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BIOLOGICAL PROPERTIES AND CHEMICAL COMPOSITION OF *JATROPHA NEOPAUCIFLORA* PAX

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Abstract

Background: Ethnopharmacological relevance. *Jatropha neopauciflora* (Pax) is an endemic species of the Tehuacan-Cuicatlan Valley, Mexico. This species has long been used as a remedy to alleviate illnesses of bacterial, fungal and viral origin. Aim of the study. Experimentally test the traditional use of *Jatropha neopauciflora* in Mexican traditional medicine.
Materials and methods. The methanol extract (MeOH1), of *Jatropha neopauciflora* (Euphorbiaceae) was obtained by maceration. Next, the methanol (MeOH2) and hexane (H) fractions were obtained. The essential oil was obtained by hydro-distillation. The extract, fractions and essential oil were analyzed by GC-MS. The antimicrobial activity was measured by the disc diffusion agar and radial inhibition growth methods.
Results: The extract and fractions showed antibacterial activity against eleven strains (five Gram-positive and six Gram-negative) and a bacteriostatic effect in the survival curves for *Staphylococcus aureus* and *Vibrio cholerae*. The extract and fractions were also shown to have antifungal activity, particularly against *Trichophyton mentagrophytes* (CF_{50} = MeOH1: 1.07 mg/mL, MeOH2: 1.32 mg/mL and H: 1.08 mg/mL). The antioxidant activity of MeOH1 (68.6 µg/mL) was higher than for MeOH2 (108.1 µg/mL). The main compounds of the essential oil were β-pinene, 1,3,8-*p*-menthatriene, ledene, *m*-menthane, linalyl acetate and 3-carene. The main compounds of MeOH1 were β-sitosterol, lupeol and pyrogallol; the main compounds of MeOH2 were β-sitosterol, spathulenol, coniferyl alcohol and lupeol; and the main compounds of H were β-sitostenone, γ-sitosterol and stigmasterol.
Conclusions: This study indicates that *Jatropha neopauciflora* is a potential antibacterial and antifungal agent.

Keywords: Euphorbiaceae; *Jatropha*; Medicinal plants; Antimicrobial activity

Introduction

Medicinal plants are an important element of indigenous medicinal systems in Mexico. More than 6000 species are used for therapeutic purposes in the country; however, only 10% have been studied at the pharmacological, microbiological and phytochemical levels (Aguilar et al., 1994). Mexico has the highest rates of endemic species, one of which is *Jatropha neopauciflora* Pax (Euphorbiaceae). This species is used in the treatment of sores and infections of the oral cavity, loose teeth, athlete's foot and wounds (Canales et al., 2006 and 2005; Arias et al., 2001).
The family Euphorbiaceae has been extensively studied because it presents medicinal properties in the relief of various ailments. *Croton lechleri* latex has shown antibacterial activity, especially against Gram-positive bacteria (Ubillas et al., 1994). *Croton urucurana* Baillon extract was tested against five dermatophyte fungi and showed significant antifungal activity (Gurgel et al., 2005).

The genus *Jatropha* has been studied phytochemically, and in some cases, the biological activity has been determined, e.g., the root extract of *Jatropha curcas* L. showed antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus* and *B. subtilis* (Arreola et al., 2010). Additionally, *J. gossypiifolia* L. and *J. dioica* Sessé ex Cerv. showed

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antibacterial activity (Chariandy et al., 1999), and the methanol extract of the leaves of *Jatropha gaumeri* Greenm. showed significant antioxidant activity (Can-Ake et al., 2004).

In particular, *J. neopauciflora* (common name: "sangre de grado") is an endemic species of the Tehuacan-Cuicatlan Valley, Mexico. This species is used by the residents of San Rafael to alleviate illnesses of bacterial origin (e.g., skin eruptions, toothache) (Canales et al., 2005). There have been few phytochemical studies, but García and Delgado in 2006 (a,b) obtained sesquiterpenes from the bark.

In this study, we experimentally validated the traditional use of *Jatropha neopauciflora* in Mexican traditional medicine. It is important to preserve and disseminate this knowledge regarding the sustainable use of these endemic species of our country.

Materials and Methods

Plant material

Plant material was collected in March 2013 in San Rafael, Coxcatlan, Puebla. The specimens were collected in the field with permission from the "Secretaria de Medio Ambiente y Recursos Naturales" (SGPA/DGVS/1266). Voucher specimens were deposited in the National Herbarium of Mexico (MEXU) at the Universidad Nacional Autonoma de Mexico and the herbarium IZTA at the Facultad de Estudios Superiores Iztacala (voucher no. 29284).

