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Antibacterial activity and chemical composition of essential oil of *Lippia microphylla* Cham

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ABSTRACT. The essential oil from the fresh leaves of *Lippia microphylla* Cham. was obtained by hydrodistillation and characterized by gas chromatography-mass spectrometry (GC-MS). Major constituents of the oil were 1.8-cineole (18.12%), β-ocimene (15.20%), bicyclogermacrene (11.63%) and caryophyllene oxide (8.32%). Antimicrobial activity of the oil against Gram-positive and Gram-negative bacteria was determined by gel diffusion method. The oil showed good antibacterial activity against *Shigella flexneri*, *Escherichia coli* and *Streptococcus pyogenes* and very good antibacterial activity against *Staphylococcus aureus*.

Keywords: antibacterial activity, 1,8-cineole, verbenaceae, *Staphylococcus aureus*.

RESUMO. Atividade antibacteriana e composição química do óleo essencial de *Lippia microphylla* Cham. O óleo essencial das folhas frescas de *Lippia microphylla* Cham. foi obtido por hidrodestilação e caracterizado por cromatografia gasosa acoplada a espectrometria de massas (CG-EM). O constituinte majoritário do óleo foi 1.8-cineol (18,12%), β-ocimeno (15,20%), bicyclogermacreno (11,63%) e óxido de caryophylleno (8,32%). A atividade antimicrobiana do óleo frente às bactérias Gram-positivas e Gram-negativas foi determinada pelo método de difusão em gel. O óleo apresentou uma boa atividade antibacteriana frente a *Shigella flexneri*, *Escherichia coli* e *Streptococcus pyogenes* sendo o melhor resultado frente a *Staphylococcus aureus.*

Palavras-chave: atividade antibacteriana, 1,8-cineol, verbenaceae, *Staphylococcus aureus*.

Introduction

The genus *Lippia* comprises about 200 species distributed mainly in South and Central America and Tropical Africa territories (SANTOS et al., 2003). This genus has yielded a great number of medicinal and economically important species that are frequently used in folk medicine as remedies against many diseases, particularly for the treatment of coughs, bronchitis, indigestion, liver disorders, hypertension, dysentery, larvicidal and for certain skin disease. Most have shown interesting biological activities, including antiviral, antimalarial, anti-inflammatory, analgesic and antipyretic as well as antimicrobial and cytotoxic (COSTA et al., 2005).

Lippia microphylla Cham., popularly known as "Alecrim-de-tabuleiro", is a subshrub plant used in traditional medicine of Brazil to treat flu, cough, nasal congestion and bronchitis (MATOS, 1994; 2000). In a previous study (CHANH et al., 1988), it was reported the antimicrobial properties from a plant collected in Maranhão State, Brazil. This paper reports the chemical composition and antibacterial activity of essential oil from *L. microphylla* leaves collected in Crato, Ceará State, Brazil.

Material and methods

Plant material

The leaves of *L. microphylla* were collected from Horto de Plantas Medicinais e Aromáticas - Universidade Regional do Cariri (Crato, Brazil) in March, 2007. The plant material was identified by Dr Arlene Pessoa and a voucher specimen was deposited with the number 1660 at the Herbarium "Dárdano de Andrade Lima" of Universidade Regional do Cariri - URCA.

Oil isolation

The fresh leaves of *L. microphylla* (200 g) were triturated, and the oil extracted by hydrodistillation for two hours using a Clevenger modified apparatus (GOTTLIEB; MAGALHÃES, 1960). The oil was collected and then dried using anhydrous sodium

sulfate, and subsequently stored under low light conditions at < 4ºC until analysis. The oil yield was found to be 1.2% (ww^{-1}).

