Antioxidant, Antiproliferative and Antibacterial Activity of *Phoradendron californicum* Extracts; a Parasitic Plant from Northwestern Mexico

Actividad Antioxidante, Antiproliferativa y Antibacteriana de Extractos de *Phoradendron californicum*; una Planta Parásita del Noroeste de México

**ABSTRACT**

*Phoradendron californicum* is a plant native of northwestern Mexico that has been used as a therapeutic alternative. In the present study, methanolic and chloroformic extracts of *P. californicum* were prepared and evaluated for their antioxidant activities, phenolic and flavonoid content, as well as their antiproliferative activity in cancer cells, and antibacterial activity. The methanolic extract presented higher antioxidant activity (IC$_{50}$: 47.62 ± 2.90 μg/mL) and higher total phenol content (186.45 ± 4.58 mg EQ/g of extract) compared to the chloroformic extract (IC$_{50}$ > 400 μg/mL and 13.54 ± 1.57 mg EAG/g of extract, respectively); both extracts presented similar content of total flavonoids (19.92 ± 1.84 and 25.55 ± 0.73 mg EQ/g of extract, respectively). On the other hand, the chloroformic extract showed higher antiproliferative activity against evaluated cell lines (HeLa, PC3 and L929) compared to the methanolic extract, while in the antibacterial activity the chloroformic extract presented higher activity against *Escherichia coli* and *Listeria monocytogenes*, and the methanolic extract was more active against *Salmonella enterica*. The clear difference in the biological activities of both extracts may be due to variations in the chemical composition of each due to the extraction method used. *P. californicum* has potential for the search of new compounds with biological activity.

**Keywords:** *Phoradendron californicum*; Antiproliferative Activity; Antioxidant Activity; Natural Extracts; Mexican Plants.

**INTRODUCTION**

In Mexico, different types of native plants have been used for their pharmacological potential, to treat different diseases and constitute part of the traditional pharmacopoeia of various ethnic groups (Alonso-Castro *et al.*, 2011; Sharma *et al.*, 2017). Northwest Mexico has a wide variety of medicinal plants with potential biological activities. *Phoradendron californicum*, known as Toji, is an autotrophic hemiparasitic mistletoe, native of the south of California, Nevada and Arizona in the USA, as well as the states of Sonora, Sinaloa and Baja California in Mexico. It is usually found infecting *Prosopis* trees and has been used for treatment of digestive disorders like diarrhea, stomachache, vomiting and kidney stones (Hawksworth and Wiens, 1994; Spurrier and Smith, 2007). Few studies have demonstrated the biological activity of *P. californicum*, such as antiinflammatory, antiproliferative and antibacterial activities, as well as of its chemical composition and antioxidant activity after extraction with different types of solvents (Iluki-Assanga *et al.*, 2015). This makes *P. californicum* an unexplored and potential source of biologically active compounds.

Reactive oxygen species (ROS) are molecules produced by the mitochondria through normal metabolic processes.
The most common oxidative species are, hydrogen peroxide ($\text{H}_2\text{O}_2$), hydroxyl radicals (•OH), superoxide radicals ($\text{O}_2^{-}$), and singlet oxygen (‘O$_2$‘). Normally, there is an intracellular balance between ROS generation and scavenging, mainly mediated by enzymatic and non-enzymatic systems such as, superoxide dismutase, glutathione peroxidase, catalase, thioredoxin, glutathione, ascorbic acid, and tocopherol (Carrocho and Ferreira, 2013; Prasad et al., 2017; Kim et al., 2019). Nevertheless, an excess of ROS can be produced by different sources such as, infection, stress, exercise, inflammation, etc., which leads to an imbalance in ROS levels known as oxidative stress (Carrocho and Ferreira, 2013).