San Rafael is a village in the municipality of Coxcatlan, which is located southeast of the Tehuacan-Cuicatlan Valley at 18°12' to 18°14' north and 97°07' to 97°09' west, 957 m above sea level. The climate is dry or arid with summer rains and a mean temperature of 22°C (Fernández, 1999).

Chemical analysis of essential oil

The essential oil was obtained by the hydro-distillation of 1 kg of fresh plant for 60 minutes in a Clevenger-type apparatus. The essential oil of *J. neopauciflora* was analyzed using a Hewlett-Packard 5890-II (Wilmington, DE USA) gas chromatograph equipped with a DB WAX ultra-column (22.50 m x 0.20 mm). The temperature of the column was programmed starting at 40ºC for 1 min, then increased by 7°C/min to 305ºC/10 min. The injector and detector temperatures were 290ºC. The gas carrier was helium, at a flow rate of 1 mL/min. The peak areas were measured by electronic integration. The relative amounts of the individual components were based on the peak areas. GC-MS analysis was performed on a Jeol AX50HA mass spectrometer. The mass spectra were recorded at 70 eV. The retention time was recorded in minutes. The oil components were identified by comparing their retention indices and mass spectra with the NIST/EPA7NIH Mass Spectral Library.

Preparation and chemical analysis of extracts and fractions

The methanol extract (MeOH1) was obtained by maceration from the dry aerial part of the plant (3 kg). After filtration, the solvent was evaporated under reduced pressure to generate the methanol extract (MeOH1). The yield of MeOH1 was 250.0 g (8.3%). Then, 120.0 g of MeOH1 was dissolved in methanol (500 mL) and hexane (500 mL) and placed in a separatory funnel. After solvent–solvent extraction, the methanol fraction (MeOH2) was removed from the hexane (H). After removing the solvent, 96.0 g of MeOH2 (80.0%) and 24.0 g of H (20.0%) were obtained.

MeOH1, MeOH2 and H were injected into a gas chromatograph AGILENT 6850 (China) equipped with a RTX column (30 m x 0.25 mm i.d., film thickness 0.25 µm). The temperature of the column was programmed starting at 70°C for 2 min, then increased by 8°C/min to 270°C. At 270°C, a programmed linear gradient increased the temperature by 10°C/min to 290°C. The injector and detector temperatures were 250°C and 290°C, respectively. The gas carrier was helium at a flow rate of 0.9 mL/min. The peak areas were measured by electronic integration. The relative amounts of the individual components were based on the peak areas. GC-MS analysis was performed on an AGILENT 5975C (China) mass spectrometer. The mass spectra were recorded at 70 eV. The components were identified by comparing the retention indices and the mass spectra with the NIST/EPA/NIH Mass Spectral Library.

Test microorganisms

Bacterial strains: *Staphylococcus aureus* (clinical case, cc) and ATCC 29213, *S. epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Micrococcus luteus*, *Vibrio cholerae* CDC V 12 El Tor, *Vibrio cholerae* INDRE 206, *Vibrio cholerae* (clinical case), *Enterobacter aerogenes*, *Yersinia enterocolitica*, *Escherichia coli* ATCC 53218, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis*, *Enterobacter aerogenes* and *Enterobacter cloacae*.

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Fungal strains: Yeast: *Candida albicans* ATCC 14065, *Candida albicans* ATCC 10231, *Candida albicans* ATCC 14065 (clinical case) and *Cryptococcus neoformans*. Filamentous fungi: *Aspergillus niger* CDBB-H-179, *Aspergillus* sp., *Trichophyton mentagrophytes* CDBB-H-1112, *Fusarium sporotrichioides* ATTC NRLL3299, *Fusarium moniliforme* CDBB-H-265 and *Rhizoctonia lilacina* CDBB-H-306.

Each bacterial strain was suspended in nutrient broth and incubated for 24 h before testing at 37°C. Müeller Hinton agar and Potato-Dextrose agar (PDA) were used in testing the antibacterial and antifungal activity, respectively.

