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Antimicrobial and Cytotoxic Activities from *Jatropha dioica* Roots

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Abstract: The antimicrobial and cytotoxic activities of organic extracts obtained from roots of the medicinal plant *Jatropha dioica* (Euphorbiaceae) were investigated. In order to evaluate their antimicrobial activity, the organic extracts were tested against clinical isolates of the human pathogens *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Salmonella typhimurium*, *Cryptococcus neoformans*, *Candida albicans*, *Candida parapsilosis* and *Sporothrix schenckii*. Results revealed that the hexane extract possess the stronger activity and a broader microbicide spectrum compared to the acetone and ethanol extracts. The activity of hexane extract may be attributed in part to the presence of β -sitosterol, the major compound identified by bioautography. The hexane extract, as well as the bioactive fraction were not cytotoxic when assays were profiled against the normal cell lines Chang, OK and LLC PK-1 ($IC_{50} > 1000 \mu\text{g mL}^{-1}$).

Key words: *Jatropha dioica*, Euphorbiaceae, antimicrobial, cytotoxicity, β -sitosterol

INTRODUCTION

One of the most alarming recent trends in infectious diseases has been the increasing frequency of drug resistance among microorganisms. Numerous classes of antimicrobial agents have become less effective as a result of the selective pressure of antimicrobial usage (Galan *et al.*, 2013). The use of plant extracts and phytochemicals could be useful for therapeutic treatments of common infectious diseases and overcome the problems of resistance and side effects of the currently available antimicrobial agents (Savoia, 2012).

According to the World Health Organization, about 80% of individuals from developed countries use traditional medicine which include the use of medicinal plant extracts and teas (Akerle, 1993). In accordance to this, during the last years, scientists from the entire world have made efforts in order to validate the medicinal properties, safety and efficiency of plants used as ethnobotanic remedies.

In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Rojas *et al.*, 2006; Talib and Mahasneh, 2010; Abu-Darwish *et al.*, 2012). Many studies have been made

in Mexico, a country with near to 4,000 plant species used for medicinal purposes (Ruiz-Bustos *et al.*, 2009). For example, *Jatropha dioica* (Euphorbiaceae) which is widely distributed in Mexico, is used as an antidiarrheic, antiseptic, anti-inflammatory, antirheumatic, anticancerogenic and also to treat buccal diseases and cutaneous eruptions (Youngken, 1951). However, scientific studies validating these uses are scarce. Considering the above said, the aim of this study was to assess the antimicrobial activity of this medicinal plant against pathogenic bacteria and fungi which possess clinical importance. Additionally, the cytotoxicity of bioactive samples on normal cell lines was tested.

MATERIALS AND METHODS

Jatropha dioica (Euphorbiaceae) was collected in Santa Catarina, Nuevo León state, México, during June 2009. The plant was identified by Dr. Marcela González Alvarez and a plant specimen was deposited in the ethnobotanical collection of the herbarium from the Biological Sciences School at UANL, San Nicolás de los Garza, NL (voucher herbarium specimen number: 023709).

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The roots of the plant were dried at room temperature and 150 mg were sequentially extracted by maceration with hexane, acetone and ethanol (7 days each). The plant: solvent ratio was 1: 5 (w/v). Organic extracts were concentrated *in vacuo* to dryness; all extracts were stored at 4°C until use. The percentage yield of extracts from *J. dioica* was: hexane (0.5), acetone (2.4) and methanol (3.1).

Clinical isolates of *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Salmonella typhimurium*, *Cryptococcus neoformans*, *Candida albicans*, *Candida parapsilosis* and *Sporothrix schenckii* were tested. By using cultures of the above mentioned microorganisms, a bacterial suspension (after 18-24 h of incubation) was prepared and turbidity was adjusted to 0.5 McFarland standards which corresponded to 10⁶ colony forming U mL⁻¹ (CFU mL⁻¹) (Ripa *et al.*, 2010). In the case of fungi, after 4-7 days of culture, the mycelia was aseptically scraped, suspended thoroughly in sterile distilled water and adjusted spectrophotometrically to an absorbance of 0.600 at 450 nm (0.5-2.5×10³ cells mL⁻¹), this suspension was used as inoculum for the tests of antifungal susceptibility (Beatriz *et al.*, 2012). The inhibition assays were performed by using the disk diffusion agar method. Test plates were prepared with 20 mL of sterile Mueller Hinton (bacterial) or Sabouraud Dextrose (fungi) media. The standardized microorganisms suspension was applied on the solidified culture medium by using sterile cotton swabs and allowed to dry for 5 min. A sterile paper disk (Whatman AA disk, 6 mm) was impregnated with 10 µL of a stock solution (50 mg mL⁻¹) from each crude extract. Disks containing 500 µg of sample were aseptically transferred on the inoculated agar plates and incubated at 37°C/18-24 h for bacteria and 25°C/4-7 days for fungi. Antimicrobial activity was determined by measuring clear zones of inhibition around the test crude extract disks. The clear zones indicated the biocide effect. Gentamicin (40 µg) and fluconazole (50 µg) impregnated disks were used as a standard reference or positive controls for bacteria and fungi respectively and the solvent or empty disks were used as negative controls (Roy *et al.*, 2011). All assays were performed in triplicate; the data were analyzed by the Kruskal Wallis and Mann Whitney statistical tests and expressed as the average±standard deviation. Significant differences were considered when p = 0.05. The SPSS software (version 10.0) was used for statistical analysis.