Reactive oxygen species can damage several cellular structures, such as lipids, proteins, DNA, and membranes, and are responsible for different pathologies like neurodegenerative disease, vascular disease, inflammation, diabetes, and cancer. Chronic oxidative stress can promote oncogenic signaling pathways, inhibit apoptosis, and enhance genomic instability, all of which are hallmarks of cancer. Additionally, ROS can activate pro-inflammatory pathways, creating a favorable microenvironment for tumor growth and metastasis (Sabharwal and Schumacker, 2014). Cancer cells often exhibit increased levels of ROS due to their rapid metabolism and mitochondrial dysfunction, further exacerbating oxidative stress (Sabharwal and Schumacker, 2014; DeBerardinis and Chandel, 2016). To counteract the effects of oxidative stress, antioxidant molecules from different external sources, such as dietary supplements and natural products, have been used (Heim et al., 2002; Babich et al., 2011; Silva et al., 2017).

In this work, we obtained the methanolic and chloroformic extracts of $\text{Phoradendron californicum}$, and determined their chemical composition, antioxidant, antiproliferative and antibacterial activities, to better establish its possible therapeutic potential.

MATERIAL AND METHODS
Sample collection and extracts preparation
Samples of $\text{Phoradendron californicum}$ attached to Prosopis sp. were collected from the area known as “El Arenoso” (N 31°02.18′, W 112°02.58′; Lat: 31.038, Lon: -112.049) between the municipalities of Caborca and Altar in the state of Sonora, Mexico.

For extraction, stems were dried at room temperature and grounded, then mixed with methanol or chloroform in a proportion of 1:10 (sample: solvent) for three days. Samples were concentrated in a rotary evaporator and stored at -20 °C until use.

Phytochemical screening and total phenolic and flavonoids content
Phytochemical screening
The phytochemical screening was determined following standard protocols previously reported (Savithramma et al., 2011). The qualitative assessment of the presence or absence of secondary metabolites was determined based on color change upon reaction (Savithramma et al., 2011; Uddin et al., 2011).

Total phenolic content
Total phenolic content was determined by the Folin-Ciocalteu method as previously described by Singleton and Rossy (Singleton and Rossy, 1965), with modifications. Briefly, 10 µL of sample (adjusted to 2.5 mg/mL) were mixed with 60 µL of sodium carbonate 7 % (w/v), 40 µL of Folin reagent (0.2 N) and 90 µL of milliQ water and placed in a 96-well microplate. The plate was incubated in the dark for 1 h, and then the absorbance measured at 750 nm by an ELISA plate reader (Multiskan EX, ThermoLabSystem, Waltham, MA, USA). A standard curve was prepared using different concentrations of gallic acid (0.8 – 0.006 mg/mL), and the results were expressed in terms of mg of gallic acid equivalent per gram of dry extract.

Total flavonoid content
Content of flavonoids was determined by the aluminum chloride method with modifications (Popova et al., 2004). Briefly, 10 µL of extract (adjusted to 2.5 mg/mL) were mixed with 130 µL of methanol and 10 µL of a 5 % (w/v) AlCl$_3$ solution in a 96-well microplate. The plate was incubated for 30 minutes in the dark and read at a wavelength of 412 nm using an ELISA plate reader (Multiskan EX, ThermoLabSystem, Waltham, MA, USA). A standard curve was prepared using different concentrations of quercetin (1 – 0.2 mg/mL), and the results were expressed in terms of mg of quercetin equivalent per gram of dry extract.

Antioxidant activity by DPPH assay
The antioxidant activity of $\text{P. californicum}$ methanolic and chloroformic extracts was determined by the DPPH assay as previously reported (Usía et al., 2002) with modifications. The samples were prepared in methanol (100 µL) at different concentrations (3.12-400 µg/mL) and mixed with 300 µM of a DPPH solution (100 µL) in a 96-well microplate (Costar, Corning, NY, USA), then the plates were incubated in the dark for 30 minutes, and the absorbance measured at 517 nm in an ELISA plate reader (Multiskan EX, ThermoScientific, Waltham, MA, USA). Ascorbic acid (70 µM) was used as antioxidant control. The antioxidant activity or free-radical scavenging activity is reported as percentage of decrease compared to ascorbic acid.

