

Chemical Composition and Aminoglycosides Synergistic Effect of *Lantana montevidensis* Briq. (Verbenaceae) Essential Oil

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Abstract: The leaves of *Lantana montevidensis* Briq. (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 19 identified components, β -caryophyllene (31.50%), germacrene D (27.50%) and bicyclogermacrene (13.93%) were the main constituents. The essential oil volatile constituents inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a MID o 0.25 and > 1 mg/L, respectively. The activity of the antibiotics gentamicin and amikacin was reinforced against *S. aureus* and *P. aeruginosa* (with a 102% of amikacin activity against *P. aeruginosa*) after contact with the volatile components, showing that this oil influences the activity of the antibiotic and may be used as an adjuvant in the antibiotic therapy of respiratory tract bacterial pathogens.

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

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1. Plant Source

Lantana is a genus of about 150 species of perennial flowering plants popularly used as antirheumatic, stimulant, antibacterial, biologic control and as ornamental plant [1]. The essential oil of *Lantana* species leaves is constituted mainly by sesquiterpenes, specially caryophyllene, β -caryophyllene, (E)-caryophyllene, isocaryophyllene, germacrene D and bicylogermacrene [2,3,4,5].

Lantana montevidensis Briq. (Verbenaceae), shrub native to Brazil and Uruguay, is popularly known as “cambará” and it was introduced to many countries as an ornamental plant [1]. The plant has been used in treatment of scratching, stomachache, rheumatism, wound healing, biliary fever, toothache, bronchitis, and antiseptic [1,6].

2. Previous Studies

The leaves methanolic extract presented antiproliferative activity against tumor cells and the flavonoid fraction from the leaves showed antiproliferative activity against human gastric adenocarcinoma, human uterus carcinoma and murine melanoma cells *in vitro* [7], but there is no previous report regarding to modulatory activity of the essential oil, by gaseous contact.

3. Present Study

Samples of fresh leaves (400 g) were triturated and submitted to hydrodistillation process, in a Clevenger-type apparatus for 2 h, resulting in essential oil yield of 0.13%. The collected essential oil was subsequently dried by anhydrous sodium sulfate (Na_2SO_4), and stored under refrigeration at < 4 °C until be tested.

The essential oil was carried out on a Hewlett-Packard Model 5971 GC/MS using a non-polar DB-1 fused silica capillary column (30 m x 0.25 mm i.d., 0.25 μm 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 °C/min and then 180 °C to 250 °C at 10 °C/min. Mass spectra were recorded from 30 – 450 m/z . Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-built library and two other computer libraries MS searches using retention indices as a pre-selection routine, as well as by visual comparison of the fragmentation pattern with those reported in the literature [8].

The antibacterial activity of *L. montevidensis* essential oil was analyzed by the gaseous contact method [9]. In this assay, two standard strains (*S. aureus* - ATCC 12692; *P. aeruginosa* - ATCC 15442), were obtained from Fundação Oswaldo Cruz – FIOCRUZ, were used.

The antibiotic modifying activity of the gaseous component was determined using the same method and the same concentrations (50, 25, 12.5, 6.25 $\mu\text{g/mL}$). 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.

The GC/MS analysis permitted the identification and quantification of eighteen constituents (98.25%), with predominance of sesquiterpenes (83.66%) and a little amount of monoterpenes (16.66%), Table 1.

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

IR ^a	IR ^b	Constituents	(%)
971	971	sabinene	0.22
1080	1081	linalool	1.83
1366	1376	α -copaene	2.70
1385	1393	β -elemene	2.93
1414	1414	β -caryophyllene	31.50
1440	1440	camphor	0.34
1441	1443	alloaromadrene	1.37
1448	1455	α -humulene	2.68
1473	1474	germacrene D	27.50
1490	1491	bicyclogermacrene	13.93
1491	1491	β -sabinene	0.73
1508	1505	germacrene A	1.08
1516	1472	β -cadinene	2.59
1543	1547	germacrene B	1.43
1572	1576	spathulenol	3.37
1571	1569	caryophyllene oxide	2.21
1636	1630	torreyol	1.33
1652	1652	α -cadinol	0.51
		Total identified	98.25
		Monoterpernes	16.66
		Sesquiterpenes	83.33

^a relative retention indices experimental: n-alkanes were used as reference points in the calculation of relative retention indices.

^b relative retention indices (literature values)

The results of antibacterial tests show that *P. aeruginosa* was less susceptible to essential oil volatile constituents, and *S. aureus* was more susceptible (MID 0.25 mg/L air). The antibacterial screening using *L. camara* showed an effective activity against *P. aeruginosa* and *S. aureus* [10]

The synergistic effect of gentamicin and amikacin activities against *S. aureus* was observed in the presence of the essential oil constituent. The amikacin zone inhibition diameter was increased (29%), Table 2. 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, Table 2.

Table 2. Modification of the antibiotic activity of the volatile compounds of *L. montevidensis* essential oil by gaseous contact on *S. aureus*.

Treatment	<i>Staphylococcus aureus</i> ATCC 12692 (mm ± DP %)			
	Gentamicin	Enhancement (%)	Amikacin	Enhancement (%)
No Treatment	16.3±0.6	-	17.3±0.6	-
DMSO	16.7±0.6	-	17.3±0.6	-
EOLm 50%	18.0±1.0	10	22.3±0.6*	29
EOLm 25%	17.3±0.6	6	21.0±0.0*	21
EOLm 12.5%	17.0±0.0	4	19.0±1.0	10
EOLm 6.25%	16.3±0.6	0	17.3±0.6	0

EOLm - Essential Oil of *L. montevidensis*; *The mean values of inhibition zones 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).

Tables 2 and 3 show that more significative synergic effects are associated to a increase of essential oil volatile constituents concentrations, and this is statistically significant ($p < 0.05$) in comparison with controls (antibiotics and DMSO).

Table 3. Modification of the antibiotic activity of the volatile compounds of *L. montevidensis* essential oil by gaseous contact on *P. aeruginosa*.

Treatment	<i>Pseudomonas aeruginosa</i> ATCC 15442 (mm ± DP %)			
	Gentamicin	Enhancement (%)	Amikacin	Enhancement (%)
No treatment	14.3±0.0	-	15.0±0.0	-
DMSO	14.0±0.0	-	15.3±0.6	-
EOLm 50%	16.0±0.0	12	30.3±0.6*	102
EOLm 25%	15.0±1.0	5	29.0±0.0*	93
EOLm 12.5%	14.3±0.6	0	28.0±1.0*	87
EOLm 6.25%	14.3±0.6	0	26.0±1.0*	73

EOLm - Essential Oil of *L. montevidensis*; *The mean values of inhibition zones 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).

The results obtained here show that *L. montevidensis* 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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