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Antimicrobial activity of essential oil of *Cordia globosa*

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Cordia globosa **is used to treat gastrointestinal diseases in San Rafael Coxcatlan, Puebla, Mexico; however, its medicinal properties have not been investigated. This study reports the chemical composition and the antimicrobial effect of *C. globosa* essential oil. The essential oil of aerial parts was obtained by steam distillation and 25 compounds were identified by gas chromatography-mass spectrometry (GC-MS). The major constituent was α-pinene (38.4%). Antimicrobial activities were tested on 6 bacterial and 9 fungi strains. The most sensitive strain was *Vibrio cholera* (minimum inhibitory concentration [MIC], 0.060 mg/ml). These results show the chemical composition and biological properties of essential oil of *C. globosa*. The results validate the medicinal use of *C. globosa*.**

Key words: *Cordia globosa*, Boraginaceae, antimicrobial activity, essential oil.

INTRODUCTION

Cordia globosa (Jacq.) Kunth (Boraginaceae) is a shrub that grows in tropical lands of America. This plant is known as “blood herb” in San Rafael Coxcatlán, Puebla, Mexico and the infusion of aerial parts is used for diseases like skin fungal infections, gastrointestinal and throat of posible infectious origin as well as for its antitussive, astringent, hemostatic and tonic (Hernández et al., 2003).

Chemical analysis of many plants of the genus *Cordia*, including *Cordia alba, Cordia alliodora* (Manners and Jurd, 1977; Ioset et al., 2000a), *Cordia millenii* (Moir and Thomson, 1973), and *Cordia curassavica* (Ioset et al., 2000b; Hernández et al., 2007) have demonstrated they contain sesquiterpenes, triterpenes, flavanoids, chromens, quinones, and hydroquinones. Phytochemical studies of *C. globosa* include the isolation of meroterpenoid benzoquinones with cytotoxic activity against several cancer cell lines (Menezes et al., 2005)

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and flavonoids (Souza et al., 2004).

Searching for new drugs with high activity is very important, especially considering that in México infectious diseases constitute a serious public health problem (INEGI, 2011). The biosphere is a potential source of many unknown bioactive molecules, especially with the great biodiversity found in México. All these factors justify continued research for antimicrobial substances from plant origin.

There are few studies on the chemical composition of the volatile compounds of *Cordia* species. The aim of this study was to elucidate the composition of the essential oil of *C. globosa* as well as to determine their antimicrobial activity against pathogenic bacteria and fungi.

MATERIALS AND METHODS

Plant

C. globosa were collected in August 2009, in San Rafael, Coxcatlán, Puebla. A voucher specimen was deposited in the IZTA herbarium (Voucher no. HCM60/2009).

Isolation of essential oils

The essential oils were obtained from aerial parts of *C. globosa* by steam distillation (2730 g of fresh plant) for 4 h in a Cleavenger-type apparatus (Cassel et al., 2009) and stored at 4°C until tested and analyzed. The yield of the essential oil was 0.067% (w/w), d^{25} = 0.79 g/ml.

Gas chromatograph-mass spectrometry (GC-MS) analysis conditions

The essential oil was analyzed in an Agilent Technologies 6850 gas chromatograph equipped with a HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The temperature of the column was 325°C. Injector and detector temperatures were set at 230 and 280°C, respectively. Oven temperature was kept at 70°C for 2 min, and then programmed to 280°C at a rate of 8°C/min. Helium was the carrier gas at a flow rate of 1 ml/min. A volume sample of 1 µl was manually injected in the split mode. Peak areas were measured by electronic integration. The relative amount of the individual components was based on the peak areas. Mass analysis was performed on an Agilent Technologies 5975C mass spectrometer. The temperature of the column and the injector were the same as those of the GC. Mass spectra were recorded at 70 eV. The oil components were identified by comparison of their retention indices and mass spectra with the NIST08.L Mass Spectral of the internal device library (Match ≥ 90%). Retention indices were calculated by linear interpolation relative to retention times, of a series of *n*-alkanes (alkane's standards Sigma-Aldrich) and through the determination of the respective Kovats retention indices (KI). The KI were compared with those reported in literature (NIST, 2011).

