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Chemical Composition of Essential Oil of *Lantana camara* L. (Verbenaceae) and Synergistic Effect of the Aminoglycosides Gentamicin and Amikacin

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Abstract: The leaves of *Lantana camara* L. (Verbenaceae) were subjected to hydrodistillation, and the essential oil extracted was examined with respect to chemical composition, antibacterial and antibiotic modifying activity by gaseous contact. Among the 25 identified components, bicyclogermacrene (26.1%), β-caryophyllene (24.4%), germacrene D (19.2%) and valecene (12.0%) were the main constituents. The essential oil volatile constituents inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with MIC of 1 and > 1 mg/L, respectively. The activity of the antibiotic amikacin was increased by 65% against *S. aureus* and *P. aeruginosa* after contact with the volatile components.

Keywords: *Lantana camara*; chemical composition; antibacterial and modulatory activities.

1. Introduction

Pseudomonas aeruginosa is an opportunistic affection that usually affects hospitatalized or immunocompromised persons. Usually occurs infection of the airways by *P. aeruginosa* occurs commonly in patients with cystic fibrosis but also occurs in patients with other forms of bronchiectasis [1]. In view of the high antibiotic resistance and virulence, the infections associated to *P. aeruginosa* are considered to have difficult management [2].

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In Germany, analysis of the sputum from patients with Cystic Fibrosis during a period of 12 months showed the presence of *P. aeruginosa* in 50% of these individuals and *Staphylococcus aureus* in 63.3% are the most prevalent pathogens [3-4]. *S. aureus* has emerged as one of the most important human pathogens, and it can be found in 20-40% of adult superior aerial vias [5].

Lantana is a genus of about 150 species of perennial flowering plants popularly used as antirheumatic, stimulant, antibacterial, biologic control and as ornamental plant [6]. Phytochemical studies of *Lantana* species lead to identification and isolation of terpenoids, flavonoids, phenylethanoid glycosides, furanonaphthoquinones, iridoid glycosides and steroids [6-8].

Lantana camara L. (camará) is a shrub that belongs to Verbenaceae family and it's native from America and Africa. Different parts of the plant, mainly the leaves, have been used in treatment of scratching, stomachache, rheumatism, wound healing, biliary fever, toothache, bronchitis, antiseptic and other affections [9].

L. camara is a rich source of many bioactive molecules and the phytochemical studies have resulted in the isolation of many triterpenes, steroids and flavonoids [10-11]. This plant has been claimed to present activities antiprotozoal [12], antibacterial and antifungical [9,13], antioxidant [14], insecticidal [15], antiviral [16], allelopathic properties [17] among others activities, but there is no previous report regarding to modulatory activity of the essential oil by gaseous contact.

In this work, we report the chemical composition, antibacterial activity and antimicrobial modulatory activity of *L. camara* essential oil from Cariri Cearense, Brazilian Northeast, by the minimal inhibitory concentrations and gaseous contact methods.

2. Materials and Methods

2.1. Plant Material

Leaves of *Lantana camara* L. were collected in April, 2009, from the Small Aromatic and Medicinal Plants Garden of the Natural Products Research Laboratory (LPPN) at University Regional do Cariri (URCA), Crato - Ceara state, Brazil. A voucher specimen (#1662) was deposited in the "Herbário Caririense Dárdaro de Andrade Lima" of Regional University of Cariri, Crato.

2.2 Isolation of the essential oil

Samples of fresh leaves (400 g) were triturated and submitted to hydrodistillation process, in a Clevenger-type apparatus for 2 hours, resulting in essential oil yield of 0.18%. The collected essential oil was subsequently dried by anhydrous sodium sulfate (Na_2SO_4), and stored under refrigeration at < 4 ºC until be tested.

2.3 GC and GC/MS analysis

Analysis by CG/MS of the essential oil was carried out on a Shimadzu GC-17 A/ MS QP5050A (GC/MS system) using a DB-5HT fused silica capillary column (30 m x 0.25 mm i.d., 0.25 m film thickness); carrier gas helium, flow rate 1.7 mL/min in split mode. The injection port and detector temperature were 270 ºC and 290 ºC, respectively. The column temperature was programmed from 35 ºC to 180 ºC at 4 ºC/min and then 180 ºC to 250 ºC at 10 ºC/min. Mass spectra were recorded from 30 – 450 *m/z*. injected volume: 1 µL of 5 µg/mL solution in ethyl acetate. Solvent cut time was 3 min. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-built library [18] as well as by visual comparison of the fragmentation pattern with those reported in the literature [19].

The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID), also set at 250 °C. *n*-Alkanes (C_9-C_{24}) 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. GC analyses were performed on a Hewlett Packard 5890 SERIES II equipped with a flame ionization detector (FID) and a J & W Scientific DB-5 fused silica capillary column (30 m x .25 mm x 0.25 µm). GC oven temperature and conditions were as described above. Injector and detector temperatures were 270°C and 290°C, respectively. Hydrogen was used as carrier gas, flow rate 1.0 mL/min, split mode (1:10).