Antibacterial activity assay

The antibacterial activity was measured by the Kirby-Baüer disc diffusion agar method (Vanden Berghe y Vlietinck, 1991). The bacterial inoculum was incubated in 10 mL Müeller Hinton broth at 37°C for 24 hours. The cultures were adjusted to a turbidity comparable to McFarland standard no. 0.5 with a sterile saline solution ($1.5x10^8$ CFU/mL). The microbial suspensions were plated on Müeller Hinton agar plates (Bioxon). Five mm diameter discs (Whatman no. 5) were impregnated with 10 µL of MeOH, MeOH2 or H (final dose per disc: 2 mg) and 5 µL of the essential oil. Discs containing only methanol or hexane served as the negative control, whereas discs with 25 µg chloramphenicol were used as positive controls. The tests were performed in triplicate.

The minimal inhibitory concentration (MIC) was estimated using the broth dilution method (Vanden Berghe and Vlietinck, 1991). Diluted plant extracts, fractions and essential oil (10.0 to 0.125 mg/mL) were used. For the essential oil assay, DMSO (0.1%) was used as an oil-in-water emulsifier. The tubes were inoculated with microorganism suspension of 10^5 CFU/mL. The MIC values were defined as the lowest extract concentration that prevented visible bacterial growth after 24 h of incubation at 37°C. Each experiment was repeated at least three times. The bactericidal kinetic assay was performed using the appropriate concentrations of the extract (corresponding to ½MIC, MIC and MBC) (Muroi et al., 1993).

Antifungal activity assay

For qualitative analysis, the method of radial growth inhibition (Wang and Bun, 2002) was used. Petri dishes with PDA agar were inoculated by point deposition of mycelium (5 mm diameter) and incubated at 28°C for 72 hours. After mycelium growth, paper filter discs saturated with 2 mg of MeOH1, MeOH2 and H were placed over these plates.

Ketoconazole was used as a positive control (disc with 7.0 µg), and 10 µL applications of the solvents (methanol and hexane) were used as negative controls. Mycelium reduction or changes in color, sporulation or morphology was reported as antifungal activity. No antifungal activity was reported when the growth of the fungi was similar to the control and grew over the discs. The tests were carried out in triplicate.

The medium fungicidal concentration (FC_{50}) was determined according a modified version of the Wang and Bun (2002) method. Each well in a 24-well culture plate was filled with 1.5 mL of PDA with the following extract concentrations: 4.0, 3.0, 2.0, 1.0, 0.50, 0.25 mg/mL, and a point (1 mm diameter) deposition of each strain inoculum was placed in the agar at the center of the well. Ketoconazol (40, 50, 60, 70, 80, 90 and 100 µg/mL) was used as a positive control, and agar with DMSO (≤ 0.1%) was used as a negative control. Standards with 1.5 mL of agar were also used. The plates were incubated at 23°C for 72 hours or until mycelium growth was observed. The tests were performed by triplicate.

The regressions were measured to determine the CI_{50}, or the extract concentration that delayed the colony radial extension by 50%.

Antioxidant activity

The antioxidant activity of the extract and fractions of *J. neopauciflora* were determined spectrophotometrically at 517 nm by the decoloration of a methanol solution of DPPH (free radical, 2,2-diphenyl-1-picrylhydrazyl) (Murillo, 2006). Ninety-six-well ELISA plates were filled with extract concentrations ranging from 1 to 100 µg/mL. Each concentration was performed by triplicate. HPLC-grade methanol served as a blank sample, and a DPPH solution (100 µM) served as a control. The plates were incubated for 30 min at 37°C, and the absorbance values were determined at 540 nm using an ELISA plate reader. The antioxidant activity values were determined according to the following equation: % inhibition = [(absorbance of control-absorbance of sample)/ absorbance of control]*100. The concentration leading to 50% inhibition (SC_{50}) was determined graphically. Quercetin was used as a reference (positive control).

General toxicity assay

The general toxicity *in vitro* was determined using brine shrimp *Artemia salina* (Leach) larvae, according to the methodology described by McLaughlin (1991). MeOH1, MeOH2 and H were tested at 1000, 100 and 10 ppm and also

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evaluated in triplicate. Samples were prepared by dissolving the extracts in DMSO. The final DMSO concentration did not exceed 1%, which has been shown to have no harmful effects on the larvae. Gallic acid was used as a positive control (LC_{50} = 321.5 µg/mL), and DMSO was used as a negative control. Survivors were counted after 24 h, and LC_{50} was determined from the 24-h counts. The general toxicity was considered weak when the LC_{50} was between 500 and 1000 µg/mL, moderate when the LC_{50} was between 100 and 500 µg/mL, and strong when the LC_{50} ranged from 0 to 100 µg/mL (Padmaja et al., 2002).