Microbial strains

The following strains of bacteria were used: *Staphylococcus aureus* ATCC 12398, *Bacillus subtilis* donated by FES-Cuautitlán, *Streptococcus pneumoniae* isolated from a clinical case, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio cholera* isolated from a clinical case. These strains were maintained at 4°C in Mueller Hinton agar (Bioxon), and were subcultured every month.

The yeasts tested were: *Candida albicans* ATCC 10231, *C. albicans* ATCC 14065, *C. albicans* isolated from a clinical case donated by the Clinical Analysis Laboratory of FES-Iztacala, *Candida glabrata, Candida tropicalis* isolated from a clinical case donated by Hospital Angeles (Metropolitano) and *Cryptococcus neoformans* donated by FES-Cuautitlán. The filamentous fungi pathogens used were: *Trichophyton mentagrophytes* CDBB-H-1112, *Aspergillus niger* CDBB-H-179, *Fusarium sporotrichoides* NRLL3299 and *Rhizoctonia lilacina* CDBB-H-306. The stock culture was maintained on CzapekDox agar (Sigma).

Antibacterial activity

Antibacterial activity was measured by the disk-diffusion method (Van der Berghe and Vlietinck, 1991). Microorganisms were grown overnight at 37°C in 10 ml of Muller Hinton Broth (Bioxon). The cultures were adjusted to turbidity comparable to that of McFarland No. 0.5 standard (1.0×10^8 CFU/ml) with sterile saline solution (Christoph et al., 2000). Petri dishes containing Muller Hinton agar were inoculated with these microbial suspensions. Disks (Whatman No. 5) of 5 mm diameter were impregnated with 4 µl (3.16 mg) of essential oil. Disks with chloramphenicol (25 µg) were used as positive controls. The plates were incubated overnight at 37°C and the diameters of any resulting zones of growth inhibition (mm) were measured. Each experiment was made three times.

The estimation of the minimal inhibitory concentration (MIC) and the minimal bactericide concentration (MBC) were carried out by the broth dilution method (Van der Berghe and Vlietinck, 1991). Dilutions from 1.5 to 0.062 mg/ml of essential oil were used. Tubes were inoculated with 10^5 CFU/ml of microorganism suspension. MIC values were defined as the lowest extract concentration that prevents visible bacteria growth after 24 h of incubation at 37°C. Chloramphenicol was used as reference, and appropriated controls with no essential oil were used. Each experiment was repeated at last three times. The inactivation broth death kinetic method was performed using appropriate concentrations of essential oil (corresponding to ½ MIC, MIC and MBC). Death kinetics was expressed in $\log_{10}$ reduction time kill plots (Christoph et al., 2000).

Antifungal activity

For *Candida* strains and *C. neoformans,* the same protocol used for bacterial strains was followed in PDA agar. Nystatin (30 µg/disk) was used as a positive control against yeast. The assay of antifungal activity (filamentous fungi) was carried out in Petri dishes containing CzapekDox agar (20 ml). After the mycelia colony had developed, disks impregnated with 4 µl (3.16 mg) of essential oil, were placed at a distance of 0.5 cm away from the rim of the mycelia colony. Petri dishes were incubated at 23°C for 72 h until mycelia growth had developed. Disks containing crescents of inhibition were considered to contain antifungal activity (Wang and Ng, 2007). Ketoconazole (25 µg/disk) was used as a positive control.

For quantitative assays, a culture plate of 24 wells was used. Six dilutions of essential oil (1.50, 1.00, 0.50, 0.25, 0.125, and 0.0625 mg/ml) were added to CzapekDox agar (5 ml) at 45°C, these being mixed rapidly and poured into three wells of a culture plate. After the agar had cooled down to room temperature a small amount (1 × 1 mm) of mycelia was inoculated. After incubation at 23°C for 72 h, the area of the mycelia colony was measured and the inhibition of fungal growth was determined in percentage; concentration-response curves were constructed with the data to calculate the

Table 1. Chemical composition of essential oil of *Cordia globosa*.