2.4 Gaseous contact

The antibacterial activity of *L. camara* essential oil was analyzed by the gaseous contact method (indirect contact) [20]. In this assay, two standard strains (*S. aureus* - ATCC 12692; *P. aeruginosa* - ATCC 15442), were obtained from Fundação Oswaldo Cruz – FIOCRUZ, were used. Petri dishes containing gar Brain Heart Infusion agar (BHI) were inoculated with the strains (24 h; 35±2 ºC). The concentration of each inoculum was confirmed by viable count on Plate Count Agar (PCA). After this, appropriate dilutions (0,5 scale McFarlland, 1 x 10^8 CFU/mL) were plated onto Plate Count Agar (PCA).

An amount of 50 µg of oil was dissolved in 50 µL of DMSO (1:1). The assay was performed in triplicate and a dilution series of this essential oil solution was prepared: 50, 25, 12.5 and 6.25 µg of oil. 100 µL of each dilution was placed inside the upper part of Petri dishs, in order to promote an interaction between the volatiles constituents of the essential oil and the antibiotics. Petri dishes were incubated at 35 ± 2 ºC (24 h).

The minimal inhibitory concentration (MIC) was defined as the minimal inhibitory dose per unit space required to suppress the growth of microorganism in a closed system. The MIC values were expressed as weight per unit volume (mg/L air), where the solution with 50 µg equals 1 mg/L air [20].

2.5 Antibiotic modulatory activity evaluation

The antibiotic modifying activity of the gaseous component was determined using the same method and the solution with a total of 50, 25, 12.5 and 6.25 µg of oil was used. In these plates, antibiotics disks with gentamicin and amikacin were used to determine changes in the inhibition zone diameter of *P. aeruginosa* and *S. aureus.* Plates without the essential oil and with DMSO alone were used as control.

2.6 Statistical analysis

The average inhibition zones obtained were submitted the statistical analysis using Analysis of Variance (ANOVA) followed by the Student-Newman Keuls-test Multiple Comparisons. The results with $p < 0.05$ were considered to be significant.

3. Results and Discussion

Table 1 summarizes the chemical composition and retention indices (RI) found using the Hewlett-Packard Model 5971 GC/MS. The average essential oil yield of the experiments was 0.18 %. Essential oil GC/MS analysis permitted the identification and quantification of twenty-two constituents (100.0 %). Bicyclogermacrene (26.1%), β-caryophyllene (24.4%), germacrene D (19.2%) and valecene (12.0%) were the main constituents identified (Table 1). Previous reports of the essential oil of *L. camara* leaves is constituted mainly by sesquiterpenes, specially, β-caryophyllene, isocaryophyllene, germacrene D and bicyclogermacrene [21-25].

Caryophyllene isomers were present between the main constituents of essential oil *L. camara* from Brazil Northeastern in different daytime [26]. In the evaluation seasonal of the essential oil of *L. camara* collected in Madagascar [27], concentration of caryophyllene has been reported to be consistently high throughout the year, independent of sampling seasons.

Table 1. Chemical constituents of essential oil of the leaves of *L. camara*.

Order	Constituents	RI[a]	RI[b]	(%)
1	α-pinene	937	938	0.1
2	sabinene	969	973	0.2
3	β-pinene	973	978	0.1
4	terpinolene	1082	1084	0.1
5	terpinene-4-ol	1175	1177	0.1
6	α-terpineol	1186	1189	0.1
7	cis-3-hexenyl isovalerate	1216	1217	0.1
8	δ-elemene	1337	1337	0.3
9	α-copaene	1371	1376	1.1
10	β-elemene	1382	1385	1.6
11	β-caryophyllene	1416	1417	24.4
12	γ-elemene	1429	1433	5.4
13	aromadendrene	1431	1439	0.8
14	germacrene-D	1473	1474	19.2
15	bicyclogermacrene	1490	1491	26.1
16	valencene	1497	1496	12.0
17	δ-cadinene	1520	1524	1.2
18	germacrene B	1558	1560	1.2
19	spathulenol	1575	1576	1.3
20	caryophyllene oxide	1582	1581	0.2
21	viridiflorol	1591	1590	4.1
22	δ-cadinol	1635	1636	0.3
	Total identified			100

[a] relative retention indices experimental: n-alkanes (C_9-C_{24}) were used as reference points in the calculation of relative retention indices. [b] relative retention indices [19].

The results of antibacterial tests by gaseous contact show that *P. aeruginosa* no was susceptible to essential oil volatile constituents, and *S. aureus* was susceptible (MIC 1 mg/L air). Other study showed that *S. aureus* was more susceptible (MIC 0.25 mg/L air) to the volatile constituents of the essential oil of *L. montevidensis* Briq. [28]. In previous reports was verified the antibacterial activity of *L. camara* essential oil against *S. aureus* by direct contact method [23-25], but there is no previous report regarding to antibacterial activity by indirect contact.

