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Chemical composition of the essential oils of *Lantana camara* L. and *Lantana montevidensis* Briq. and their synergistic antibiotic effects on aminoglycosides

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Recently, several plants have been evaluated not only for antimicrobial activity but also for resistance-modifying action. In this work, the chemical composition and antibacterial and antibiotic-modulatory activities of the essential oils from *Lantana camara* L. and *Lantana montevidensis* Briq. were analyzed. The essential oils extracted from the leaves by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) were characterized by a high percentage of sesquiterpene hydrocarbons. Among the 34 constituents identified, bicyclogermacrene (19.4%), isocaryophyllene (16.7%), valencene (12.9%) and germacrene D (12.3%) were the main constituents of the oil from *L. camara*, while in the oil from *L. montevidensis*, β-caryophyllene (31.5%), germacrene D (27.5%) and bicyclogermacrene (13.9%) predominated. The essential oils were examined for antibiotic activities alone and in combination with aminoglycosides by a microdilution assay utilizing five bacterial strains. They exhibited significant antibacterial activities, mainly against *Proteus vulgaris* (MIC 64 μg/mL, *L. camara*; MIC 128 μg/mL, *L. montevidensis*). Both oils also showed a synergistic effect on the activity of aminoglycoside antibiotics. Thus, the essential oils of *L. camara* and *L. montevidensis* could be used as a source of plant-derived natural products with resistance-modifying antibacterial activity.

Keywords: essential oil composition; *Lantana*; antibacterial activity; aminoglycosides; synergism

Introduction

Several new antibacterial agents are currently being developed in response to the emergence of bacterial resistance to existing drugs. New plant sources showing antimicrobial activity and low toxicity could be a viable alternative (1). Of particular importance, with increased incidence of resistance to antibiotic, natural products from plants have been evaluated not only for direct antimicrobial activity but also as resistance-modifying agents in combination with conventional antibiotics (2, 3).

Many natural products from plants possess antibacterial properties and drug resistance-modifying activity, which can produce a synergistic or antagonistic effect. Synergism is defined as a phenomenon in which different compounds are combined to enhance their individual activity. If the combination results in a worse effect, it is called antagonism (4). Compounds with such activity are classified as modifiers of antibiotic activity, and can represent a progress against resistance mechanisms of aminoglycosides (5).

Lantana camara L. is a shrub native to the Americas and Africa and has been cultivated as an ornamental plant in other countries. Infusions of the leaves have been used in the treatment of pruritis, stomach ache, rheumatism, wound healing, biliary fever, toothache and bronchitis and as an antiseptic (5, 6). Their roots are used in the treatment of malaria, rheumatism and rash (7).

Lantana montevidensis Briq. is a shrub native to Brazil and Uruguay, and an infusion of the dried leaves has been used in folk medicine for the same purposes as *L. camara*. The methanolic leaf extract has shown antiproliferative activity against tumor cells, and a flavonoid-rich fraction was found to be effective against human gastric adenocarcinoma, human uterine carcinoma and melanoma cell lines (8).

The aim of this work was to analyze the chemical composition of the essential oils of *Lantana camara* and *L. montevidensis* and to determine their potentiation of the antibiotic activity of aminoglycosides, in continuation of our earlier studies on medicinal properties of essential oils of Cariri Cearense species, northeast Brazil.

Experimental

Plant material

Leaves of *Lantana camara* L. and *L. montevidensis* Briq. were collected in May 2011, in the Small Aromatic and Medicinal Plants Garden of the Natural

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Products Research Laboratory (LPPN) at University Regional do Cariri (URCA), Crato County, Ceará State, Brazil. A voucher of each specimen was sent to the Herbarium Caririense Dárdano de Andrade Lima (HCDAL), Department of Biological Sciences (URCA), and deposited under registry numbers 1662 and 1619, respectively for *L. camara* and *L. montevidensis*.

Essential oil isolation

Several samples of fresh leaves (500 g) were triturated and submitted to a hydrodistillation process in a Clevenger-type apparatus for 2 hours. The essential oils collected were subsequently dried with anhydrous sodium sulfate (Na_2SO_4), and both volatile oils were stored in a refrigerator at < 4°C until used.

