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Temporal variation of chemical composition and antimicrobial activity of the essential oil of *Cordia curassavica* (Jacq.) Roemer and Schultes: Boraginaceae

[Variación temporal de la composición química y la actividad antimicrobiana del aceite esencial de *Cordia curassavica* (Jacq.) Roemer y Schultes: Boraginaceae]

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Abstract: The people of San Rafael Coxcatlán use *Cordia curassavica* to treat gastrointestinal and respiratory disorders. The aim of this work was to investigate the temporal variation of chemical composition and antimicrobial activity of the essential oil of *C. curassavica* in two seasons of the year (dry and rainy). The essential oil of aerial parts was obtained by steam distillation, 12 and 17 compounds were identified for dry and rainy seasons respectively by GC-MS. The major component was for the dry season 1,7,7-trimethyl tricyclo (2.2.1.0(2,6)) heptane (20.3%), and for the rainy season was germacrene (24.41%). The antibacterial activity of essential oils varies temporarily because was active in nine strains in the dry season and four in the wet. The more sensitive strains were *Stapahylococcus epidermidis* and *Vibrio cholera*. The essential oil obtained in dry season presented a MIC of 0.75 and 0.125 mg/mL, and for the rainy season a MIC of 1.00 and 0.375 mg/mL respectively. *Rhizoctonia solani* was the more sensitive fungi strain (IC25 0.1300 mg/mL) in the rainy season. These results show that the chemical composition and biological properties of essential oil of the *C. curassavica* have temporal variation.

Keywords: Antimicrobial activity, Boraginaceae, *Cordia curassavica*, essential oils, traditional medicine.

Resumen: La población de San Rafael para tratar padecimientos gastrointestinales y respiratorios emplean *Cordia curassavica*. El objetivo del presente trabajo fue investigar la variación temporal de la composición química y la actividad antimicrobiana del aceite esencial de *C. curassavica* colectada en dos épocas del año (sequía y lluvias). El aceite esencial se obtuvo mediante la técnica de arrastre de vapor y los componentes se identificaron por CG-MS. En la época de secas se detectaron 12 compuestos y 17 en la de lluvias. El principal componente para la época de secas fue el 1,7,7 trimetil triciclo (2.2.1.0(2,6)) heptano (20.3%) y para la temporada de lluvias fue el germacrano (24.41%). La actividad antibacteriana del aceite esencial varía temporalmente porque fue activo sobre nueve cepas en la época seca y sobre cuatro cepas en la época lluviosa. Las cepas más sensibles para ambas estaciones fueron *Stapahylococcus epidermidis* y *Vibrio cholerae*. En la época de seca, el aceite esencial presentó la concentración mínima inhibitoria (CMI) de 0.75 y 0.125mg/mL, mientras que en la época de lluvias fueron de 1.00 y 0.375 mg/mL respectivamente. La cepa fúngica más sensible fue *Rhizoctonia solani* (IC25 0.130 mg/mL) en la época de lluvia. Estos resultados muestran que la composición química y las propiedades biológicas de los aceites esenciales de *Cordia curassavica* varian de acuerdo a la época del año.

Palabras Clave: Actividad antimicrobiana, aceites esenciales, Boraginaceae, *Cordia curassavica*, medicina tradicional

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INTRODUCTION

Infusions of the aerial parts of *Cordia curassavica* are used in traditional medicine for the treatment of various diseases. This plant is widely distributed in the Tehuacán-Cuicatlán Valley where is used mainly in the dry season for its medicinal properties. In our ethnobotanical study, we reported that it is recognized among the eight species of greater relative importance in the treatment of gastrointestinal diseases in Zapotitlán Salinas, Puebla (Hernández *et al.,* 2003). *C. curassavica* is a perennial plant that blooms in the rainy season (Argueta and Cano, 1994). In San Rafael Coxcatlán people use the plant to treat their ailments. Some studies have identified some compounds of the essential oil of *C. curassavica* as: β-terpinene, α and β-pinene, caryophyllene, carvacrol, β-eudesmol, spathulenol, and bicyclogermacrene (Gómez *et al.,* 1999; Hernández *et al.,* 2007; Santos *et al.,* 2006). Other studies have also investigated various biological properties such as antimicrobial activity of the essential oil of *C. curassavica*. Hernández *et al.,* (2003 and 2007) reports the antibacterial activity of extracts and essential oil against Gram-positive and Gram-negative bacteria and the antifungal activity on *Rhizoctonia solani* and *Trichophyton mentagrophytes* (Hernández *et al.,* 2007). Ioset *et al.,* (2000) studied the antifungal activity on *Cladosporium cucumerinum* and *Candida albicans* and toxic properties against larvae of the *Aedes aegypti*.