Cell culture
The human cervix (HeLa), human prostate (PC3) adenocarcinoma cell lines, and non-cancerous murine subcutaneous connective tissue (L-929) cell line were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). All cells were cultured in DMEM media supplemented with L-glutamine solution (200 mM), sodium pyruvate solution (100 mM), L-asparagine (98 %), L-arginine monohydrochloride (≥ 98 %), penicillin-streptomycin solution (1000 U/L U per mL) and 5 % FBS (D5F) at 37 °C and 5 % of CO$_2$.

Antiproliferative activity
The antiproliferative activity was determined using the MTT assay (Mosmann, 1983). Initially, 50 µL of cells were seeded...
into a 96-well plate (1x10^4 cells) (Costar, Corning, NY, USA) and incubated for 24 h at 37 °C and 5 % CO₂. Next, 50 µL of extract samples at different concentrations were added to each well, and plates were incubated for 48 h. During the last 4 h of treatment, 10 µL of an MTT solution (5 mg/mL) were added. The formed formazan crystals were dissolved using 100 µL of acidic isopropyl alcohol, and the absorbance of the plates were measured by an ELISA plate reader (Multiskan EX, ThermoLabSystem, Waltham, MA, USA) at 570 nm with a reference wavelength of 630 nm. DMSO was used as a solvent control.

**Antibacterial activity**
The antibacterial activity was evaluated following the protocols established by the CLSI (Clinical and Laboratory Standards Institute, 2023). Briefly, the strains of *E. coli* ATCC 25922, *S. enterica* ATCC 14028 and *L. monocytogenes* ATCC 19115 were incubated for 18 h in LB media. Next, the strains were adjusted to the 0.5 McFarland scale (1x10^8 UFC/mL) and then diluted 1:20 in LB media. Then, 10 µL of the inoculum were deposited in each well of a 96-well plate containing 100 µL of *P. californicum* extract at different concentrations (800 - 50 µg/mL). The plates were incubated for 24 h at 37 °C and measured by an ELISA plate reader (Multiskan EX, ThermoLabSystem, Waltham, MA, USA) at 620 nm.

**Statistical analysis**
The results shown were obtained by at least three independent experiments carried out in triplicate. Data was analyzed using GraphPad Prism 7.0 and statistical significance (p < 0.05; p < 0.001; p < 0.0001) was determined by two-way ANOVA with Bonferroni’s test.

**RESULTS**

**Phytochemical profile and total phenolic and flavonoids content**
The biological activities and antioxidant activity of natural extracts could be due to the presence of polyphenolic compounds such as flavonoids. Nevertheless, their presence can depend on the extraction procedure of the sample. Here, we determined the phytochemical profile and quantified the amount of total phenolics and flavonoids present in both *P. californicum* extracts by spectrophotometric techniques. Table 1 shows the phytochemical profile of methanolic and chloroformic extracts, finding that methanolic extract had presence of saponins, quinones, phenolics and free sugars, while the chloroformic extract was only positive to the presence of saponins. Table 2 shows the total phenolic and flavonoid content of both extracts. We observed that the methanolic extract has a higher amount of phenolic compounds (186.45 ± 4.58 mg EAG/g of extract) compared to the chloroformic extract (13.54 ± 1.57 mg EAG/g of extract) (p < 0.001). Nevertheless, the flavonoid content of both methanolic and chloroformic extracts is similar (19.92 ± 1.84 mgEQ/g of extract and 25.55 ± 0.73 mgEQ/g of extract, respectively) (p < 0.05).

**Antioxidant activity**
Antioxidant activity of the methanolic and chloroformic extracts of *P. californicum* was evaluated by the DPPH assay. We can observe that the methanolic extract has potent antioxidant activity with an IC₅₀ of 47.62 ± 2.90 µg/mL (Table 2), and higher concentrations of 100 to 400 µg/mL are comparable to the antioxidant activity of ascorbic acid, which is a strong antioxidant compound. In comparison, the chloroformic extract presented a weak antioxidant activity even at the higher concentration of 400 µg/mL, where its antioxidant activity reaches a maximum of ~ 25 % (Figure 1).

