

Enhancement of the antibiotic activity of erythromycin by volatile compounds of *Lippia alba* (Mill.) N.E. Brown against *Staphylococcus aureus*

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ABSTRACT

Background: *Lippia alba* (Mill.) N.E. Brown, popularly known as “erva-cidreira,” is commonly found in northeastern Brazil. The leaves tea is used to treat digestive disturbances, nausea, cough, and bronchitis. **Objective:** This work reports the chemical composition and erythromycin-modifying activity by gaseous contact against *Staphylococcus aureus*. **Materials and Methods:** The leaves of *L. alba* were subjected to hydrodistillation, and the essential oil extracted was examined with respect to the chemical composition, by gas chromatography-mass spectrometry (GC-MS), and the essential oil extracted was evaluated for antibacterial and antibiotic-modifying activity by gaseous contact. **Results:** The overall yield of essential oil obtained by hydrodistillation was 0.52%. The GC-MS analysis has led to the identification of the main components: geranial (31.4%) and neral (29.5%). It was verified that the essential oil interfered with erythromycin antibiotic activity against *S. aureus* ATCC 25923 was enhanced (221.4%) in the presence of 12% essential oil. The 3% essential oil increased the effect against *S. aureus* ATCC 25923 (41.6%) and *S. aureus* ATCC 6538 (58.3%). **Conclusion:** The essential oil of *L. alba* influences the activity of erythromycin and may be used as an adjuvant in antibiotic therapy against respiratory tract bacterial pathogens.

Key words: Chemical composition, erythromycin, essential oil, *Lippia alba*, modulatory activity

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INTRODUCTION

Knowledge on medicinal plants sometimes means the only therapeutic resource of some communities and ethnic groups.^[1] Although the antibacterial properties of essential oils have been long recognized, the recent interest in alternative naturally derived antimicrobials has led to a renewed scientific interest in these substances.^[2]

The genus *Lippia* (Verbenaceae) comprises about 200 species of herbs, shrubs, and small trees.^[3] *Lippia alba* (Mill.) N.E. Brown, popularly known as “erva-cidreira,”

is commonly found in northeast Brazil. In many cases, leaves and flowers infusions or decoctions are employed orally to treat digestive disturbances, nausea, cough, and bronchitis.^[4,5]

Erythromycin belongs to the class of drugs known as macrolides and it acts on the ribosomal 50S subunit inhibiting the protein synthesis.^[6] It is estimated that 15% of *Staphylococcus aureus* strains are resistant to erythromycin and this drug is not recommended for empiric treatment of staphylococcal skin and soft tissues infections. A relatively high incidence of nausea and vomiting (oral formulation) or phlebitis (endovenous formulation) is reported.^[7] Recent studies^[8] showed that *S. aureus* is the most frequent microorganism in nasopharynx of children and 14% of the isolates were no susceptible to erythromycin.

This work reports the modulatory antimicrobial activity of the *Lippia alba* (Mill.) N. E. Brown essential volatile components by the gaseous contact method.

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MATERIALS AND METHODS

Plant material

Leaves of *Lippia alba* (Mill.) N.E. Brown were collected in March, 2010, from the Small Aromatic and Medicinal Plants Garden of the Natural Products Research Laboratory (LPPN) at University Regional do Cariri (URCA), Crato of county, Ceará state, Brazil. A voucher specimen was sent to the Herbarium Caririense Dárdano de Andrade - Lima – HCDAL, Department of Biological Sciences (URCA), which is deposited on the registration n° 5059.

Obtention of the essential oil

Samples of fresh leaves (236 g) were triturated and submitted to hydrodistillation process, in a Clevenger-type apparatus for 2 hours, resulting in essential oil yield of 0.52%. The collected essential oil was subsequently dried by anhydrous sodium sulfate (Na_2SO_4), and stored under refrigeration at $<4^\circ\text{C}$ until be tested.

Analysis of the essential oil

Analysis by CG/MS of the essential oil was carried out on a Hewlett-Packard Model 5971 GC/MS using a nonpolar DB-1-fused silica capillary column (30 m x 0.25 mm i.d., 0.25 m film thickness); carrier gas helium, flow rate 0.8 ml/min and with split mode. The injector temperature and detector temperature were 250°C and 200°C , respectively. The column temperature was programmed from 35°C to 180°C at $4^\circ\text{C}/\text{min}$ and then 180°C to 250°C at $10^\circ\text{C}/\text{min}$. Mass spectra were recorded from 30 to 450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer database using the Wiley L-built library and two other computer libraries MS searches using retention indices as a preselection routine,^[9] as well as by visual comparison of the fragmentation pattern with those reported in the literature.^[10]

Modulatory activity by gaseous contact

Antibacterial activity of the essential oil from *L. alba* (EOLA) was assayed using gaseous contact. An amount of 50 μg of oil was dissolved in 50 μl of DMSO (1 : 1). A two-fold dilution series of this essential oil solution was prepared: 50, 25, 12, 6, and 3 $\mu\text{g}/\text{ml}$ of oil.^[11]

Different *S. aureus* strains—ATCC 12692, ATCC 25923, and ATCC 6538—were used as standard. Brain heart infusion broth was used for bacterial growth (24 hours, 37°C). 10^5 CFU/ml of the inoculum from overnight culture of each bacterial species were inoculated in Mueller Hinton Petri dishes (90 mm diameter) by streaking method. Disks containing erythromycin (15 μg , LABORCLIN®) were used to determine changes in diameter of the zone of inhibition of studied strains.