Essential oils are volatile, natural, complex compounds, and it is known their antiseptic and medicinal properties and their fragrance. In nature, essential oils play an important role in the protection of the plants to bacterial, fungal and viral infections, also against herbivores. They attract some insects to favor pollens and seeds dispersion (Bakkali *et al.,* 2008). Several studies report the variation of chemical composition of the essential oil of diverse species according to environmental conditions such as climate, soil, solar radiation, etc. (Viljoen *et al.,* 2005; Van Vuuren *et al.,* 2007; Bakki *et al.,* 2008; Bourgou *et al.,* 2012). In this study, we compare the temporal variation of chemical composition and antimicrobial activity of the essential oils of *C. curassavica* in two seasons (dry and rainy) of San Rafael-Coxcatlán, Puebla in México.

MATERIALS AND METHODS

Plant Material

Cordia curassavica were collected at two times of the year, the first in dry season and the second in rainy season (February and October respectively) 2008, in San Rafael, Coxcatlán, Puebla. A voucher specimen was deposited in the IZTA herbarium (Voucher no. HCM5-2/2008 and HMC5-10/2008).

Isolation of essential oils

The essential oils were obtained by steam distillation (1.000 g of fresh plant) during 4 h in the Cleavenger-type apparatus and stored at 4 °C until tested and analyzed. The yield of the essential oil of the dry season was about 0.3491% (w/w), d^{25}= 0.86 g/mL, and the yield of the rainy season was 0.1839% (w/w), d^{25}= 0.78 g/mL.

GC-MS analysis conditions

The analysis of the essential oils was performed using a Hewlett Packard 5890-II gas chromatograph equipped with DB WAX column (30 m x 0.32 mm). The temperature of the column was programmed from 80 to 220° C at 8° C/min. The injector and detector temperatures were 225° C. The gas carrier was He, at a flow rate of 1 mL/min. Peak areas were measured by electronic integration. The relative amount of the individual components was based on the peak areas. GC-MS analysis was performed on a JEOL AX50HA using a DB Wax (30 m x 0.32 mm) capillary column. The temperature of the column and the injector were the same as those from GC. Mass spectra were recorded at 70 eV. The oil components were identified by comparison of their retention indices and mass spectra with the NIST/EPA/NIH Mass Spectral Library (Match ≥ 90%) and through the determination of the respective Kováts retention indices (KI) (alkanes standards provided by Sigma-Aldrich) (Charlwood and Charlwood, 1991). The KI were compared with those reported in the NIST (2011) database and Pherobase (2011).

Microbial Strains

The following strains of bacteria were used: *Staphylococcus epidermidis, Bacillus subtilis, Sarcina lutea, Enterobacter aerogenes* and *Yersinia enterocolitica* (all donated by the Clinical Analysis Laboratory of FES Iztacala). *Staphylococcus aureus* ATCC 12398, *Enterobacter agglomerans* ATCC 27155, *Salmonella typhi* ATCC 19430, *Escherichia*

coli ATCC 25922, *Vibrio cholera* INDRE 206 (isolated from polluted water), *Vibrio cholera* isolated from a clinical case, *Vibrio cholera* (clinical strain pertaining to 01 group, "Inaba" serotype, "El Tor" biotype, and enterotoxin producer), and *Vibrio cholera* CDC V12. These strains were maintained at 4° C in Mueller Hinton agar (Bioxon), submitted to sensitivity tests (multidiscs Bigaux) and were subcultured every month.

One strain of yeast was tested: *Candida albicans* isolated from a clinical case, donated by the Clinical Analysis Laboratory of FES-Iztacala. The fungal pathogens used were: *Fusarium moniliforme* CDBB-H-265, *Trichophyton mentagrophytes* CDBB-H-1112, *Aspergillus niger* CDBB-H-179 and *Rhizoctonia solani* donated by the INIFAP, Celaya, México. The stock culture was maintained on Czapek Dox agar (Sigma).

Antibacterial Activity

The antibacterial activity was measured by the disk-diffusion method (Van der Berghe and Vlietinck, 1991). The 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 (Lennette *et al.,* 1987). Petri dishes containing Muller Hinton agar were inoculated with these microbial suspensions. Disks (Whatman Nº 5) of 5 mm diameter were impregnated with 5 µL (4.3 mg and 3.9 mg dry and rainy seasons) 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 inhibition zones (mm) of growth were measured. Each experiment was made three times.