**Antiproliferative activity against cancer cells**
The antiproliferative activity of methanolic and chloroformic extracts of *P. californicum* was evaluated against HeLa and PC3 cancer cell lines, as well as an L929 normal cell line, by an MTT assay (Figure 2). A dose-response effect can be observed, especially for HeLa and PC3 cell lines treated with chloroformic extract. *Phoradendron californicum* chloroformic extract exhibited higher antiproliferative activity against all three tested cell lines in comparison to the methanolic extract. The most susceptible cell line was PC3 (IC₅₀: 167.67 ± 5.08 µg/mL), followed by HeLa (IC₅₀: 215.62 ± 14.70 µg/mL) and L929 (IC₅₀:

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**Table 1.** Phytochemical profile of *P. californicum* extracts.

<table>
<thead>
<tr>
<th>Phytochemical Profile</th>
<th>Methanolic</th>
<th>Chloroformic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Phenolics</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Free aminoacids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Free sugars</td>
<td>+</td>
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</tr>
</tbody>
</table>

**Table 2.** Total flavonoids, phenolic content, and antioxidant activity (DPPH) of *P. californicum* methanolic and chloroformic extracts.

<table>
<thead>
<tr>
<th>Phytochemical Profile</th>
<th>Methanolic</th>
<th>Chloroformic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Phenolics</td>
<td>186.45 ± 4.58 a</td>
<td>13.54 ± 1.57 a</td>
</tr>
<tr>
<td>Total Flavonoids</td>
<td>19.92 ± 1.84 b</td>
<td>25.55 ± 0.73 b</td>
</tr>
<tr>
<td>DPPH – IC₅₀(µg/mL)</td>
<td>47.62 ± 2.90</td>
<td>&gt; 400</td>
</tr>
</tbody>
</table>

*Expressed as mg equivalents of gallic acid/g of dry extract (mg EAG/g of extract) *Expressed as mg equivalents of quercetin/g of dry extract (mg EQ/g of extract) *Value of p < 0.001 of significance from the corresponding Total phenolics of methanolic to the Total phenolics of chloroformic extract. **Value of p < 0.005 of significance from the corresponding Total flavonoids of methanolic to the Total flavonoids of chloroformic extract. **Total Flavonoids 19.92 ± 1.84 b 25.55 ± 0.73 b **Total Phenolics 186.45 ± 4.58 a 13.54 ± 1.57 a **DPPH – IC₅₀(µg/mL) 47.62 ± 2.90 400 **
43.68 ± 10.54 µg/mL) (p < 0.001). For the methanolic extract, the IC₅₀ against PC3 and HeLa were similar (340 ± 11.58 µg/mL and 352.51 ± 9.87 µg/mL, respectively) with no statistical significance between them (p < 0.05), meanwhile, for L929 cell line the IC₅₀ was higher than 400 µg/mL (Table 3).

**Antibacterial activity**

The antibacterial activity of methanolic and chloroformic extracts was evaluated against three different bacterial strains; *Escherichia coli* ATCC 25922, *Salmonella enterica* (serovar typhimurium) ATCC 14028 and *Listeria monocytogenes* ATCC 19115. As observed in Figure 3, the antibacterial activity depends on the strain and type of extract evaluated. *S. enterica* was more susceptible to the methanolic extract compared to the chloroformic extract, on the other hand, *E. coli* was more susceptible to the chloroformic extract compared to methanolic extract. For *L. monocytogenes* the chloroformic extract was more effective at the higher concentration evaluated (800 µg/mL) with a viability of around 60%.