Results

The physicochemical data of the essential oil were as follows: *J. neopauciflora* d^{25} = 0.85 g/mL, and 0.013% (v/w) of oil from fresh weight was obtained. Nine compounds of the essential oil of *J. neopauciflora* were identified by GC-MS analysis, representing 100.00% (Table 1). Of these compounds, seven were monoterpenes and only two sesquiterpenes. The main compounds with concentrations higher than 10% as a percentage of peak area were β-pinene (21.04%), 1,3,8-*p*-menthatriene (14.98%), ledene (14.29%), *m*-menthane (12.77%), linalyl acetate (12.03%) and 3-carene (10.14%).

The GC-MS of MeOH1 showed five compounds, and the main ones were β-sitosterol, lupeol and pyrogallol; for MeOH2, the main compounds were β-sitosterol, spathulenol, coniferyl alcohol and lupeol; and for H, the main compounds were β-sitostenone, γ-sitosterol and stigmasterol (Table 1, Fig. 2).

Table 1: Composition of essential oil, MeOH1, MeOH2 and H of *Jatropha neopauciflora.* The components were identified by comparing their retention indices and mass spectra with the NIST/EPA7NIH Mass Spectral Library.

Compounds	RT (min)	Abundance (%)
	Essential oil	
α-Pinene	6.547	7.12
Camphene	6.965	3.78
Linalyl acetate	7.777	12.03
β-Pinene	8.060	21.04
1,3,8-*p* -Menthatriene	9.081	14.98
m-Menthane	9.179	12.77
3-Carene	9.499	10.14
(-)-Spathulenol	21.455	3.85
(+)-Ledene	22.844	14.29
	MeOH1	
pyrogallol	10.76	6.56
Arabinol	17.90	14.34
β-sitosterol	27.25	16.01
Lupeol	30.28	22.08
Stigmasterol	33.72	3.39
	MeOH2	
Spathulenol	13.45	1.37
coniferyl alcohol	15.19	0.58
β-sitosterol	27.99	16.54
Lupeol	29.67	21.82
	H	
γ-sitosterol	28.34	3.48
β-sitostenon	28.91	0.70
Stigmasterol	29.10	2.09

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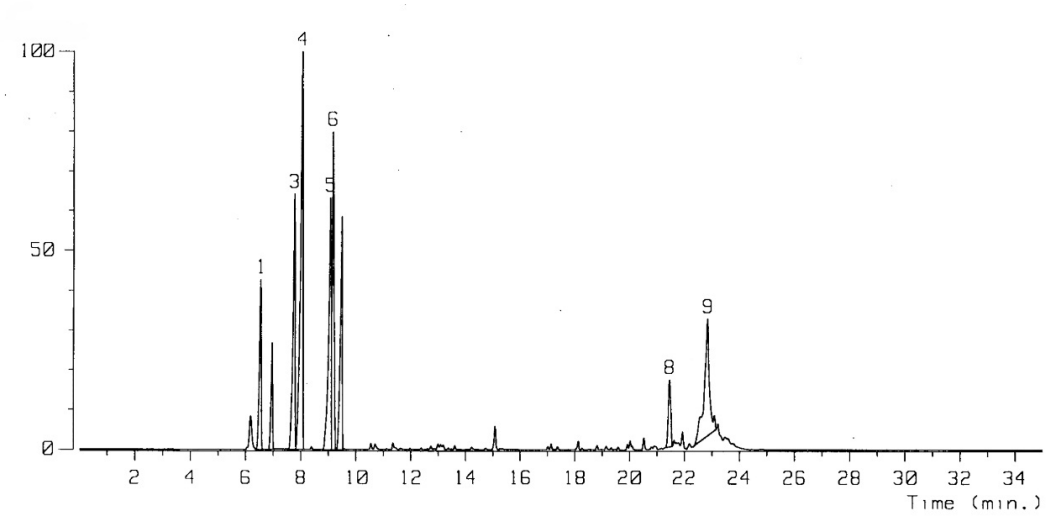

Figure 1: Chromatogram plot of *J. neopauciflora* essential oil.