The estimation of the minimal inhibitory concentration (MIC) was carried out by the broth dilution method (Van der Berghe and Vlietinck, 1991). Dilutions of essential oil from 3.0 to 0.062 mg/mL were used. The tubes were inoculated with microorganism suspension of 10^5 CFU/mL. 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 expressed in $\log_{10}$ reduction time kill plots (Lennette *et al.*, 1987; Christoph *et al.*, 2000).

Antifungal Activity

The assay of antifungal activity was carried out in Petri dishes containing of Czapek Dox agar (20 mL). After the mycelia colony had developed, disks impregnated with 5 µL (4.3 mg and 3.9 mg dry and rainy seasons) of essential oil, were placed at a distance of 0.5 cm away from the rim of the mycelia colony. The petri dishes were incubated at 23° C for 72 h until mycelia growth had enveloped. Disks containing crescents of inhibition were considerate with antifungal activity (Ye *et al.,* 1999). Ketoconazol (25 µg/disk) was used as a positive control.

For quantitative assays, a cultive plate of 24 wells was used. Six dilutions of essential oil (2.00, 1.50, 1.00, 0.50, 0.25 and 0.125) were added to Czapek Dox agar (5 mL) at 45° C, these being mixed rapidly and poured into three wells of a cultive plate. After the agar had cooled down to room temperature a small amount (1 x 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 at hence the IC_{25} was determined. Ketoconazole was used as reference and appropriate controls with no essential oil were used. Each experiment was reported 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 analyzed with a Kruskal-Wallis analysis. The IC_{25} values were calculated by lineal model.

RESULTS AND DISCUSSION

As shown in Table 1, 12 compounds (81.98%) of the essential oil obtained from *C. curassavica* in dry season were identified by GC/MS analysis, 41% corresponds to monoterpenes and 59% to sesquiterpenes. Unlike the above, in the rainy season we identified 17 compounds (95.43%) in the essential oil with 41% of monoterpenes and 59% of sesquiterpenes. The main compounds for the dry season were: 1,7,7-trimethyltricyclo (2.2.1.0(2,6)) heptane (20.30%), germacrene (16.29%) and isocariophyllene (12.40%). In the rainy season the major components were: germacrene (24.41%), 1,7,7-trimethyltricyclo (2.2.1.0(2,6)) heptane (18.83%) and α-pinene (10.52%). Both essential oils are constituted mainly by sesquiterpenes.

Essential oils obtained from *C. curassavica* at two seasons in San Rafael, Coxcatlán share nine compounds: germacrene (15.29% dry; 24.41 % rainy); 1,7,7-trimethyltricyclo (2.2.1.0(2,6)) heptane (21.66% dry; 18.83% rain), α-pinene (10.18% dry; 10.52% rainy); isocariophyllene (12.40% dry; 8.18% rainy); Unknow (5.18% dry; 6.49% rainy); Unknown (1.85% dry; 3.90% rainy); selineno (6.11% dry; 2.85% rainy) and D-limonene (2.04% dry; 1.17% rainy).

Table 1
Chemical composition of essential oils *Cordia curassavica.*

Compounds	RT	KI e	Drought (%)	RT	KIe	Rain (%)	KIr
1,7,7-trimethyl tricyclo (2.2.1.0(2,6)) heptane	7.43	923	20.3	7.09	921	18.83	921
α-pinene	7.69	942	9.53	7.31	948	10.52	939
Camphene	---	---	---	7.61	949	6.75	954
α-myrcene	---	---	---	8.44	985	0.91	981
α-phellandrene	---	---	---	8.73	1003	0.91	1004
D-limonene	9.76	1024	1.90	9.23	1024	1.17	1029
4-carene	---	---	---	14.98	1035	4.28	1031
α-cubebene	---	---	---	15.18	1352	1.17	1356
Carvacrol	15.19	1357	1.90	---	---	---	1344
Unknown	---	---	---	15.82	1379	1.30	---
Unknown	16.90	1399	1.90	15.90	1398	3.90	---
Isocariophyllene	17.45	1409	12.40	16.41	1416	8.18	1404
Germacrene	17.61	1438	16.29	16.58	1437	24.41	1441
Humulene	18.02	1450	3.12	16.95	1450	0.91	1451
Unknown	18.48	1464	4.85	17.37	1463	6.49	---
Selinene	18.58	1484	5.72	17.47	1474	2.85	1487
Levomenol	---	---	---	18.22	1507	1.43	---
7-epi-α-selinene	18.71	1522	2.25	---	---	---	1512
α-bisabolol	---	---	---	20.36	1694	1.42	1704
Unknown	20.60	1772	1.82	---	---	---	
Total			**81.98**			**95.43**	