After this, 50 μl of each dilution was added to the upper Petri dish and incubated for 24 hours at room temperature. Petri dishes without the essential oil and with DMSO were used as positive and negative controls, respectively.

RESULTS AND DISCUSSION

As can be observed in Table 1, geranial (31.5%) and neral (29.5%) were identified as the main components of *L. alba* essential oil. This chemical composition profile indicates that the natural product presents antimicrobial activity. According to Schuck *et al.*,^[12] citral has been shown to be more efficient than nystatin and benzalkonium chloride against *Candida albicans* strains. As stated by Onawunmi,^[13] alkaline pH increased citral activity and it can be used combined with others agents to enhance their antimicrobial activity.

As shown in Table 2, the synergic effect was verified for all strains tested. The most expressive effect was the 221.4% increase of the diameter of the zone of inhibition to erythromycin on *S. aureus* ATCC 25923 in the presence of 12% EOLA. 3% EOLA increased the erythromycin activity against *S. aureus* ATCC 25923 and ATCC 6538 (12.5% and 35.7%, respectively).

The antimicrobial activity of *L. alba* extracts and essential oil has been described in some studies;^[14-16] however, there is no report about the synergic effect between the essential oil and antibiotics by the gaseous contact method.

Essential oils may interact with and affect the plasma membrane, interfering with respiratory chain activity and energy production.^[17] Besides this, the lipophilic structure of plasma membrane facilitates the antibiotic entrance into the bacteria cell and a consequent increase in its activity.^[18,19] Impairment of bacterial enzyme systems

Table 1: Chemical composition (%) of the essential oil of *Lippia alba*

Component	RT* (min)	KI**	(%)
α -pinene	5.2	934	0.5
Limonene	7.7	1026	3.0
Eucalyptol	7.8	1030	0.6
cis-limonene oxide	11.0	1132	0.7
Neral	14.6	1236	29.5
Geranial	15.6	1261	31.5
Geranyl acetate	17.8	1372	13.3
β -elemene	18.9	1392	16.5
E-caryophyllene	22.9	1416	1.2
germacrene D	24.7	1473	1.3
Total			98.1

** : Kovats indices, *: Retention time

Table 2: Modification of the antibiotic activity of the volatile constituents of *L. alba* essential oil by gaseous contact on *S. aureus* ATCC 12692, ATCC 25923, ATCC 6538

Treatment	Erythromycin (mm ± SD%)					
	<i>S. aureus</i> ¹	Increase %	<i>S. aureus</i> ²	Increase %	<i>S. aureus</i> ³	Increase %
No treatment	28 ± 0.7	-	28 ± 0.7	-	28 ± 0.7	-
DMSO	28 ± 0.0	-	28 ± 0.0	-	28 ± 0.0	-
50%	≥90 ± 0.0	≥221.4	≥90 ± 0.0	≥221.4	≥90 ± 0.0	≥221.4
25%	87.5 ± 1.4	212.5	≥90 ± 1.4	≥221.4	≥90 ± 0.0	≥221.4
12%	63 ± 1.4	125	≥90 ± 0.7	≥221.4	66 ± 0.7	135.7
6%	30 ± 0.0	7.1	60.5 ± 0.7	116.1	47 ± 1.4	67.8
3%	27.5 ± 0.0	-	31.5 ± 1.4	12.5	38 ± 1.4	35.7

SD: standard deviation, *S. aureus*¹: ATCC 12692, *S. aureus*²: ATCC 25923 and *S. aureus*³: ATCC 6538

may also be a potential mechanism of action due to the combination of direct vapor absorption by the microorganisms and vapor absorption through the medium.^[20]

Several studies have reported the antimicrobial activity of essential oils and plant extracts. The essential oil of the *Mentha piperita* was effective against Gram-positive and Gram-negative strains, and antioxidant activity.^[21] Tannins, alkaloids, and flavonoids in the ethanol extracts from *Lantana montevidensis*, *Zanthoxylum rhoifolium*, and *Croton zehntneri* were responsible for the antimicrobial activity demonstrated.^[22] These and other studies confirm the popular use of natural products from medicinal plants to combat several diseases.

Most of *Lippia* species are used to treat respiratory affections. In Central and South America (Guatemala, Venezuela, Brazil), *L. alba* is also employed as a remedy for colds, gripe, bronchitis, coughs, and asthma.^[3] Essential oils, such as eucalyptus (*Eucalyptus globulus* Labill), act on the respiratory system, and possess, by oral and inhalatory ways, expectorant, fluidizing, and antiseptic activities of bronchial secretion.^[23]

It is known that it is very common to use plants as self-medication supply concomitant to allopathic drugs (55.9%).^[23] Another study^[24] demonstrate that 41.7% of people have associated medicinal plants with some drug, before searching for a medical service. Because of this, it is necessary to improve the studies about the possible interactions between natural products and allopathic drugs.

Plant-derived natural products, in some cases, can interfere with the effectiveness of antibiotics being used for the clinical treatment.^[25-29] The present article demonstrates that the volatile components of EOLA, in association with erythromycin, are able to promote the enhancement of the antibiotic activity.

The results are relevant and promising, considering the synergic association between *L. alba* essential oil and erythromycin as a possible therapeutic alternative to treat respiratory infections. So, new researches must be propounded in order to elucidate the possible action mechanisms involved.

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