Essential oil analysis

Oil analysis was performed using a Shimadzu GC-17 A/ MS QP5050A (GC/MS system): DB-5HT capillary column (30 m x 0.251 mm, 0.1 μm film thickness); helium carrier gas at 1.7 mL $min.^{-1}$; injector temperature 270ºC; detector temperature 290ºC; column temperature 60ºC (2 min.) – 180ºC (1 min.) at 4ºC $min.^{-1}$, then 180º – 260ºC at 10ºC $min.^{-1}$ (10 min.). Scanning speed was 0.5 scan/sec from m/z 40 to 450. Split ratio (1:30). Injected volume: 1 μL of 5 mg mL^{-1} solution ethyl acetate. Solvent cut time = 3 minutes. The mass spectrometer operated using 70 eV of ionization energy. Identification of individual components was based on their mass spectral fragmentation based on two computer library MS searches (Wiley 229), retention indices, and comparison with published data (ADAMS, 2001; ALENCAR et al., 1990). The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID), also set at 250ºC. *n*-Alkanes were used as reference points in the calculation of relative retention indices. The concentration of the identified compounds was computed from the GC peak area without any correction factor.

Antibacterial Assay

Antibacterial activity of the oil from *L. microphylla* was examined using the gel diffusion method (BAUER et al., 1966; KONEMAM et al., 1993; ROMERO, 2001), based document M7-A6 (NCCLS, 2003). Standard bacterial lines *Shigella flexneri* (ATCC 12022), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus aureus* (ATCC 10390) and *Streptococcus pyogenes* (ATCC 19165) were furnished by the Oswaldo Cruz Foundation (Fiocruz-Brazil). These microorganisms were cultivated in Brain Heart Infusion media (BHI) and incubated at 37ºC for 24 hours. After this period they were replicated on Petri dishes containing Muller–Hilton (MH) agar. The plates containing the microorganisms were then perforated with the aid of a stainless steel handle (5 mm) and the cavities filled with 30 μL of the oil solutions at 10, 5, 2.5, 1.25, 0.62 and 0.31% concentrations. Trials were performed in triplicate and commercial antibiotic disks of chloramphenicol (30 μg $disk^{-1}$) and amikacin (30 μg $disk^{-1}$) (LABORCLIN) were employed as positive controls, while water and Tween 80 served as negative controls. Inhibition halos were measured 24 and 48 hours after initial exposure.

Results and discussion

The hydrodistillation of fresh leaves of *L. microphylla* presented yield of essential oil 1.2% of their total fresh weight. Twenty one components were identified representing 86.99% of the total oil. Data about qualitative and quantitative essential oil composition are presented in Table 1, where compounds are listed in order of their elution on the DB-1 column. The major constituents of the oil were 1.8-cineole (18.12%), β-ocimene (15.20%), bicyclogermacrene (11.63%) and caryophyllene oxide (8.32%). While in a species from Maranhão State (Brazil) the major constituents were 1.8-cineole (36.0%), thymol (11.1%) and α-pinene (10.9%) (COSTA et al., 2005). Differences in essential oil composition according to area of collection are well documented in Brazil, which depends upon genetic factors, environmental factors, and stage of the plant development (MORAIS et al., 2007).

Table 1. Chemical composition (%) of the Essential oil of *Lippia microphylla*.

Component	RT (min.)	Leaf oil (%)	Component	RT (min.)	Essential oil (%)
α-pinene	5.9	1.18	bicyclo-germancrene	33.2	11.63
β-pinene	7.4	1.06	δ-cadinene	34.6	1.94
mircene	8.0	1.07	valencene	35.3	2.15
β-ocimene	8.7	15.20	spathulenol	37.1	1.54
p-cimene	9.3	0.93	caryophylenne oxide	37.3	8.32
1,8-cineole	9.6	18.12	Ledol	38.3	3.36
Terpinolene	12.1	1.48	humulene oxide	38.5	0.91
β–caryophyllene	29.2	5.05	viridiflorol	40.1	0.89
aromadendrene	30.2	2.95	Globulol	40.7	1.59
α–humulene	30.9	2.16	aromadendrene oxide	41.2	1.31
germacrene D	32.4	4.15			

The essential oil was tested against five Gram-positive and Gram-negative bacteria. The results of the bioassays (Table 2) showed that the oil exhibited moderate to strong antibacterial activity against all the tested bacteria, especially *S. aureus*. The present study revealed that the essential oil of *L. microphylla* at different dilutions also showed a similar inhibitory activity to that of standard antibiotics. It is especially important for *S. aureus* that showed a halo of 15 mm at a concentration of 10%.

