum* belong to sesquiterpenes series, except for nonadecane found in the leaves. The leaf oil was characterized by the abundance of germacrene-D (20.0%), β-caryophyllene (19.6%) and 1,10-di-epi-cubenol (15.4%). β-Caryophyllene (34.9%), germacrene-D (26.5%) and 1,10-di-epi-cubenol (5.9%) were also the main constituents in the branches while β-caryophyllene (25.5%), α-humulene (12.7%) and caryophyllene oxide (10.7%) were the major representatives in the roots. It was observed that β-caryophyllene had a significant presence in the oils analyzed, thus being a true chemical marker for the *A. fastigiatum* essential oil.

Table 1. Main components of the essential oils from *Ageratum fastigiatum*

Compound	Retention Indices*	Yield (%)			Method of identification
		Leaves	Branches	Roots	
δ-elemene	1334	3.1	4.3	-	RI, GC-MS
α-copaene	1377	-	2.0	-	RI, GC-MS
β-elemene	1390	0.9	3.4	5.0	RI, GC-MS
cyperene	1401	-	-	4.0	RI, GC-MS
β-caryophyllene	1420	19.6	34.9	25.5	RI, GC-MS
β-cedrene	1422	1.9	2.9	-	RI, GC-MS
β-gurjunene	1434	0.9	1.4	-	RI, GC-MS
α-humulene	1455	4.9	9.6	12.7	RI, GC-MS
γ-muurolene	1477	0.9	-	-	RI, GC-MS
germacrene-D	1481	20.0	26.5	4.6	RI, GC-MS
β-selinene	1489	3.9	4.5	-	RI, GC-MS
valencene	1496	1.1	-	-	RI, GC-MS
γ-cadinene	1518	1.0	-	-	RI, GC-MS
cis-calamenene	1520	0.9	-	-	RI, GC-MS
δ-cadinene	1522	2.2	1.0	-	RI, GC-MS
elemol	1547	-	1.0	6.1	RI, GC-MS
spathulenol	1575	1.0	-	4.8	RI, GC-MS
caryophyllene oxide	1578	1.3	1.3	10.7	RI, GC-MS
viridiflorol	1592	0.8	-	-	RI, GC-MS
humulene epoxide II	1605	-	-	3.5	RI, GC-MS
1,10-di-epi-cubenol	1616	15.4	5.9	4.4	RI, GC-MS
hinesol	1637	-	-	3.3	RI, GC-MS
epi-α-cadinol	1641	1.7	-	-	RI, GC-MS
cedr-8(15)-en-9-α-ol	1652	-	-	4.8	RI, GC-MS
α-muurolol	1646	-	-	5.9	RI, GC-MS
himachalol	1650	-	-	2.0	RI, GC-MS
α-bisabolol	1686	3.8	-	-	RI, GC-MS
(<8S,14>) cedrane-diol	1890	-	-	2.6	RI, GC-MS
nonadecane	1900	14.6	-	-	RI, GC-MS
Total yield (%)		99.9	98.7	99.9	

*in HP-5 Column

The essential oils were evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria strains. Using agar diffusion method, the essential oils were actives against *S. aureus*, *S. mutans*, *S. faecalis*, *E. coli*, and *S. typhosa* but not on *P. aeruginosa* and the yeasts *C. albicans* and *C. tropicalis* (Table 2). The inhibition zones varied between 6 and 12 mm. The MIC values confirmed the activity against the tested microorganism, as shown in Table 3. The MIC values of oils ranged from 5.0 to 18.0 mg/mL, while the MIC values for chloramphenicol varied from 1.25 to 5.00 mg/mL.

Table 2. Antimicrobial activities of essential oils of *Ageratum fastigiatum*

Microorganisms	Inhibition zone (mm)									
	Leaves (mg)			Branches (mg)			Roots (mg)			Control
	10	25	50	10	25	50	10	25	50	
S. aureus	-	7	9	-	8	10	7	8	12	33
S. mutans	-	7	8	-	6	6	-	6	8	30
S. faecalis	-	-	7	-	6	8	-	7	8	31
E. coli	6	7	8	6	7	8	-	6	7	25
S. typhosa	-	-	6	-	6	7	-	6	6	29
P. aeruginosa	-	-	-	-	-	-	-	-	-	26
C. albicans	-	-	-	-	-	-	-	-	-	20
C. tropicalis	-	-	-	-	-	-	-	-	-	21

Experiments were done in duplicate and results are mean values.

The essential oils evaluated in this study presented a great variety of sesquiterpenes that could be considered as answerable for the antimicrobial activity. Although they usually occur as complex mixtures, their activity may generally account for in terms of their major components. Antibacterial activity was also reported with essential oils in plants belonging Asteraceae family [16, 17]. Probably, similar components such as germacrene-D and β-caryophyllene detected in our experiments could be responsible for this property [18]. β-Caryophyllene, for example, was active against *S. aureus* [19]. α-Humulene, caryophyllene oxide, β-selinene and elemol, which were found in appreciable amounts in the oils characterized in this study, have been associated with the antimicrobial activity [18, 20-21]. α-Bisabolol, although a minor constituents, seems to be responsible by antibacterial activity in *Lantana achyranthifolia* [23]. However, it should be considered that minor and major components, as well as possible interactions between the substances, could contribute with the antimicrobial properties studied. In addition, these results may justify the use of *A. fastigiatum* in traditional medicine [10]. Therefore, the essential oils from this species are potential candidates to be used as antimicrobial agents in new drugs for therapy of infectious diseases. Further toxicological and clinical studies are required to prove the safety of the oil as a medicine.

Table 3. Minimal inhibitory concentration (MIC) exhibited by the *Ageratum fastigiatum* oils

Microorganisms	MIC (mg/mL)			
	Leaves	Branches	Roots	Control
S. aureus	9.0	5.0	5.0	2.5
S. mutans	12.5	9.0	6.25	1.25
S. faecalis	18.0	12.5	6.25	2.5
E. coli	12.5	9.0	6.25	5.0
S. typhosa	18.0	6.25	6.25	5.0
P. aeruginosa	-	-	-	5.0

Experiments were done in duplicate and results are mean values.
Control: Chloramphenicol (mg/mL x 10^{-3}).

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