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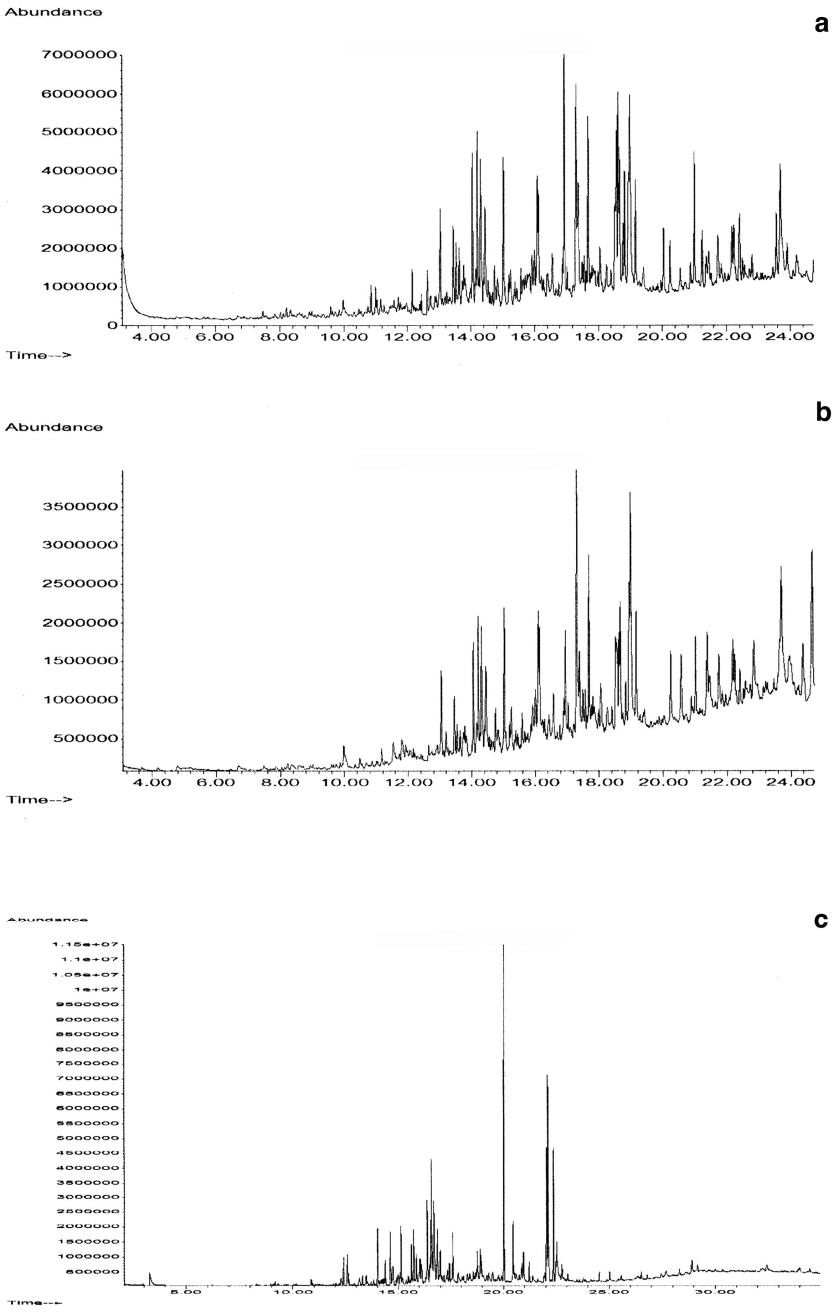

Figure 2: Chromatogram plots of *J. neopauciflora*. a) methanol extract (MeOH1), b) methanol fraction (MeOH2), c) hexane fraction (H).

The essential oil alone was tested against *S. aureus* (inhibition halos 9.0 ± 0.5 mm; MIC 2.0 mg/mL) and *Vibrio cholera* (inhibition halos 18.0 ± 0.5 mm; MIC 1.0 mg/mL). MeOH1 exhibited inhibitory effects against five Gram-positive bacteria and three Gram-negative bacteria. MeOH2 exhibited inhibitory effects against four Gram-positive bacteria and one Gram-negative bacteria, and H showed activity against three Gram-positive and four Gram-negative bacteria. *E. coli* showed the highest diameter inhibition zones for MeOH1 (8.33 mm), MeOH2 (12.66 mm) and H (10.0 mm). All strains were susceptible to at least one extract or fraction; however, *Staphylococcus aureus* (cc) and the *E. coli* strains showed sensitivity to both the extract and fractions (Table 2).