No	Compounds	RI	RIr	Percentage
1	α-Pinene	917	917	38.4
2	Camphene	932	933	9.0
3	β-Pinene	956	964	1.3
4	α-Phellandrene	982	985	0.9
5	Limonene	1005	1014	4.6
6	γ-Terpinene	1059	1060	0.4
7	α-Terpinolene	1086	1093	0.4
8	Bornylacetate	1287	1287	0.5
9	α-Cubebene	1335	1339	0.6
10	α-Funebrene	1377	1399	0.6
11	β-Elemene	1437	1432	2.4
12	β-Bourbonene	1394	1391	0.3
13	γ-Elemene	1437	1432	8.0
14	Caryophyllene	1441	1444	4.3
15	γ-Cadinene	1456	1493	0.6
16	Calamenene	1465	1496	1.1
17	α-Caryophyllene	1470	1477	1.3
18	α-Gurjunene	1478	1408	0.9
19	β-Bisabolene	1496	1489	4.0
20	α-Selinene	1499	1494	3.1
21	Valencene	1503	1496	1.0
22	δ-Cadinene	1511	1519	2.3
23	*cis*-α-Bisabolene	1520	1511	3.1
24	3,7(11)-Selinadiene	1543	1542	1.9
25	α-Bisabolol	1690	1688	1.0
	Total	-	-	92.0

[a]Compounds listed in order of elution from a non-polar HP-5 MS capillary column. RI: Retention indices relative to *n*-alkanes on non-polar HP-5MS column. RIr: Kováts Index references (NIST, 2011).

medial fungicidal concentration (FC_{50}). Ketoconazole was used as reference and appropriate controls with no essential oil were used. Each experiment was repeated three times (Wang and Ng, 2007).

Statistical analysis

All experiments were performed in triplicate. The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for experiment was compared using multifactorial analysis of variance (ANOVA) and the Tukey honest significant difference (HSD) test. The FC_{50} values were calculated by linear model.

RESULTS AND DISCUSSION

Constituents (25), comprising 92.0% of the oil from aerial parts of *C. globosa* were characterized by GC-MS (Table 1). α-Pinene was the major monoterpene constituent in the essential oil comprising 38.4% of the total. Hydrocarbon monoterpenes as well as camphene and limonene constituted 9.0%, and 4.6% of the oil, respectively. Essential oils are characterized by 56.1% of monoterpenes and 35.9% of sesquiterpenes. Generally, these compounds determine the biological properties of the oils (Bakkali et al., 2008). This is the first report of the composition of the essential oils of *C. globosa* collected in México.

Bioassays of antimicrobial activity showed that the essential oil of *C. globosa* was active and inhibited the growth of most of the microbial strains evaluated (Table 2). The more sensitive bacterial strains were *V. cholera, S. pneumoniae* and *P. aeruginosa* with a MIC of 0.060, 0.250, and 0.750 mg/ml, respectively. *S. pneumoniae* and *P. aeruginosa* infect the respiratory tract. *V. cholerae* is the responsible agent of severe gastrointestinal infections (cholera). The fungus most sensible to essential oil of *C. globosa* was *T. mentagrophytes* (IC_{50} 0.350 mg/ml). This microorganism causes athlete's foot. The results of bioassays with the essential oil of *C. globosa* are consistent with the popular use of this herb for the treatment of diarrheal diseases, respiratory tract and epidermal fungi infections. Clinical studies will be required to confirm the efficacy of essential oil of *C. globosa*.

Table 2. Antimicrobial activity of essential oil of *Cordia globosa.*

Organism	Positive controls					Essential oil		
	Inhibition zone (mm)			MIC (mg/ml)	FC_{50} (mg/ml) Ketoconazole	Inhibition zone (mm)	MIC (mg/ml)	FC_{50} (mg/ml)
	Chloramphenicol (25µg/disk)	Nystatin (30µg/disk)	Ketoconazol (25µg/disk)			3.16 mg/disk		
Sa	24.00±0.82	--	--	0.001	--	10.00±0.58	1.000	--
Spn cc	8.33±0.57	--	--	0.016	--	7.00±0.00	0.250	--
Bs	28.00±1.63	--	--	0.002	--	15.00±1.41	3.000	--
Ec	21.67±0.50	--	--	0.004	--	7.00±0.000	3.000	--
Vch cc	27.67±0.47	--	--	0.001	--	13.00±2.83	0.060	--
Pae	22.60±0.11	--	--	0.008	--	8.50±0.70	0.750	--
Ca 14065	--	11.83±2.02	--	0.011	--	NA	NA	--
Ca 10231	--	9.67±0.58	--	0.004	--	7.00±0.00	3.00	--
Cg	--	7.67±0.58	--	0.008	--	8.50±0.70	3.00	--
Ct	--	9.00±1.00	--	0.008	--	7.00±0.00	3.00	--
Cneo	--	8.67±0.58	--	0.004	--	7.00±0.00	1.00	--
An	--	--	++	--	0.0153	++	--	1.650
Fs	--	--	++	--	0.0040	++	--	0.760
Tm	--	--	++	--	0.0012	++	--	0.350
Rl	--	--	++	--	0.0215	++	--	0.820