Analysis of the essential oils

Analysis by gas chromatography–mass spectrometry (GC–MS) of the essential oils was carried out on a Shimadzu GC-17 A/MS QP5050A (GC–MS system) using a DB-5HT fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness); helium gas was the carrier, with a flow rate of 1.7 mL/minute and split ratio of 1:30. The injector temperature was 270°C and the íon-source temperature was 290°C. The column temperature was programmed from 35°C to 180°C at 4 °C/minute and then 180°C to 250°C at 10°C/minute. Scanning speed was 0.5 scan/second, with mass spectra recorded from 30 to 450 *m/z*, and the sample was injected as 1 μL of 5 mg/mL solution in ethyl acetate. Solvent cut time was 3 minutes. Individual components were identified by matching their 70 eV (electron impact mode) mass spectra, with those of the spectrometer database using the Wiley L-built library and two other computer MS library 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 (9).

The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID). *n*-Alkanes (C_9–C_{24}) were used as reference points in the calculation of relative retention indices. The concentration of the compounds identified 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 and a J & W Scientific DB-5 fused silica capillary column (30 m × 25 mm × 0.25 μm). GC oven temperature and conditions were as described earlier. Injector and detector temperatures were 270°C and 290°C, respectively. Hydrogen was the carrier gas, using a flow rate of 1.0 mL/minute and split mode (1:10).

Antibacterial assay and minimal inhibitory concentration (MIC)

The antibacterial assay for minimal inhibitory concentration (MIC) determination of the essential oils was performed by a microdilution assay (10). The assay was carried out with bacterial species obtained from Fundação Oswaldo Cruz – FIOCRUZ: *Staphylococcus aureus* ATCC 6538, *Proteus vulgaris* ATCC 13135, *Pseudomonas aeruginosa* ATCC 5442, *Vibrio cholareal* ATCC 15748, and *Escherichia coli* ATCC 2992.

Brain heart infusion (BHI) broth (Difco Laboratories Ltd.) at 3.8% was used for bacterial growth (24 hours, 35 ± 2°C). The inoculum was an overnight culture of each bacterial species in BHI broth diluted in the same medium to a final concentration of approximately 1×10^8 CFU/mL (0.5 nephelometric turbidity units – McFarland scale). Afterwards, the suspension was diluted to 1×10^5 CFU/mL in 10% BHI broth. A total of 100 μL of each dilution were distributed in 96-well plates with each oil, with a final inoculum of 5×10^5 CFU/mL.

The initial solution of each essential oil was prepared using 10 mg oil dissolved in 1 mL of dimethyl sulfoxide (DMSO) to obtain an initial concentration of 10 mg/mL. From this concentration, several dilutions were made in distilled water to obtain a stock solution of 1024 μg/mL. Further serial dilutions were performed by addition of BHI broth to obtain a final concentration in the range of 8 to 512 μg/mL.

The experiments were performed in triplicate and the microdilution trays were incubated at 35 ± 2°C for 24 hours. Antibacterial activity of the essential oils was detected using a colorimetric method by adding 25 μL of an aqueous solution of resazurin stain (0.01%) to each well at the end of the incubation period. The MIC was defined as the lowest concentration of oil able to inhibit bacterial growth, as indicated by resazurin staining (dead bacterial cells are not able to change the color from blue to red on visual observation).

Antibiotic modifying assay

To evaluate the essential oils with respect to modulatory effect on antibiotic resistance, MIC of the antibiotics neomycin, kanamycin, amikacin and gentamicin (Sigma Chemical Co) against *Proteus vulgaris* ATCC 13315 and *Staphylococcus aureus* ATCC 10390 strains were determined in the presence or absence of the essential oils at sub-inhibitory concentrations (MIC/8). The experiments were performed in triplicate by a microdilution assay (10), utilizing suspensions of 10^5 CFU/mL in BHI broth (10%) and an antibiotic concentration range of 0.0012 to 2.5 mg/mL (two-fold serial dilutions). The plates were incubated for 24 hours at

37°C and controls using DMSO in MIC determination and antibiotic modulation activity tests were performed.

Results and discussion

Hydrodistillation of the leaves of *Lantana camara* and *L. montevidensis* for 2 hours yielded their essential oil (the yields were, respectively, 0.1% and 0.1% (w/w). The volatile components of the essential oils, their percentage composition and retention index (RI) are listed in Table 1. Analysis of essential oils by GC and GC–MS led to the identification and quantification of thirty-four constituents, which accounted for 99.7% (*L. camara*, 25 components) and 98.2% (*L. montevidensis*, 18 components) of the total oils, with significant predominance of sesquiterpene hydrocarbons in both species. Previous reports showed a large percentage of sesquiterpene hydrocarbon constituents in essential oils of *Lantana* species (11, 12).

Table 1. Chemical constituents of *Lantana camara* and *L. montevidensis* leaves essential oils.