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ANOVA testing revealed significant differences between the two bacterial groups (Gram-positive and Gram-negative bacteria) (F=418.81, p=0.0001): the Gram-positive bacteria showed higher inhibition zones than Gram-negative bacteria. Additionally, *E. coli* was the bacterium with the lowest MIC for MeOH1 (<<0.25 mg/mL), MeOH2 (0.25 mg/mL) and H (0.5 mg/mL) (Table 3).

Table 2: Antibacterial activity of extract and fractions of *J. neopauciflora*

	Inhibition halos (mm)			
Bacteria	Methanol extract (MeOH1)	Methanol fraction (MeOH2)	Hexane fraction (H)	Positive control chloramphenicol
S. aureus (cc)	7.3 ± 0.5	6.6 ± 0.5	7.6 ± 0.5	28.0 ± 0.0
S. epidermidis FES-C	na	na	na	30.3 ± 0.5
B. subtilis FES-C	7.3 ± 0.5	na	na	32.6 ± 0.5
B. subtilis	7.0 ± 0.0	7.0 ± 0.0	na	24.0 ± 1.0
S.aureus 29213	na	4.0 ± 3.4	7.0 ± 0.0	20.0 ± 0.0
E. feacalis	na	na	na	24.3 ± 0.5
M. luteus	8.0 ± 0.0	na	na	25.3 ± 0.5
S.pneumonie	7.0 ± 0.0	9.0 ± 1.0	10.0 ± 0.0	30.0 ± 0.0
P. mirabilis	na	na	na	17.3 ± 0.5
P.mirabilis CC	na	na	na	14.6 ± 0.5
E. aerogenes	na	na	7.0 ± 1.0	22.0 ± 0.0
V. cholerae El Tor	7.3 ± 0.5	na	7.0 ± 0.0	25.0 ± 0.0
V. cholerae. CC	na	na	na	23.3 ± 1.0
V.cholerae. Agua	na	na	6.3 ± 0.5	22.0 ± 0.0
E. coli. 53218	8.3 ± 0.5	12.6±0.5	10.0 ± 2.6	30.0 ± 0.0
P. aeruginosa	7.0 ± 0.0	na	na	20.0 ± 0.0

na: no activity. Each assay was performed by triplicate. The data show the media and standard deviation.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract and fractions of *Jatropha neopauciflora.*

BACTERIA	Extract and fractions of *J. neopauciflora* (mg/mL)					
	MeOH1		MeOH2		H	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus (cc)	0.1	0.2	4.0	6.0	2.0	4.0
S. epidermidis FES-C	0.1	0.2	na	na	na	na
B. subtilis FES-C	1.0	2.0	na	na	na	na
B. subtilis	0.12	4.0	4.0	6.0	na	na
S. aureus 29213	4.0	>4.0	4.0	6.0	6.0	>6.0
E. feacalis	na	na	na	na	na	na
M. luteus	na	na	na	na	na	na
S.pneumonie	0.5	1.0	4.0	6.0	2.0	4.0
P. mirabilis	na	na	na	na	na	na
P. mirabilis CC	na	na	na	na	na	na
E. aerogenes	2.0	>4.0	na	na	2.0	4.0
V.cholearae El Tor	2.0	>4.0	na	na	6.0	>6.0
V.cholearae CC	na	na	na	na	na	na
V.cholearae Agua	na	na	na	na	2.0	4.0
E. coli 53218	<<0.2	<0.2	0.2	0.5	0.5	1.0
P. aeruginosa	4.0	>4.0	na	na	na	na

na: no activity.

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Figures 3 and 4 show the effect of MeOH1 on the survival curve for a Gram-positive (*S. aureus* cc) and a Gram-negative bacterium (*V. cholerae* El Tor). In both survival curves, all MeOH1 concentrations (MBC, MIC and ½ MIC) reduced bacterial growth; however, the growth of *V. cholerae* was more affected than the growth of *S. aureus*.