KIe: Kováts Index experimental and KI; KIr: Kováts Index references

Essential oils are characterized by two or three major components at fairly high concentrations (≥ 20%). Generally, these compounds determine the biological properties of the oils (Bakkali *et al.*, 2008). The production of terpenes in the essential oils is often modified by external factors such as climate and internal like plant phenology. The chemical composition of a plant is thus subject to quantitative and qualitative variation (Van Vuuren *et al.*, 2007). In this study, *C. curassavica* was collected in the same zone but at different times of the year (dry and rainy). Compared both seasons, the differences in the yield, quality, quantity and composition of the essential oils can be attributed to this factor.

As the chemical composition of an essential oil potentially affects the biological properties, the antibacterial and antifungal activities were recorded. The results obtained in the evaluation of the antibacterial activity of the essential oils of *C. curassavica* are shown in Table 2. Both essential oils exhibited antibacterial activity in Gram-positive and Gram-negative bacteria. The antibacterial activity of essential oils varies temporarily because was active in nine strains in the dry season and four in the wet. The more sensitive strain was *V. cholerae* with MIC 0.037 mg/mL rainy season and 0.125 mg/mL dry season. *S.*

epidermidis followed, in dry season presented a MIC of 0.75 mg/mL, and 1.00 mg/mL for the rainy season. The yeast *C. albicans* was no sensible, but the essential oils were active in four strains of fungi. *R. solani* showed the highest sensitivity of all fungi studied, having an IC_{25} of 0.13 mg/mL. Our results show that the essential oils extracted from *C. curassavica* collected at different time periods in San Rafael Coxcatlán, not exerts significant difference between in the percentages of inhibition in the antifungal activity. The results of the antimicrobial activity are in accordance with a previous study, where we evaluate the essential oil and extracts of *C. curassavica* of Zapotitlan de las Salinas, Puebla. The oil and extracts present antimicrobial activity against Gram positive and Gram-negative bacteria and five fungal strains (Hernandez *et al.,* 2007). Because of the great number of constituents, essential oils seem to have no specific cellular targets. 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 permeabilize them (Sikkema *et al.,* 1994; Juven *et al.,* 1994; Ultee *et al.,* 1999; Dorman and Deans, 2000; Guynot *et al.,* 2003; Bakkali *et al.,* 2008). In bacteria, the permeabilization of the membrane is associated with loss of ions and reduction of membrane potential. Essential oils can coagulate the cytoplasm and damage lipids and proteins (Bakkali *et al.,* 2008). Sensibility of fungi to essential oil may be due to some of the compounds to induce changes in cellular permeability push between fatty membranes, phospholipids acyl chains causing increased permeability and the fluid. The degrees of variation in fluent correspond to the position of the bilayer lipid, which depends on the hydrophobicity of the compound (Hammer *et al.,* 2004; Carson *et al.,* 2006). Essential oils can provoke depolarization of mitochondrial membranes and other ionic channels affecting the proton pump and the ATP pool (Bakkali *et al.,* 2008). Another mechanism of action of the oils in fungi was inhibiting the synthesis of ergosterol and sterol uptake (Inouye *et al.,* 2000; Inouye *et al.,* 2001; Ricci *et al.,* 2005; Bakkali *et al.,* 2008).

