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Antibacterial Activity of *Lippia alba* (Lemon Herb)

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SUMMARY. The essential oil of fresh leaves of *Lippia alba* (Mill.) N. E. Brown (Verbenaceae) popularly used in Brazil as a remedy for stomach disorders was analyzed by GC/MS and evaluated for antibacterial activity. The results showed that the essential oil was found to be effective against the tested microorganisms and confirm its traditional uses.

RESUMEN. "Actividad antibacteriana de *Lippia alba"*. *Lippia alba* (Mill.) N. E. Brown. (Verbenaceae) es una especie ampliamente utilizada en la medicina popular en Brasil como digestivo y antiséptico. La caracterización preliminar de los aceites esenciales obtenidos de las partes aéreas de la planta fue realizada por CG/EM. Con estos aceites esenciales se llevaron a cabo los análisis de la actividad antibacteriana. Los resultados encontrados mostraron una amplia actividad antibacteriana, confirmando así el uso de esta planta en la medicina popular.

INTRODUCTION

Leaves of *Lippia alba* (Mill.) N. E. Brown (Verbenaceae), popularly known as "erva cidreira" (lemon herb) in Brazil, was collected in January 2004 from South (Cascavel, Paraná) and North (Ji-Paraná, Rondonia) regions of Brazil. Authenticated vouchers, n° 2281 South region and n° 2282 North region, respectively, were deposited in the herbarium of the University (HUNOP).

In the traditional medicine of Brazil the species *L. alba* (Mill.) N.E. Brown is used as a remedy for stomach disorders [1,2], antibacterial and antiseptic for infectious diseases influenza, measles, rashes, and headaches [3,4]. The plant presents a great morphological and chemical variability that Matos [5] suggested the division into different chemotypes. So far, at least 12 chemotypes have been described: citral [5], linalool [6], carvone [5], limonene [7], γ-terpinene [8], citral-myrcene [6], citral-limonene [6], citral-β-caryophyllene [9], citral-germacrene-D [10], carvone-limonene [6], 1,8-cineol-camphor [11], 1,8-cineol-limonene [10], limonene-piperitone [12]. GC analyses of essential oils from *L. alba* revealed the predominance of monoterpene type compounds such as citral, β-myrcene, limonene and carvone [13].

MATERIAL AND METHODS

The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus. A gas chromatograph (Shimadzu QP2000A) equipped with flame ionization detection (FID), split/splitless injector (1:30 split ratio), and a data system (NIST/EPA/MSDC Mass Spectral Database) was used for GC analysis of essential oils. The detector and injector temperatures were set at 250 °C and 220 °C, respectively. A capillary column SE-30 (polidimethylsiloxane) 60 m coated with (0.20 μm film thickness) was used. The oven temperature was programmed from 35 °C (15 min) to 250 °C (40 min) at 3 °C

KEY WORDS: Antibacterial activity, Essential oil, *Lippia alba*.
PALABRAS CLAVE: Actividad antibacteriana, Aceite esencial, *Lippia alba*.

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min^{-1}. Helium (99.995%) was used as carrier gas. Hydrogen and air at 30 and 300 mL $min.^{-1}$, respectively, were used in the FID, with nitrogen (30 mL $min.^{-1}$) as a make-up gas. Compound identification was based on mass spectra (EI 70 eV) by comparison of mass spectra with those in a spectral database and retention indices. The antibacterial activity was performed by the disk diffusion method [14] using bacteria isolated in hospital environment from clinical patients some of them multi-drug resistant, except *Lactobacillus casei* isolated from Yakult 40®. For the antibacterial assay 5.0; 2.5; 1.25; 0.6 and 0.3 µg/µL of the oils were applied to paper disc of 5 mm of diameter. The inoculum was prepared by culturing each organism tryptona soya Agar (TSA, Oxoid) at 37 °C to turbidity equivalent to McFarland 0.5 standard (1,5 x 10^8 CFU/mL). One microliter of each dilutes inoculum (10^4 - 10^6 CFU) was applied onto Mueller Hinton Agar medium (MHA-DIFCO) and distributed over plates contend impregnated paper disc. The plates were incubated overnight at 37 °C. Ciclopirox olamine was used as positive control.