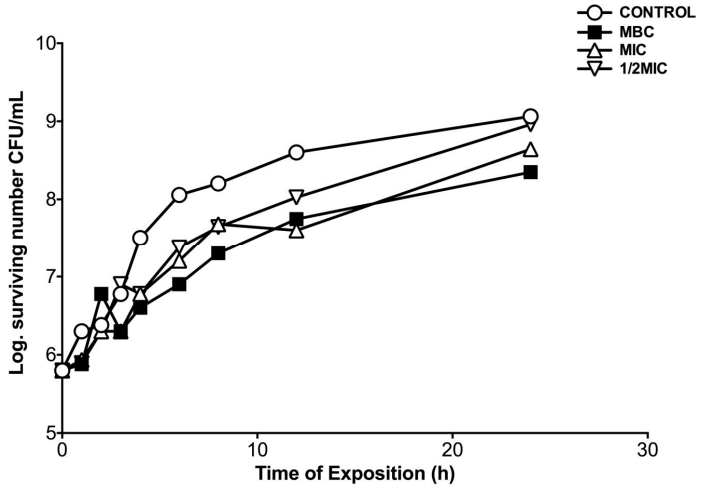

Figure 3: Effect of MeOH1 of *J. neopauciflora* on the growth curve of *S. aureus* (cc): 1/2 MIC: 0.062 mg/mL; MIC: 0.125 mg/mL; MBC: 0.250 mg/mL. The MBC only decreases the bacterial growth.

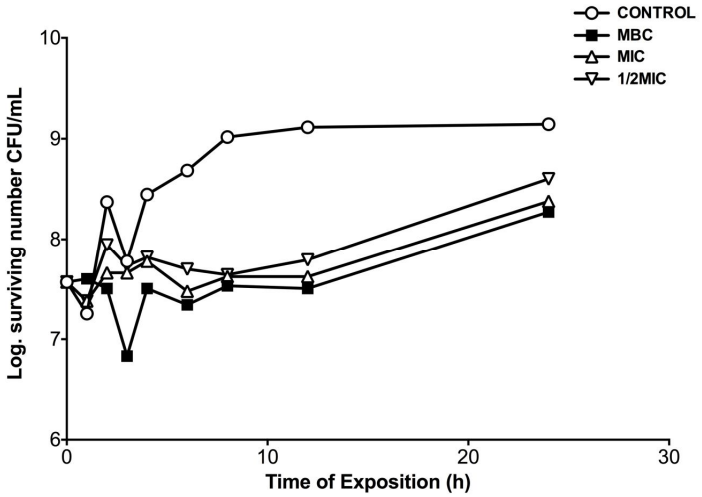

Figure 4: Effect of MeOH1 of *J. neopauciflora* on the growth curve of *Vibrio cholerae* El Tor. 1/2 MIC: 1 mg/mL; MIC: 2 mg/mL; MBC: 4 mg/mL. The three concentrations decrease the bacterial growth. The qualitative tests demonstrated that MeOH1, MeOH2 and H had no activity against the yeasts but affected all of the strains of filamentous fungi. The highest inhibition values were for *Fusarium moniliforme* and *Trichophyton mentagrophytes* (MeOH1 IC_{50}= 2.76, 1.07 mg/mL; MeOH2 IC_{50}= 1.69, 1.32 mg/mL; H IC_{50}= 0.99, 1.08 mg/mL) (Table 4).

Table 4: IC_{50} of the extract and fractions of *J. neopauciflora.*

	Jatropha neopauciflora (mg/ mL)			Ketoconazole (µg/mL)
FUNGUS	MeOH 1	MeOH 2	H	C^+
Aspergillus sp.	>4.0	>4.0	>4.0	9.7
A. niger.	>4.0	>4.0	>4.0	15.2
F. sporotrichoides	2.9	2.5	1.2	3.9
R. lilacina	0.7	2.8	1.5	21.5
F. moniliforme	2.7	1.6	0.9	7.5
T. mentagrophytes	1.0	1.3	1.0	1.1

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In terms of antioxidant activity, MeOH1 had SC_{50}=68.50 µg/mL and MeOH2 had SC_{50}=108.1 µg/mL compared with the SC_{50}=4.6 µg/mL of quercetin (positive control). The total phenolic content of MeOH1 was determined from the gallic acid curve to be 74.4 mg GAE/g, which represents 0.5% of the total extract. The total flavonoid content of MeOH1 was 21982.76 µg (QE)/g, which represents 0.022% per gram of MeOH1. With respect to toxicity, the extract MeOH1 (LC_{50}= 17.4 µg/mL) and fractions MeOH2 (LC_{50}= 11.2 µg/mL) and H (LC_{50}= 41.6 µg/mL) were all toxic against *A. salina*.

Discussion and conclusion

Jatropha neopauciflora is a medicinal plant and is one of the main species used in San Rafael, Coxcatlán, Puebla (Canales et al., 2005). The results obtained in this work showed the composition of the essential oil, extract and fractions as well as the antibacterial, antifungal and antioxidant activity.