Table 2
Antimicrobial activity of essential oil of *Cordia curassavica*

Organism	Positive controls				Essential oils					
	Inhibition zone (mm)		MIC (mg/mL)	IC_{50} (mg/mL)	Inhibition zone (mm)		MIC (mg/mL)		(IC_{25}) (mg/mL)	
	Chloramphenicol	Ketoconazole	Chloramphenicol	Ketoconazole	Dry	Rainy	Dry	Rainy	Dry	Rainy
Sa	24.00±0.82	---	0.001	---	9.33±0.58	9.33±0.58	1.000	1.500	---	---
Se	24.00±0.82	---	0.002	---	8.33±0.58	8.00±0.00	0.750	1.000	---	---
Sl	34.00±0.82	---	0.001	---	13.33±1.20	Na	0.500	>3.00	---	---
Bs	28.00±1.63	---	0.002	---	10.33±0.58	11.67±0-58	1.500	0.500	---	---
Vch w	35.00±0.50	---	0.001	---	7.00±0.00	Na	3.000	Na	---	---
Vch No 01	35.00±0.50	---	0.001	---	8.33±1.15	Na	3.000	Na	---	---
Vch Tor	22.67±0.47	---	0.001	---	8.00±0.00	Na	1.500	Na	---	---
Vch. cc	21.67±1.70	---	0.001	---	9.33±0.58	10.67±0.58	0.125	0.037	---	---
E.c.	21.67±1.70	---	0.004	---	8.33±0.58	Na	3.000	Na	---	---
An	---	0.015	---	0.015	---	---	---	---	0.880	0.270
Fm	---	0.008	---	0.008	---	---	---	---	19.38	5.04
Tm	---	0.002	---	0.002	---	---	---	---	5.050	1.600
Rs	---	0.020	---	0.020	---	---	---	---	0.165	0.130

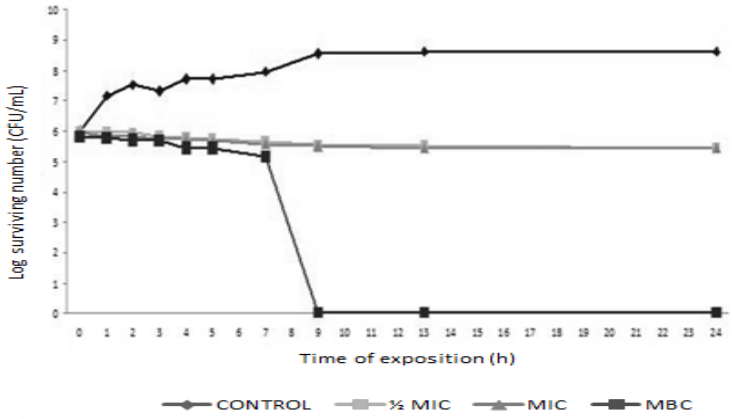
Bacteria (MICs values); Fungi (IC_{25} values); Sa, *Staphylococcus aureus*, Se, *Staphylococcus epidermidis*, Sl, *Sarcina lutea*, Bs, *Bacillus subtilis*; Vch w, *Vibrio cholerae* isolated from contaminated water, Vch No-01, *Vibrio cholerae*, Vch Tor, *Vibrio cholerae* CDC V12, Vch cc, *Vibrio cholerae* (clinical isolate), An, *Aspergillus niger*, Fm, *Fusarium moniliforme*, Fs, *Fusarium sporotichum*, Tm, *Trichophyton mentagrophytes*, Rs, *Rhizoctonia solani*, Na, no activity.

Time kill assays were performed on two pathogens *S. epidermidis* (Gram-positive) and *V. cholera* clinical isolated (Gram-negative). The figures 1a and b shows the effect of the essential oil of *C. curassavica* in both seasons in kill curve of *S. epidermidis.* ½ MIC (0.375 dry; 0.5 mg/mL rainy) and MIC (0.75 dry; 1.0 mg/ml)

of essential oils had bacteriostatic effect in both seasons. Minimum bactericidal concentration (MBC 1.0 mg/mL) has a lethal effect on the bacterial population at 9 h in dry season, but in rainy is at 13 h (MBC 1.5 mg/mL).

In both seasons, essential oils of *C. curassavica* at ½ MIC and MIC also had bacteriostatic effect in *V. cholera* clinical isolated. MCB (0.25 mg/mL) had a lethal effect at 7 h for the dry season, but in rainy was at 9 h with a (MBC 0.50 mg/mL) (Figures 2a and b).