Bacteria (MICs values); Fungi (IC_{50} values); Sa: *Staphylococcus aureus* ATCC12398; Spn cc: *Streptococcus pneumoniae* (clinical isolate); Bs: *Bacillus subtillis*; Vch cc: *Vibrio cholerae* (clinical isolate); Pae: *Pseudomonas aeruginosa* ATCC 27853; Ca 14065: *Candida albicans* ATCC 14065; Ca 10231: *Candida albicans* ATCC 10231; Cg: *Candida glabrata* (clinical isolate); Ct:*Candida tropicalis* (clinical isolate); Cneo: *Criptococcus neoformans* (clinical isolate); An: *Aspergillus niger*CDBB-H-179*;* Fs: *Fursariumsporotrichoides* NRLL3299; Tm: *Trichophytonmentagrophytes* CDBB-H-1112; Rl: *Rhizoctonia lilacina* CDBB-H-306; Na: no activity, -- No determinated, ++ radial growth inhibition ≥ 10 mm.

The essential oil molecules are lipophilic, they pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and rendering them more permeable (Dorman and Deans, 2000; Guynot et al., 2003; Bakkali et al., 2008). Essential oil exerts its antimicrobial effect at cytoplasmic membrane by altering the structure and function of microorganisms (Holley and Patel, 2005). Fei et al. (2011) demonstrated by electron microscopy, that essential oil of various plants disrupt the cellular membranes of *E. coli, S. aureus, B. subtilis* and *S. cerevisiae*.

Results of the effect of *C. globosa* essential oil at ½ MIC, MIC and CBM on cell viability (kill time) of *V. cholera* and *P. neumoniae* are shown in Figures 1 and 2, respectively. The oil caused a drop in viable cell number (CFU) in comparison to the control treatment at all assayed concentrations. In *V. cholerae*, a bactericidal effect was found after 10 min of exposure to the oil at MIC and CBM doses. After 10 min, the oil at ½ MIC decreased the cell count to <5 log CFU, the oil provided a bacteriostatic effect along the evaluated intervals (Figure 1). In *P. pneumoniae* the oil caused a significant decrease in the bacterial count in comparison to the control assay at 2 and 3 hours of exposure with MIC and CBM doses, in both cases the oil occurring bactericidal effect; at ½ MIC decreased the viable cells count to <3 log CFU (Figure 2). These findings show an interesting inhibitory effect of *C. globosa* essential oil toward the cell viability of *V. cholera* and *P. pneumoniae*, with a fast and steady bacterial kill rate. Time kill curve showed a clear relationship of the extent of inhibition and the oil concentration and time of exposure.

The antibacterial activity showed by the essential oil can be attributed to the presence of some components such as α-pinene, β-pinene, limonene, etc. (Rivas et al., 2012), which are