Table 2. Antimicrobial activity of the essential oil of *Lippia microphylla*.

Lines	Concentration of *L. microphylla* oil (%) Inhibition halo diameter (mm)							
	10	5	2.5	1.25	0.62	0.3	AMC⋆	AMP⋆
S. pyogenes	10 ± 0.4	9 ± 0.01	7 ± 0.8	-	-	-	23 ± 0.0	-
S. aureus	15 ± 0.8	12 ± 0.4	10 ± 0.5	8 ± 0.8	7 ± 0.0	-	19 ± 0.0	16 ± 0.0
S. flexneri	12 ± 0.5	10 ± 0.0	10 ± 0.8	-	-	-		
E. coli	7 ± 0.0	-	-	-	-	-	15 ± 0.3	12 ± 0.3
K. pneumoniae	12 ± 0.8	10 ± 0.5	8 ± 0.5	-	-	-	16 ± 0.3	14 ± 0.3

⋆Concentration used: (AMC) Amikacin 30 μg; (AMP) Ampicillin 30 μg.

Insensitive

Numerous studies have demonstrated that the essential oils of *Lippia* species are among the most potent essential oils with regard to antimicrobial properties (BOTELHO et al., 2007; SALGUEIRO et al., 2003; YILJOEN et al., 2005). This was confirmed and extended in the present study. According to the results, the essential oil had good *in vitro* antimicrobial activities against all five bacteria, except against the *E. coli*, which was sensitive only to the concentration of 10%. These results are comparable to previously published activities for *Lippia* species (AGUIAR et al., 2008).

The relative tolerance of Gram-negative bacteria to essential oils has been ascribed to the presence of a hydrophilic outer layer, which can block the penetration of hydrophobic components through the target cell membrane. The oils rich in monoterpenes compounds are reported to exhibit high levels of antimicrobial activity (LAKUSICI et al., 2008).

The antibacterial activity of *L. microphylla* is probably related to the presence of the monoterpenes β-ocimene (15.20%) and 1,8-cineole (18.12%), which are compounds that have antimicrobial activity and synergism with the other constituents. (RODRIGUES et al., 2009; SONBOLI et al., 2005). The major component of *L. microphylla* was the monoterpene 1.8-cineole. Since the active antimicrobial compounds of essential oils are terpenes, it seems reasonable that their mode of action might be similar to that of other compounds. Most studies on the mechanism of terpenes compounds focused on their effects on cellular membranes, altering its function, causing swelling and increasing permeability. The increases in cytoplasmic membrane permeability appear to be a consequence of the loss of the cellular pH gradient, decreased ATP levels, and loss of the proton motive force, which lead to cell death (WINKOWSKI et al., 1994).

A number of studies indicate that essential oils containing carvacrol, eugenol or thymol have the highest antimicrobial properties (LAVABRE, 1993; YOUSEFZDI et al., 2008). Therefore, the antimicrobial activities of *Lippia* species do not arise only from the 1.8-cineole content, since the oil of *L. sidoides*, which is rich in thymol and carvracol also presented relatively good activity (BOTELHO et al., 2007). Some studies reported that whole essential oils have a greater antibacterial activity than the major components mixed, which suggests that the minor components are critical to the activity and could also affect the antimicrobial properties (SANTOS et al., 2007).

Conclusion

The results show that the essential oil of *L. microphylla* showed significant antibacterial activity in vitro, this effect can be associated with the content of monoterpenes present in essential oil and its ability to penetrate in the assemblies of lipids and a consequent disruption of the lipid fractions of plasma membrane.

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