a) Dry season



b) Rainy season

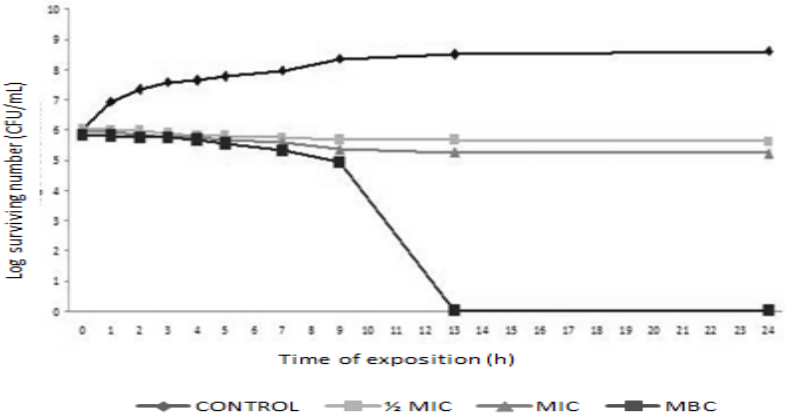
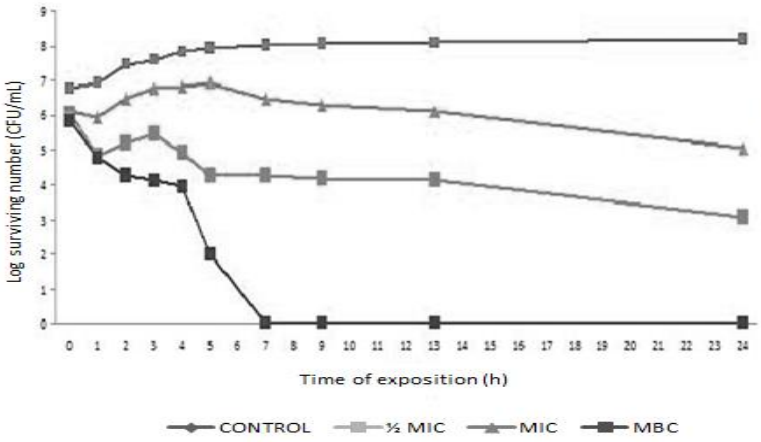


Figure 1
Survival curve *of Staphylococcus epidermidis* exposed to essential oil of *C. curassavica* collected in San Rafael Coxcatlán. The essential oil was added to each experimental culture in zero time. The concentrations used were: a) Dry season 0.375 mg/mL (½MIC), 0.750 mg/mL (MIC) and 1.0 (MBC); b) Rainy season 0.5 mg/mL (½MIC), 1.0 mg/mL (MIC) and 1.5 mg/mL (MBC). The control tube did not contain essential oil.

Many studies have reported differences in the biological activity of the same plant collected in different seasons (Bakkali *et al.*, 2008). Van Vuuren *et al.*, (2007) demonstrated seasonal and geographical variation in antimicrobial activity of *Heteropyxis natalensis* essential oils. Also Kamatou *et al.*, (2008) demonstrated the seasonal variation in essential oil composition and antibacterial activity of three *Salvia* species. In this study we report the best antibacterial activity of *C. curassavica* essential oil in the dry season. This may be explained by the presence of carvacrol (1.90%) in this season, it has antibacterial activity (Nostro and Papalia, 2012).

a) Dry season



b) Rainy season

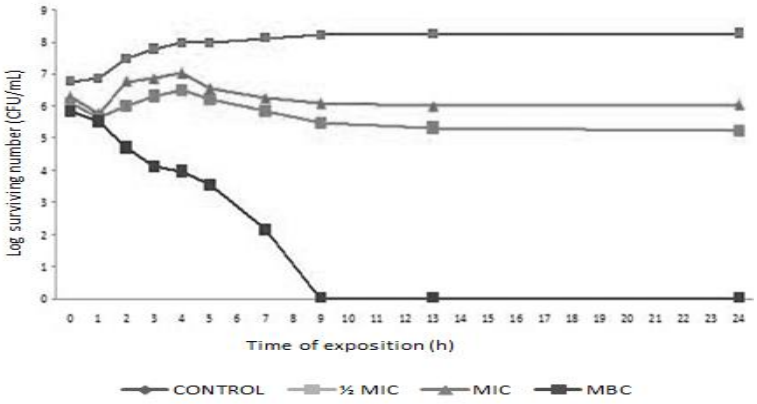


Figure 2
Survival curve of *Vibrio cholerae* (clinical case) exposed to essential oil of *C. curassavica* collected in San Rafael Coxcatlán. The essential oil was added to each experimental culture in zero time. The concentrations used were: a) Dry season 0.0625 mg/mL (½MIC), 0.125 mg/mL (MIC) and 0.250 mg/mL (MBC); b) Rainy season 0.187 mg/mL (½MIC), 0.375 mg/mL (MIC) and 0.50 mg/mL (MBC). The control tube did not contain essential oil.

CONCLUSIONS

The results obtained in this investigation, showed that the essential oil of *Cordia curassavica* collected in dry and rainy seasons have different chemical composition and biological activity and validates the use in the folk medicine, for treating in gastrointestinal diseases.

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