**DISCUSSION**

Mexico possesses a wide variety of medicinal plants commonly used in traditional medicine to treat different pathologies. Different studies have been carried out demonstrating the antimicrobial, antidiabetic, antioxidant and antiproliferative activities of different Mexican plants (Alonso-Castro...
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The free-radical scavenging capacity of both extracts was determined by a DPPH assay, and it was evident that the methanolic extract exert higher antioxidant activity compared to the chloroformic extract. Although reports on \textit{P. californicum} biological activities are scarce, some studies reporting the antioxidant activity of different extracts have been observed. Jiménez-Estrada et al. (2013) reported the antioxidant activity of the methanolic extract of various medicinal plants from northwest Mexico, including \textit{P. californicum}, nevertheless, its antioxidant activity was weaker (approximately, 30\% at 100 µg/mL) compared to our results. These differences could be attributed to the extraction process despite using the same type of solvent. On the other hand, Iloki-Assanga et al. (2015) observed an IC$_{50}$ of around 50 µg/mL for the methanolic extract of \textit{P. californicum}, very similar to the results obtained in this work. These findings remark the importance of the standardization of the extraction process regarding natural extracts for the suitable comparison of their biological activities.
Antioxidant activity of plant extracts is usually related to its phenolic and flavonoid content, which are mainly responsible due to their free-radical scavenging potential, and is strongly associated with the type of solvent used for the extraction process. Methanolic extract of *P. californicum* presented a higher content of phenolic compounds (186.45 ± 4.58 mg EAG/g of extract) compared to the chloroformic extract (13.54 ± 1.57 mg EAG/g of extract), which could be the main compounds responsible for the poor antioxidant activity of chloroformic extract. Nevertheless, the total flavonoid content was similar between the two samples. Phenolic compounds and flavonoids exert their antioxidant activity mainly by scavenging reactive oxygen species, by the electron-donating properties of their hydroxy groups, either by hydrogen atom transfer or sequential proton loss electron transfer, by modulating the activity of enzymes responsible for the formation of ROS and chelating elements needed in their synthesis (Pietta, 2000; Zeb, 2020).

There are few reports regarding the antiproliferative activity of *Phoradendron* species extracts. Gil Salido (2016) reported the antiproliferative activity of various Mexican plants extracts, including a methanolic extract of *P. californicum*. They determined an IC50 of 103.21 ± 3.01 μg/mL and 178.43 ± 3.32 μg/mL against RAW 264.7 and L929 cell lines, respectively. This activity is lower than the one we observed against HeLa and PC3 cell lines, and even against L929. Our results are more in accordance with the antiproliferative activity reported by Jimenez-Estrada et al. (2013), who observed low antiproliferative activity of methanolic extract of *P. californicum* against HeLa, Raw 264.7, L929 and M12/C3/F6 cell lines, with IC50 higher than 400 μg/mL.

Regarding the antibacterial activity, in this study the chloroformic extract was more effective against *E. coli* and *L. monocytogenes*, in contrast, the methanolic extract was more active against *S. enterica*. Reports of the antibacterial activity of *Phoradendron* species is very limited. *Phoradendron serotinum* extract has been evaluated against various gastrointestinal pathogens were the minimum inhibitory concentrations values ranged from 6.25 to 12.5 mg/mL (Science Technology and Management, 2023). Meanwhile, other studies have been highly contrasting, were extracts of *P. bollanum* have presented MIC values of 377.1 μg/mL and 5.6 μg/mL against *Xanthomonas campestris* and *Clavibacter michiganensis*, respectively (García-García et al., 2021).

The solvent used for the extraction plays a critical role in the chemical composition of the final product. Chloroform is used for the extraction of non-polar compounds like triterpenes, sterols, alkaloids and fatty acids. Valencia-Chan et al. (2022) demonstrated that four triterpenes isolated from *Phoradendron wattii* induce apoptosis and cell cycle arrest on KS62 and HL60 leukemia cells. Here, the chloroformic extract of *P. californicum* was negative for terpenes and alkaloids, nevertheless, some constituents like fatty acids and sterols have been reported in different *Phoradendron* species, such as palmitic acid, octadecadienoic acid, octadecenoic acid and stearic acid in *P. mucronatum* and hexadecane, hexaco-

**CONCLUSIONS**

*P. californicum* methanolic extract exhibited a higher antioxidant activity compared to the chloroformic extract, this correlates to its higher total phenolics content. On the other hand, the chloroformic extract showed a higher antiproliferative and antibacterial activity, which may be due to non-polar compounds present in the plant, which may have a higher bioactivity against cancer cells and bacteria.

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