

## Antimicrobial activity and toxicity of plants from northern Mexico

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The aim of this study was to evaluate antimicrobial potential of methanolic plant extracts: *Carya illinoensis* (Wangenh.) K. Koch, *Selaginella lepidophylla* (Hook. and Grev.) Spring, *Euphorbia antisiphilitica* Zucc., and *Jatropha dioica* Sesse. Plant samples were collected from northern Mexico and the extracts were tested against reference bacteria (RS): *Staphylococcus aureus* (BAA44) and *Klebsiella pneumoniae* (9180) and clinical isolated bacteria (CB): *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. Antimicrobial activity was performed with Steers Replicator, the LD<sub>50</sub> was evaluated with *Artemia salina* bioassay, and a phytochemical screening was done with colorimetric tests. The extracts showed a minimum inhibitory concentration of 500 µg/mL and LD<sub>50</sub> of 1000 µg/mL. The phytochemical tests were positive for flavonoids, lactones, quinones, triterpenes and sterols. *C. illinoensis*, *S. lepidophylla* and *J. dioica* had high correlations ( $\geq 0.969$ ) to inhibit the growth of *S. aureus* (RS and CB), *K. pneumoniae* (RS) and *K. pneumoniae* (CB) ( $p = 0.080, 0.076, 0.016$  and  $0.029$ , respectively). The results will contribute to the knowledge of plants used in Mexican traditional medicine.

**Keywords:** Medicinal plants, Methanol extracts, Steers' replicator, Antimicrobial activity.

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Medicinal plants have attracted worldwide attention through searching of new molecules for the treatment of certain infectious diseases<sup>1-3</sup>. In Mexico, the importance of medicinal plants resides in the high biodiversity and cultural acceptance of medicinal plants<sup>3-5</sup>. Plants produce different types of substances that serve as a means of defense against microorganisms, insects or predators; substances that might serve as well as antibiotics, antivirals, anti-inflammatory, anti-parasitic, anti-cancer<sup>3</sup>. Given the importance of medicinal plants' therapeutic use in Mexico, the development of more studies including clinical trials becomes necessary<sup>6</sup>. The semi-desert region has great importance due to the large area it occupies in the world, and its specific characteristics besides diversity of species present on it. As an example in the Laguna region (comprised by some cities of Coahuila and Durango states) there is a variety

of flora with healing properties used in traditional Mexican medicine for its empirical antimicrobial properties<sup>7-10</sup>; Thus, we decided to select some of the most popular medicinal plants of the Laguna region according to Williamsons' criteria (Some of these are: ethnopharmacological features, medical uses, possibility of containing antibacterial compounds, presence of active ingredients and geographic distribution)<sup>11</sup>. The objective of this study was to evaluate the antimicrobial potential of methanol leaf's extract: *Carya illinoensis* (Wangenh.) K. Koch, Juglandaceae, (Pecan); *Selaginella lepidophylla* (Hook. & Grev.) Spring, Selaginellaceae, (Evergreen); *Euphorbia antisiphilitica* Zucc., Euphorbiaceae, (Candelilla) and *Jatropha dioica* Sesse, Euphorbiaceae, (sangregrado) collected from semi-desert in northern Mexico due to their popular use as a traditional remedy in that region of Mexico.

The bacterial species *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

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are responsible for severe infections in man; So, these strains were selected for this study because they are important pathogens for man and they quickly develop resistance to current antibiotics.

## Materials and methods

### Bacterial species

Reference bacterial strains (RS) *S. aureus* (ATCC) BAA44 and *K. pneumoniae* (ATCC) 9180, and bacteria from clinical isolates (CB) *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. coli*, were donated by the Laboratorio de Química Analítica de la Facultad de Ciencias Biológicas. Universidad Autónoma de Nuevo León, México. The identification and characterization of bacterial strains were performed according to biochemical profiles and recommendations of Microbiology (published elsewhere) and maintained in liquid medium.

### Plant material collection

The fresh plants: *Carya illinoensis* (Wangenh.) K. Koch, *Selaginella lepidophylla* (Hook. & Grev.) Spring, *Euphorbia antisyphilitica* Zucc., *Jatropha dioica* Sessé were collected from different areas of Nazas and Tlahualilo cities, in Durango State, these cities are located at Chihuahuan semi-desert comprising the Laguna Region from Northern Mexico<sup>12</sup>. Their identity were authenticated by professors from Universidad Autónoma Antonio Narro Torreon, Mexico. Voucher specimens (labeled C.i.181214, S.i.181214, E.a191214., J.d.201214, respectively) are deposited at the Centro De Investigación Biomédica, Facultad De Medicina Torreón, Universidad Autónoma De Coahuila. Mexico.