A simple, direct bioautographic assay on TLC plates was used to screen compounds that showed activity against the tested microorganisms (Hamburger and Cordell, 1987). One hundred microliter of extract (50 mg mL⁻¹) were applied to the TLC plate and developed in a

petroleum:benzene:acetone:methanol (9:9:2:2) mobile phase. The suspension of a given microorganism was applied with a hyssop to the developed TLC plate until the respective agar media was wet. Plates were inoculated with 10⁶ CFU mL⁻¹ for bacteria or 0.5-2.5×10³ cells mL⁻¹ for fungus and incubated for different periods at 37 and 25°C, respectively. Afterwards, the culture plates were sprayed with 2 mg mL⁻¹ of a MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution and incubated during 4 h in a sealed container for colour development. Antibacterial compounds were identified as clear zones against a blue/purple coloured background that indicated bacterial growth.

The LLCPK-1 (Pig kidney epithelial), Chang (Human hepatocytes) and OK (Opossum kidney epithelial) normal cell lines were used in order to determine the cytotoxicity of the samples (extract and fraction) that showed relevant antimicrobial activity. Cell cultures were grown in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, 100 U mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin and were incubated at 37°C in a humidified atmosphere with 5% CO₂. The cytotoxicity of samples was measured by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In this assay, mitochondrial dehydrogenases from viable cells reduce MTT to a blue/purple formazan product which is directly related to cell viability (Talib and Mahasneh, 2010).

RESULTS AND DISCUSSION

The relative effectiveness of organic extracts is determined by comparing the diameter of the zone of inhibition with referenced values (Table 1); if the extract displays inhibition zones at 500 µg disk⁻¹, the antimicrobial activity is considered good (Ripa *et al.*, 2010; Roy *et al.*, 2011). The hexane extract of *Jatropha dioica*

Table 1: Antimicrobial activity of the extracts by the root of *J. dioica*

Microorganisms	Diameter of inhibition zone (mm)			
	Gram organic extract			
	EtOH ^b	Acetone ^c	Hexane ^a	Control*
<i>Bacillus cereus</i> G+	-	-	9±0	21±1
<i>Staphylococcus aureus</i> G+	14±1	15±2	16±1	35±4
<i>Escherichia coli</i> G-	21±3	12±1	22±3	23±3
<i>Salmonella typhimurium</i> G-	-	-	11±1	20±1
<i>Salmonella typhi</i> G-	-	-	-	23±5
<i>Enterobacter aerogenes</i> G-	-	11±2	-	34±3
<i>Enterobacter cloacae</i> G-	-	-	-	32±2
<i>Candida albicans</i>	14±2	-	12±1	9±1
<i>Candida parapsilosis</i>	-	-	7±1	30±3
<i>Cryptococcus neoformans</i>	-	-	8±0	40±5
<i>Sporothrix schenckii</i>	11±2	14±3	14±2	35±4

*Gentamicin was used as positive control for bacteria and fluconazole for fungus, p<0.05

(HE) was the most active, exhibiting significant differences when compared with the other extracts ($p > 0.05$), but was inactive against the majority of Gram negative bacteria. In this extract, an active fraction with an Rf value of 0.84 (FHE) was identified by bioautography. This fraction showed activity against *S. aureus*, *E. coli*, *C. albicans* and *C. neoformans*. However, no activity of this fraction was found on the other studied microorganisms, even when the crude extract showed antibacterial or fungicidal activity. The previous finding could be due to a synergic activity of this fraction with other components of the crude extract. The FHE isolated from the hexane extract, was analyzed using a GC-MS Perkin-Elmer AutoSystem GC equipment with a Quadrex 007.5MS column (30 m × 0.25 mm, film thickness 0.25 μm); the (3β)-stigmast-5-in-3-ol (β-sitosterol) was identified as the major constituent (>90% of relative abundance). The β-sitosterol compound has been previously reported to possess antimicrobial activity (Kiprono *et al.*, 2000). According to our results of cytotoxicity from the hexane extract and FEH on Chang, OK and LLCPK-1 normal cell lines, with IC₅₀ values >1000 μg mL⁻¹, not significant cytotoxicity was encountered. Although, it has been reported that many species of the Euphorbiaceae possess highly toxic components which are able to destroy normal cells in mammals (Zhang *et al.*, 2012), this was not the case for the Mexican *J. dioica* species.

Our results suggest that the hexane extract of *Jatropha dioica* is selective against bacteria and fungus. The antimicrobial activity of this extract may be attributed to the presence of terpenes, like β-sitosterol and other compounds which appear to be concentrated in this non-polar extract. These results provide scientific support for the use of *J. dioica* in traditional medicine.

CONCLUSION

In conclusion, the hexane extract of *J. dioica* could be a good alternative in the search for new antimicrobial agents, especially because of the multidrug resistance observed on human pathogenic microorganisms.

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