RESULTS

Essential oil (0.52% yield, south region, chemotype carvone) main constituents (GC/MS analysis): carvone (52.15%), neral (7.2%), geranial (7.8%), β-caryophyllene (6.3%), mircene (0.36%), caryophillene oxide (5.8 %), β-farnesene (3.2 %), δ-cadinol (0.29 %), linalool (0.20 %), germacrene-D (3.7%), *trans*-sabinol (1.8 %), 1-octen-3-ol (4.2%), *trans*-neral (7.0%).

Essential oil (0.48% yield, north region, chemotype citral) main constituents (GC/MS analysis): geranial (60.96%), neral (8.3%), β-caryophyllene (7.9%), mircene (5.1%), linalool (0.20%), β-farnesene (5.9%), germacrene-D (4.6%), δ-cadinol (0.21%), 1-octen-3-ol (6.3 %), nerolidol (0.23%).

The antibacterial activities of essential oils are shown in Tables 1 and 2.

Microorganisms		Control [a]	Essential oil from NORTH region [b]	Essential oil from SOUTH region [b]
		Zone of inhibition (mm) [c]		
Acinetobacter baumannii *	*Gram-*	6.0	5.0	7.0
Bacillus subtilis	*Gram+*	10.0	15.0	15.0
Escherichia coli	*Gram-*	9.0	17.0	17.0
Pseudomonas aeruginosa *	*Gram-*	5.0	n/a	n/a
Proteus mirabilis *	*Gram-*	7.0	n/a	n/a
Lactobacillus casei	*Gram+*	10.0	18.0	18.0
Staphylococcus intermedis	*Gram+*	12.0	18.0	20.0
Bacillus cereus	*Gram+*	17.0	n/a	n/a
Streptococcus mutans	*Gram+*	12.0	7.0	7.0

Table 1. Antibacterial activity of essential oil from fresh leaves of *L. alba* collected from north and south regions. [a] Ciclopirox Olamine 2 µg/disc; [b] 5 µL of crude essential oil/disc; n/a=not active; [c] average of triplicate; *= Multi-drug resistant bacteria.

Microorganisms	C [a]	Essential oil from NORTH region					Essential oil from SOUTH region				
		Zone of inhibition (mm) [b]									
		5.0	2.5	1.25	0.6	0.3	5.0	2.5	1.25	0.6	0.3
S. intermedis	12	18.0	16.0	n/a	n/a	n/a	20,0	17.0	16.0	n/a	n/a
B. subtilis	10	15.0	16.0	n/a	n/a	n/a	15,0	14.0	n/a	n/a	n/a
E. coli	9	17.0	n/a	n/a	n/a	n/a	17,0	16.0	n/a	n/a	n/a
L. casei	10	18.0	16.0	9.0	7.0	5.0	18.0	15.0	10.0	7.0	4.0

Table 2. Antibacterial activity (MIC, µg/µL) of essential oil from fresh leaves of *L. alba* collected from north and south regions against four bacteria. [a] C = Ciclopirox Olamine 2 µg/disc; n/a = not active; [b] average of triplicate.

CONCLUSIONS

Results revealed that essential oil from both regions exhibited variable levels of antibacterial activity against all tested bacterial strains. According the results, Gram-positive bacteria seemed to be more sensitive than Gram-negative bacteria. The essential oils displayed significant inhibitory activity against *Bacillus subtilis, Escherichia coli, Lactobacillus casei* and *Staphylococcus intermedis* showing inhibition zone higher than positive control. Due to the antibacterial activity observed, these microorganisms were used in the determination minimum inhibitory concentration. The results are in accordance to the folk use of this plant for infectious diseases confirm the ethnomedical use of *L. alba* (Mill) N.E. Brown against bacterial respiratory infections and its antimicrobial activity against other microorganisms. The antibacterial power of the oil can be considered to be due to the presence of geranial, germacrene-D, and linalool metabolites in the oils from both regions [15-18].

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