### Preparation of leaf extracts

The whole plants species were dried at 45 °C and ground in a mill (Wiley® model number 4). About 60 gm of the powdered materials of the leaves of each plant were extracted by maceration using methanol (CTR Scientific®). They were kept at room temperature for 24 hrs and then filtered. This process was performed three times and it was stored in a fresh place, light protected. The filtrates were then evaporated to dryness under vacuum in a rotary evaporator (rotary evaporator Büchi® R-205, Switzerland).

### Preparation of the plant extracts solutions

The extracts were prepared at concentrations of 500, 1000 and 2000 µg/ml with 10 ml of sterile distilled water and 1 % of dimethyl sulfoxide (DMSO).

### Microbiological assay with the steer's replicator method

A series of five repetitions of Mueller Hinton agar plates supplemented with extracts and 5 % defibrinated sheep blood were prepared. Bacterial inoculates were standardized to  $1 \times 10^6$  CFU rapidly and simultaneously applied on the agar surface using a Steer's replicator. The tests were examined after 24 hrs incubation at 37 °C and the minimum inhibitory concentration (MIC) of the extract was determined against the tested microorganism.

### Brine shrimp lethality bioassay

Cytotoxic activity of the extracts were evaluated using Brine shrimp of *Artemia salina* (Brine shrimp Nitro Pack®, Salt Creek, Inc., Salt Lake City, USA) lethality bioassay method where four graded doses (based on the Probit test criteria of scale 1000, 500, 100 and 10 µg/mL) were used<sup>13</sup>. Brine shrimps (*Artemia salina* Leach) *nauplii* were used as test organisms. For hatching, eggs were kept in artificial sea water with a constant oxygen supply for 48 hrs. The mature *nauplii* were then used in the experiment. Artificial seawater was used as a negative control, and potassium dichromate (400 ppm) as a positive control. The numbers of survivors were counted after 24 hrs. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation. To ensure that the mortality observed in the bioassay could be attributed to bioactive compounds and not to starvation; we compared the dead larvae in each treatment to the dead larvae in the control<sup>14</sup>. Probit statistical method was used to determine the median lethal dose (LD<sub>50</sub>)<sup>13-14</sup>. Toxicity of the extracts was determined according to the next criteria of scale: LD<sub>50</sub> > 1000 µg/mL as non-toxic,  $500 \leq 1000$  µg/mL as weakly toxic, and < 500 µg/mL as toxic<sup>13</sup>.

### Phytochemical screening

Identification of chemicals compounds was performed by colorimetric tests (Terpenoids, Flavonoids, Alkaloids, Saponins, Sterols and Tannins) according the recommendations of Kuklinsky<sup>25</sup>.

### Statistical analysis

Pearson linear regression coefficient was used to model the relationship between the effect of the extracts and the bacterial strains considering a unilateral significance with SPSS software version 21<sup>th</sup> (IBM). The experiments were developed in five repetitions.

**Results and discussion**

The descriptive statistics, of the mean inhibition of bacterial colony diameter at the MIC according to plant extracts, are shown in Table 1. The linear regression model was used to evaluate the inhibitory effect of extracts by Pearson coefficients, showing a change in inhibition halo per unit dose. In the study, we used a statistical regression model to calculate the antibacterial growth as shown in Table 2.

The extracts showed a minimum inhibitory concentration of 500 µg/mL and LD<sub>50</sub> around 1000 µg/mL. Phytochemical test results were positive for flavonoids, lactones, quinones, triterpenes and sterols a complete report is shown in Table 3. Extracts of *C. illinoensis* (Wangenh.) K.Koch, *J. dioica* Sessé, *S. lepidophylla* (Hook. & Grev.) Spring showed high correlations ( $\geq 0.969$ ) to inhibit the growth of *S. aureus* (RS and CB); *K. pneumoniae* (RS) and *K. pneumoniae* (CB) ( $p = 0.080, 0.076, 0.016, 0.029$ ), respectively. The greatest inhibition halo of bacterial growth at MIC was against *E. coli* (CB) using extracts of *C. illinoensis* (Wangenh.) K. Koch and *S. lepidophylla* (Hook. & Grev.) Spring ( $2.12 \pm 0.96$  &  $2.60 \pm 0.72$ ), respectively and *J. dioica* Sessé against *S. aureus* (RS) and *K. pneumoniae* (RS) ( $1.88 \pm 0.50$  and  $2.06 \pm 0.57$ ), respectively.

All extracts showed a MIC of 500 µg/mL. In a study with methanol extract of *Leucophyllum frutescens* (another plant from the northern Mexico) against *S. aureus* (CB) antibacterial activity was

reported at concentrations of 1000, 500 and 250 µg/mL with a MIC of 25.4 µg/ mL<sup>16</sup>. These results are consistent with those of our study but with different plants, so providing evidence of the antimicrobial activity in vitro of Northern Mexico's plants.

Another study with methanol extracts of five plants from northern Mexico semi-desert region: *Tecoma stans*, *Acacia farnesiana*, *Euphorbia antisiphilitica*, *Fouquieria splendens* and *Leucophyllum frutescens* showed activity against *S. aureus* except *A. farnesiana* that turned inactive. These results are similar to those reported in this study<sup>17</sup> in the antibacterial context.