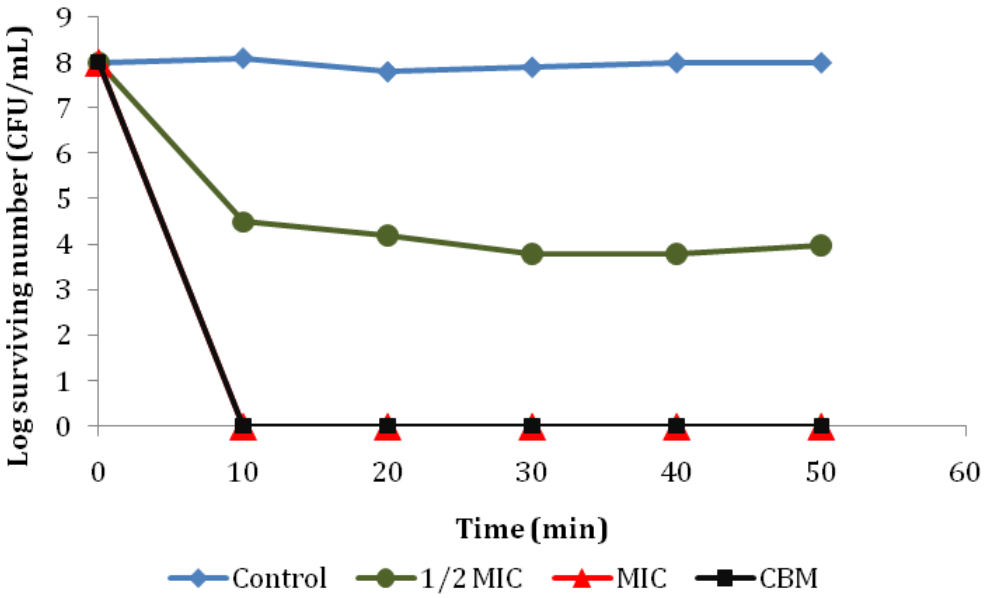

Figure 1. Survival curve of *Vibrio cholerae* exposed to essential oil of *C. globosa* collected in San Rafael Coxcatlán. The essential oil was added to each culture at time zero. The concentrations used were: 0.030 mg/ml (½MIC), 0.060 mg/ml (MIC) and 0.120 mg/ml (MBC). The control did not contain essential oil.

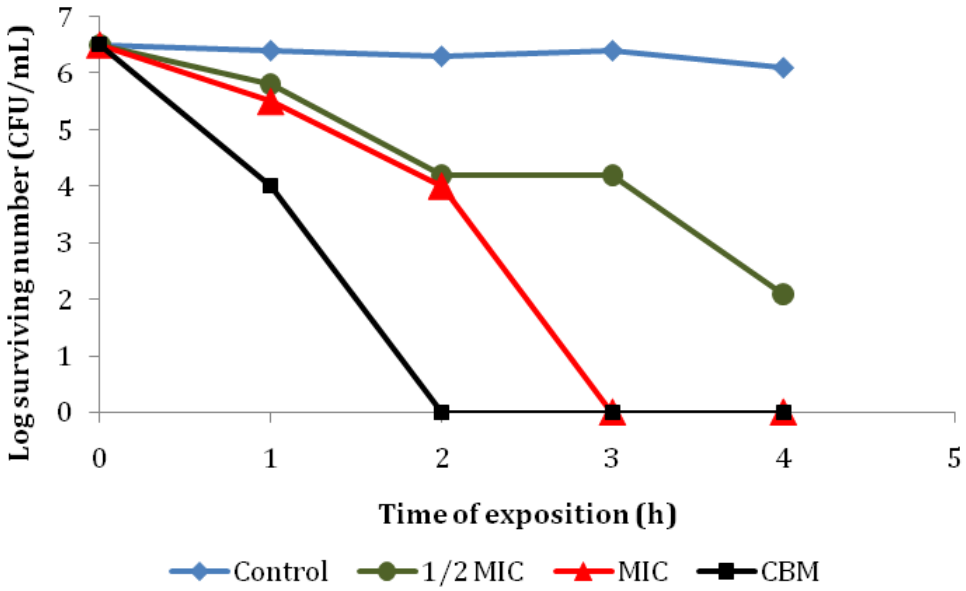

Figure 2. Survival curve of *Streptococcus pneumoniae* exposed to essential oil of *C. globosa* collected in San Rafael Coxcatlán. The essential oil was added to each culture at timezero. The concentrations used were: 0.125 mg/mL (½MIC), 0.250 mg/mL (MIC) and 0.500 mg/mL (MBC). The control did not contain essential oil.

already known to exhibit antibacterial activity and also several studies have demonstrated that whole essential oil usually have higher antibacterial activity than the principal components, suggesting that the mixes of the compounds are critical to synergistic activity (Solórzano-Santos and Miranda-Novales, 2012).

Conclusion

In summary, the major constituent of the essential oil of the aerial parts of *C. globosa* was α-pinene. The essential oil had antimicrobial activity on bacterial and fungi strains of medical importance. The oil presents antimicrobial

activity against Gram positive and Gram-negative bacteria and five fungal strains. The present study confirms the rational use in folk medicine of the aerial parts of *C. globosa* in gastrointestinal, respiratory, and dermatological diseases.

Conflict of interest

The authors have not declared any conflict of interest

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