				Oils composition (%)	
Order	Constituents	RI[a]	RI[b]	*L. camara*	*L. montevidensis*
Monoterpene hydrocarbons				*10.1*	*0.2*
1	sabinene	969	973	4.8	0.2
2	β-pinene	973	978	0.2	-
3	ß-myrcene	990	992	0.4	-
4	*p*-cymene	1020	1021	1.4	-
5	(*Z*)-ß-ocimene	1034	1037	0.9	-
6	(*E*)-ß-ocimene	1045	1050	1.0	-
7	γ-terpinene	1060	1060	0.6	-
8	terpinelene	1082	1084	0.8	-
Oxigenated monoterpenes				*5.8*	*1.8*
9	Linalool	1093	1097	-	1.8
10	cis-*p*-menth-2-en-1-ol	1103	1103	5.2	-
11	terpinene-4-ol	1175	1177	0.6	-
Sesquiterpene hydrocarbons				*79.5*	*88.7*
12	α-elemene	1337	1337	1.6	-
13	α-copaene	1371	1376	0.4	2.7
14	β-elemene	1382	1385	1.2	2.9
15	(*Z*)-β-caryophyllene	1400	1404	2.9	-
16	Isocaryophyllene	1409	1409	16.7	-
17	(*E*)-ß-caryophyllene	1416	1417	-	31.5
18	Alloaromadrene	1441	1443	2.3	1.4
19	γ-elemene	1429	1433	1.6	-
20	Camphor	1444	1446	-	0.3
21	α-humulene	1448	1455	2.1	2.7
22	germacrene D	1473	1474	12.3	27.5
23	eremophilene	1480	1486	5.6	-
24	Bicyclogermacrene	1490	1491	19.4	13.9
25	ß-sabinene	1491	1491	-	0.7
26	Valencene	1497	1496	12.9	-
27	germacrene A	1508	1505	-	1.1
28	δ-cadinene	1516	1514	0.5	-
29	ß-cadinene	1520	1524	-	2.6
30	germacrene B	1558	1560	-	1.4
Oxigenated sesquiterpenes				*4.3*	*7.4*
31	Spathulenol	1575	1576	2.6	3.4
32	caryophyllene oxide	1582	1581	1.7	2.2
33	torreyol	1636	1630	-	1.3
34	δ-cadinol	1635	1636	-	0.5
	Total identified			99.7	98.1

Notes: [a]Relative retention index experimental: *n*-alkanes (C_9–C_{24}) were used as reference points in the calculation of relative retention indices. [b]Relative retention index (9).

The sesquiterpene hydrocarbons bicyclogermacrene (19.4%), isocaryophyllene (16.7%), valecene (12.9%) and germacrene D (12.3%) were identified as the main components of the *Lantana camara* essential oil. In *L. montevidensis* the main constituents were (*E*)-ß-caryophyllene (31.5%), germacrene D (27.5%) and bicyclogermacrene (13.9%). The β-caryophyllene isomers (*E* and Z) are common constituents in essential oils of aerial parts of *Lantana* species, and are found among the main constituents of essential oil of *L. camara* from northeast Brazil at different times of day (13).

In a seasonal evaluation of the essential oil of *Lantana camara* L. collected in Madagascar, the concentration of β-caryophyllene has been shown to be consistently high throughout the year, independent of sampling seasons (14). Germacrene D and bicyclogermacrene are also common constituents in *Lantana* essential oils, and β-caryophyllene has been reported to be present in these oils, making it a good candidate for a chemical marker of *Lantana* species (11).

The essential oils were found to be inhibitory against the five bacterial strains tested (Table 2). They displayed similar inhibitory activities against *S. aureus* ATCC 10390 (MIC 256 μg/mL) and *Pseudomonas aeruginosa* ATCC 15442 (MIC 512 μg/mL) strains. The highest inhibitory activities were against *Proteus vulgaris* (MIC 64 μg/mL, *Lantana camara*; and MIC 128 μg/mL, *L. montevidensis*).

The results obtained can be due to the presence of constituents with known antibacterial activity and non-polar characteristics, such as the sesquiterpene hydrocarbons isocaryophyllene, bicyclogermacrene and β-caryophyllene (*E* and *Z*) (15, 16). The mechanisms of bacterial growth inhibition vary, but here, growth inhibition could have been due in part to the hydrophobic nature of some constituents of the essential oils. For example, they can interact with the lipid bilayer and affect the cell membrane, interfering with respiratory chain activity and energy production, or even make the cell more permeable to antibiotics, causing the interruption of vital cellular activities (17).