Essential oils play important roles in the protection of plants as antibacterials, antivirals, antifungals, and insecticides, and also by reducing the appetite of herbivores for such plants. Some essential oils appear to exhibit particular medicinal properties (Silva et al., 2003). This oil consists mainly of monoterpenes (Table 1). This activity may be attributed to the presence of β-pinene (major component, 21.04%), which presents antiviral, antibacterial, anti-inflammatory and fungicidal activity by acting on cellular integrity, inhibiting the respiration and ion transport processes, and increasing membrane permeability (Lima et al., 2005). Additionally, a number of monoterpenes and sesquiterpenes (camphene, spathulenol, linalool) have been shown to possess antimicrobial activities (Takikawa et al., 2002; Cimanga et al., 2002; Magwa et al., 2006).

The MeOH1 extract was obtained by exhaustive maceration, the purpose was to obtain all the compounds present in the plant. After this maceration, the utility of the partition liquid-liquid was to obtain one fraction with all polar compounds (MeOH2) and one fraction with the non-polar compounds (H), and then, we observed there were not significative differences between the MeOH1, MeOH2 and H with respect at the antimicrobial activity. On the other hand, this partition facilitated the chemical analysis. In the extract and fractions, the presence of pyrogallol and coniferyl acid is observed (Table 1). These compounds are probably responsible for the antibacterial activity, as the data are consistent with Kocacaliskan et al., (2006), who tested the antibacterial activity of pyrogallol against three bacteria (*Pseudomonas putida, Pseudomonas pyocyanea, Corynebacterium xerosis*) and found significant antibacterial activity. Pyrogallol showed activity against bacteria and fungi through inhibiting the function of the plasma membrane, altering its permeability, and is a good antioxidant (Muñoz et al., 2004).

The Gram-negative bacteria (*Vibrio cholera* El Tor) were the most sensitive (Table 2). Because of the great number of constituents, essential oils seem to have no specific cellular targets (Carson et al., 2002). As typical lipophiles, the cytotoxicity appears to include membrane disruption (Turina et al., 2006). However, the extract and fractions showed greater inhibition in Gram-positive bacteria, which is very important because *J. neopauciflora* inhibits many bacteria that cause various infections.

In the determination of MIC and MBC (Table 3), the extract and fractions all showed antibacterial activity. The most sensitive strains were *Staphylococcus aureus* (Gram-positive) and *Escherichia coli*, (Gram-negative), with smaller concentrations needed to have an effect. These results are consistent with several previous studies: for example, the leaf extract of *Euphorbia caracasana* showed antibacterial activity against *S. aureus* ATCC 25923 (Rojas et al., 2008), and the root extract of *Jatropha* curcas showed antibacterial activity against *S. aureus, B. cereus* and *B. subtilis* (Arreola et al., 2010).

In terms of the antifungal activity (Table 4), the most sensitive strain was *T. mentagrophytes* (MeOH1: IC_{50}=1.07, MeOH2: IC_{50}=1.32 and H: IC_{50}=1.08 mg/mL). *J. neopauciflora* is used as a treatment for athlete's foot, for which the fungus responsible is *T. mentagrophytes* (Arias et al., 2001) Additionally, several species of the family Euphorbiaceae have been reported to show antifungal activity. For example, *Croton urucurana* (Gurgel et al., 2005) showed antifungal activity against five dermatophyte fungi (*T. tonsurans, T. mentagrophytes, T. rubrum, Microsporum canis and Epidermophyton floccosum)*, and the chloroform extract of the aerial part of *Phyllanthus amarus* showed significant antifungal activity against *Microsporum gypseum* (Abad et al., 2007).

For antioxidant activity, MeOH1 showed the best antioxidant capacity (SC_{50}= 68.6 µg/mL), better than MeOH2 (SC_{50}= 108.1 µg/mL). A synergism probably exists among the compounds of the extract. The extract and fractions showed strong toxicity. These results are similar to toxicity tests performed on the stem of *Euphorbia kamerunica*, which also showed high toxicity (Ogunnusi, 2008). The leaf extract of *J. curcas* also showed high mortality rates (Karmegan et al., 1997).

The results obtained in this investigation showed that the essential oil, extract and fractions of *Jatropha neopauciflora* have antibacterial activity. The composition and antibacterial activity of the essential oil, extract and fractions of *Jatropha neopauciflora* are reported here for the first time. These results indicate that *Jatropha neopauciflora* is a potential antibacterial and antifungal agent.

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