In other study essential oil of *L. camara* show antibacterial activity by direct contact against *Arthrobacter protophormiae*, *Micrococcus luteus*, *Rhodococcus rhodochrous* and *S. aureus* with minimal bactericidal concentrations of 50, 25, 12.5 and 200 µg/mL, respectively [23].

The antibiotic activity of amikacin against *S. aureus* was enhanced in the presence of the essential oil by gaseous contact Table 2. Enhancement of antibacterial activity of amikacin and gentamicin against *P. aeruginosa* by the essential oil was verified too. The amikacin zone inhibition diameter was increased (65%), Table 2.

Table 2 show that more significative synergic effects are associated to an increase of essential oil volatile constituents concentrations, and this is statistically significant ($p < 0.05$) in comparison with controls (antibiotics and DMSO).

One study was observed synergistic effects of gentamicin and amikacin activities against *S. aureus* in the presence of the essential oil constituents of *L. montevidensis* Briq., by gaseous contact method. The amikacin zone inhibition diameter was increased (29%). Enhancement of antibacterial activity of amikacin and gentamicin against *P. aeruginosa* by the essential oil was verified too, it was verified a increasing in 102% of the amikacin activity [28].

In one study oil essential of the leaves of *L. camara* was examined to modulatory activity by microdilution test against two multiresistant strains, *E. coli* from sputum and *S. aureus* from surgical

wound, obtained from clinical material. The synergism of the essential oil on aminoglycosides was verified which showed significant reduction of MICs (1250 to 5 µg/mL) against *E. coli* [22].

In other study was verified the synergistics effects of the extracts etanolic of leaves and roots of *L. montevidensis* Briq. on aminoglycosides activity by microdilution test. The maximum effects were obtained with extract roots on gentamicin activity against multiresistant strains of *E. coli* with MIC reduction (312 to 2 µg/mL) [29].

Table 2. Modification of the antibiotic activity of the volatile constituents of *L. camara* essential oil by gaseous contact on *S. aureus* and *P. aeruginosa*.

Treatment	***Staphylococcus aureus*** (mm ± DP)				***Pseudomonas aeruginosa*** (mm ± DP)			
	Gentamicin	(%)	Amikacin	(%)	Gentamicin	(%)	Amikacin	(%)
Antibiotics	16.3±0.6	-	17.3±0.6	-	14.3±0.6	-	15.0±0.0	-
DMSO	16.7±0.6	-	17.3±0.6	-	14.0±0.0	-	15.3±0.6	-
EOLc 50 µg	16.3±0.6	0	22.3±1.1*	29	17.3±0.6*	21	24.7±0.6*	65
EOLc 25 µg	16.3±0.6	0	21.0±1.1*	21	17.0±0.6*	19	23.3±0.6*	55
EOLc 12.5 µg	16.3±1.1	0	19.3±0.6	11	16.5±0.0	15	20.0±0.0*	33
EOLc 6.25 µg	16.0±0.0	0	18.0±0.0	4	16.0±1.0	12	18.3±0.5*	22

EOLc - Essential Oil of *L. camara*; **(%)** - Percentages of enhancement on antibiotic activity; *****The mean values of inhibition zones (mm ± DP) are statistically significant when compared with controls ($p < 0.05$ – ANOVA followed by the Student-Newman Keuls-test Multiple Comparison). The results are expressed as mean ± DP (n=3).

Essential oils may interact with and affect the plasma membrane, interfering with respiratory chain activity and energy production [30]. The improvement of antibacterial activity against the gram-negative bacteria *P. aeruginosa* demonstrated a significative result, as the gram-positive bacteria are more susceptible to natural products [31].

The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds, with permeability enhancement [32]. This property can facilitate the antimicrobial agents to penetrate into cell, leading to an activity enhancement. That is a plausible explanation for the positive interactions between the sesquiterpenes constituents and the conventional antibiotics [33].

Many plants have shown no only antibacterial properties, but also the ability to interfere with the antibiotic resistance. The sesquiterpenes constituents (guaiazulene, nerolidol (mixture of the *cis* and *trans* isomers) and germacrene D in association with ciprofloxacin, erythromycin, gentamicin and vancomycin demonstrated synergic effect against *E. coli* and *S. aureus* [34].

The data cited in the literature regarding the essential oil interference, by gaseous contact, show relevant and promising results. In one study *Croton zehntneri* essential oil reinforced the gentamicin activity against *P. aeruginosa* increasing the inhibition halo (48.2%) [35].

The results obtained here show that *L. camara* volatile constituents suppress the *S. aureus* growth, pathogenic bacteria of respiratory system and could be a source of metabolites with antibacterial modifying activity to be used as adjuvants to antibiotic therapy against these pathogens. In part, this study can justify the popular use of *L. camara* to treat respiratory affections.

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