Table 2. MIC values (μg/mL) for the essential oils from the leaves of *Lantana camara* and *L. montevidensis.*

	MIC (μg/mL)	
Strains	*L. camara*	*L. montevidensis*
Proteus vulgaris ATCC 13135	64	128
Pseudomonas aeruginosa ATCC 5442	512	512
Vibrio cholareae ATCC 15748	≥ 512	≥ 512
Escherichia coli ATCC 2992	256	512
Staphylococcus aureus ATCC 6538	256	256

The growth inhibition of Gram-negative bacteria demonstrated here is an important result, since the essential oils, in general, are more able to show antibacterial activity against Gram-positive bacteria (more sensitive to antibiotics). The Gram-negative bacteria possess structural particularities that hinder the penetration of antibiotics, as the lipopolysaccharide structures containing polysaccharides of different length contribute greatly to cell surface properties, such as membrane permeability and antibiotic susceptibility (18).

In an antibacterial assay using essential oil of *Lantana achyranthiofolia* Desf., effective antibacterial activity was demonstrated against a multiresistant *Staphylococcus epidermidis* strain and several *Vibrio cholerae* strains (19). In another study, essential oil obtained from *L. camara* L. leaves and tested for antibacterial activity using the disk-diffusion method, showed moderate activity against *Staphylococcus typhi* and *Pseudomonas aeruginosa* with zone inhibition varying from 10 to 14 mm (20).

Table 3 shows the MICs of the antibiotics and the synergic effects of the essential oils when combined with the antibiotics. The MICs of the antibiotics for the bacterial strains were in the range of 156 to 625 μg/mL, and they decreased in the presence of the essential oils. The most notable effect was the potentiation of amikacin against *Proteus vulgaris* ATCC 13135 by the essential oil of *Lantana montevidensis* (64 μg/mL) with MIC decreasing from 312 to 2 μg/mL. In general, the influence of essential oils (synergism) on antibiotic action depended on the antibiotic type and bacterial strain. The control DMSO showed a MIC ≥ 1024 μg/mL and no antibiotic-modifying activity.

The mechanism of action of terpenes is not fully understood, but it is believed to involve membrane disruption by the lipophilic compounds, resulting in enhanced permeability (21). This property can facilitate the penetration of antimicrobial agents into the cell, leading to increased activity. This provides a plausible explanation for the positive interaction between the sesquiterpene constituents and conventional antibiotics (22).

The essential oil of *Lantana montevidensis* was effective in changing the resistant phenotype of the bacterium to a sensitive one, and this capacity was more evident in the Gram-negative bacteria. This result can be due to a greater concentration of sesquiterpene hydrocarbon constituents, mainly (*E*)-ß-caryophyllene (31.5%) and germacrene D (27.5%). The sesquiterpene constituents (guaiazulene, nerolidol (racemic mixture of the *cis* and *trans* isomers) and germacrene D, in combination with ciprofloxacin, erythromycin, gentamicin and vancomycin, demonstrated a synergistic effect against *Escherichia coli* and *Staphylococcus aureus* (23).

Table 3. MIC values (μg/mL) of aminoglycosides with and without the *Lantana camara* and *L. montevidensis* essential oils.

	Proteus vulgaris ATCC 13135			*Staphylococcus aureus* ATCC 6538		
		MIC combined			MIC combined	
Antibiotics	MIC alone	OELc 8 μg/mL	OELm 64 μg/mL	MIC Alone	OELc 8 μg/mL	OELm 32 μg/mL
Neomycin	312 (R)	156 (R)	5 (S)	156 (R)	156 (R)	156 (R)
Amikacin	625 (R)	312 (R)	5 (S)	312 (R)	156 (R)	78 (R)
Kanamycin	625 (R)	312 (R)	10 (S)	625 (R)	78 (R)	156 (R)
Gentamicin	312 (R)	78 (R)	2 (S)	156 (R)	20 (R)	40 (R)

Note: OELc: oil essential of *L. camara*; OELm: oil essential of *L. montevidensis*; R, phenotypic profile of resistance; S, phenotypic profile of sensitivity (17).

In conclusion, this study demonstrated that essential oils from *Lantana camara* and *L. montevidensis* leaves have antibacterial activities. It is suggested that they could be used as a source of plant-derived natural products with resistance-modifying activity with regard to aminoglycosides. Both essential oils have the potential to be used for medical purposes as antibiotic adjuvants.

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