Methanol extracts of semi-desert plants *Lysiloma acapulcensis*, *Miconia mexicana*, *Hibiscus sabdariffa* showed activity against *S. aureus*, *S. faecalis*, *E. coli* and *K. pneumoniae*. The results were similar to those

Table 3—Identification of chemical compounds and secondary metabolites of plants extracts.

Chemical compounds	Methanol extracts of plants			
	<i>S. lepidophylla</i>	<i>C. illinoensis</i>	<i>J. dioica</i>	<i>E. antisiphilitica</i>
Alkaloids	-	-	-	-
Flavonoids	+	+	+	-
Saponins	-	-	-	+
Tannins	-	-	-	-
Lactons	+	+	+	-
Quinons	-	+	+	+
Terpenes/sterols	+	-	+	-

Table 1—Statistical mean inhibition of bacterial colony diameter at the MIC

Methanol extracts of plants	Bacteria					
	<i>S. aureus</i>		<i>K. pneumoniae</i>		<i>P. aeruginosa</i>	<i>E. coli</i>
	RS (mm)	CB (mm)	RS (mm)	CB (mm)	CB (mm)	CB (mm)
<i>C. illinoensis</i>	0.54 ± 0.52	0.46 ± 0.52	1.04 ± 1.03	1.42 ± 1.02	1.54 ± 0.92	2.12 ± 0.96
<i>S. lepidophylla</i>	1.05 ± 0.76	0.40 ± 0.45	1.18 ± 0.67	1.45 ± 0.61	1.90 ± 0.75	2.60 ± 0.72
<i>E. antisiphilitica</i>	1.25 ± 0.72	0.10 ± 0.50	1.45 ± 0.76	-0.05 ± 0.68	1.60 ± 0.72	0.25 ± 0.55
<i>J. dioica</i>	1.88 ± 0.50	1.03 ± 0.74	2.06 ± 0.57	0.88 ± 0.59	0.28 ± 0.41	1.16 ± 0.81

Mean ± DS. RS Reference strain, Cb clinical bacteria.

Table 2—Linear regression analysis for the halo inhibitory effect against bacterial strains studied

Methanol extracts of plants	Bacteria					
	<i>S. aureus</i>		<i>K. pneumoniae</i>		<i>P. aeruginosa</i>	<i>E. coli</i>
	RS	CB	RS	CB	CB	CB
<i>C. illinoensis</i>	0.969 (0.080)	0.972 (0.076)	0.509 (0.330)	-0.966 (0.083)	-0.913 (0.134)	-0.411 (0.365)
<i>S. lepidophylla</i>	0.628 (0.284)	0.866 (0.167)	0.999 (0.016)	0.745 (0.233)	0.820 (0.194)	0.590 (0.299)
<i>E. antisiphilitica</i>	0.756 (0.227)	-0.189 (0.439)	0.786 (0.212)	0.877 (0.159)	0.317 (0.397)	0.786 (0.212)
<i>J. dioica</i>	0.831 (0.188)	0.546 (0.316)	0.262 (0.416)	0.996 (0.029)	0.891 (0.150)	0.564 (0.309)

R Person Coefficient (p value). RS reference strain. CB clinical bacteria.

found in our study but with different plant species that are easily found in the same region where we collected our plant samples providing more in vitro evidence of the empiric and traditional knowledge of the anti bacterial activity of the northern Mexico's plants. In another study with semi-desert plants from Northern Mexico, *Schinus molle* and *Cyperus alternifolius*, methanolic extracts were active against *S. aureus* with a MIC of 62.5 and 250 µg/mL, respectively<sup>18</sup>. Besides, all the plant extracts showed information that indicate that the extracts were non-toxic according to the criteria used in the materials and methods section of this manuscript. This work found a LD<sub>50</sub> ≥ 1000 µg/mL. The methanol extracts of *Carya illinoensis*, *Sellaginella lepidophilla*, *Euphorbia antisiphilitica* and *Jatropha dioica* presented three main groups of secondary metabolites: Isoprenoids such as terpenes and saponins, phenolic derivatives such as phenols, phenolic acids, flavonoids, anthocyanins and alkaloids. This is consistent with other authors<sup>19</sup>.

### Conclusion

The methanol extracts of the four studied plants from northern Mexico showed antimicrobial activity against bacteria (RS and CB). A MIC of 500 µg/mL showed no toxicity against *A. salina*. Phytochemical analysis revealed the presence of flavonoids, lactons, quinons, triterpens and sterols. These results contribute to the knowledge of plants used in traditional medicine in northern Mexico and could be the basis for future studies to isolate active compounds from plants and test for effectiveness against other microorganisms.

### Conflict of Interests

The authors declare that they have no conflicts of interests.

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