Histopathological findings in Anisakidae nematodes exposed to aqueous plant extracts with nematicidal capacity in vitro

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ABSTRACT

Objective. This study was performed to expose nematodes of the Anisakidae family to different aqueous extracts and identify those extracts with nematicidal capacity. Material and methods. The concentrations of bioactive compounds in the aqueous extracts of epazote (Dysphania ambrosioides), onion (Allium cepa), sempre viva (Kalanchoe pinnata), and xoconostle (Opuntia oligacantha) were identified. Live parasites of the Anisakidae family were obtained from Lisa fish (Mugilidae) and exposed to different concentrations of these extracts. Results. K. pinnata and O. oligacantha exhibited higher concentrations of bioactive compounds, including polyphenols, flavonoids, and tannins, as well as greater antioxidant activity in DPPH and ABTS assays. The parasite mortality rate was 100% at 48 hours with a concentration of 855 mg/mL of K. pinnata. For O. oligacantha, the pulp and whole fruit extracts caused 66% mortality at 72 hours. The main histopathological changes induced by K. pinnata were muscle vacuoles. In O. oligacantha, the whole fruit caused degeneration and vacuolization of the intestinal epithelium, while the seed extract caused edema, intestinal degeneration, and vacuolization. Conclusions. These results suggest that aqueous extracts of K. pinnata and O. oligacantha are effective nematicidal agents against nematodes of the Anisakidae family.

Keywords: Anisakidae, Kalanchoe, Mugilidae, Opuntia, parasite (Source: CAB).

RESUMEN

Objetivo. Exponer nematodos de la familia Anisakidae a diferentes extractos acuosos para determinar su capacidad nematicida, mediante la identificación de sus componentes bioactivos y la actividad antioxidante. Material y métodos. Se identificaron las concentraciones de los compuestos bioactivos de los extractos acuosos de epazote (Dysphania ambrosioides), cebolla (Allium cepa), siempre viva (Kalanchoe pinnata) y xoconostle (Opuntia oligacantha). Se obtuvieron parásitos vivos de la familia...
Anisakidae de peces Lisa (Mugilidae), para ser expuestos a diferentes concentraciones de extractos acuosos. **Resultados.** *K. pinnata* y *O. oligocantha* presentaron mayor cantidad de componentes bioactivos de polifenoles, flavonoides y taninos; así como en la actividad antioxidante de DPPH y ABTS. La mortalidad de los parásitos ocurrió en la concentración de 855 mg/mL para *K. pinnata* del 100% a las 48 horas; y en pulpa y fruto entero de *O. oligocantha*, con mortalidades del 66% a las 72 horas. Los principales cambios histopatológicos causados por *K. pinnata* fueron vacuolas musculares; el fruto entero de *O. oligocantha* degeneración del epitelio intestinal y vacuolización; la semilla provocó edema, degeneración intestinal y vacuolización. **Conclusiones.** Los resultados obtenidos indican que, el uso de extractos acuosos de *K. pinnata* y *O. oligocantha* sobre nematodos de la familia Anisakidae, son una opción para su uso como agentes nematicidas.

**Palabras clave:** Anisakidae; Mugilidae; Opuntia; parásito; Kalanchoe (Fuente: CAB).

**INTRODUCTION**

Anisakidae parasites in marine foods pose a public health risk for consumers. These parasites are found in various fish products, including sardines (*Sardina pilchardus*), sole (*Solea solea*), red grouper (*Epinephelus morio*), and mullet (*Mugilidae*), among others (1). Widely distributed among different marine organisms, these parasites complete their life cycle in waterfowl, which serve as the primary hosts. The adult phase (L4) of the parasite releases eggs containing L1 larvae in feces into the marine environment (2). Small crustaceans and copepods facilitate the molting process from L1 to L2 larvae. When these crustaceans are consumed by paratenic hosts (such as fish, squid, and octopuses), the larvae molt from L2 to L3. Humans acquire the parasite by consuming these paratenic hosts raw or semi-raw, although the molting from L3 to L4 does not occur in humans (3).

Ichthyozoonosis caused by these parasites is identified in two ways: anisakidosis (when caused by the genera *Anisakis* spp., *Contracaeca* spp., and *Pseudoterranova* spp.) and anisakiasis (caused specifically by *Anisakis simplex*) (4). When humans consume the live parasite, it can survive for a short period attached to the surface of the gastrointestinal tract (5). The live or dead parasite in the human body can trigger various clinical manifestations such as allergies, angioedema, bronchospasms, anaphylactic shock (6), epigastralgia, nausea, vomiting, diarrhea, chronic abdominal pain, and granuloma formation (5). However, there is no antiparasitic that can be administered to fish prior to consumption to prevent zoonosis.

Plants can produce bioactive elements as a defense method against herbivorous insects (7). Aqueous extracts made from plants have shown bioactive properties and have been used as anthelmintic agents (8). For instance, antiparasitic effects of epazote (*Dysphania ambrosioides*) have been reported (9). Onion (*Allium cepa*) has demonstrated anthelmintic activity against the nematodes *Toxocara canis* and *Ancylostoma caninum* (10). The succulent plant *Kalanchoe pinnata* has shown antiparasitic capacity against protozoa such as *Plasmodium berghei* and *Leishmania amazonensis* (11). Additionally, its consumption by indigenous peoples for the treatment of parasitic diseases has been reported (12).

Accidental consumption of larvae from the Anisakidae family and the resultant clinical manifestations can lead to incorrect diagnoses. Thus, it is necessary to safeguard public health by ensuring the production of safe marine products for consumption. The objective of this investigation was to expose nematodes of the Anisakidae family to different aqueous plant extracts to determine their nematicidal capacity by identifying their bioactive components and antioxidant activity.

**MATERIALS AND METHOD**

**Fish.** Mullet fish (*Mugil curema*) were purchased at a local market. Fresh fish were selected based on criteria such as a good smell, firm meat consistency, shiny eyes, red gills, and overall healthy skin appearance. The fish were placed in a cooler at 4°C and transferred to the Parasitology Laboratory at the Institute of Agricultural Sciences, Autonomous University of the State of Hidalgo (13).

Necropsy and management of parasites based on necropsy technique described by Noga (14). To collect the live parasites, the internal organs of the fish were dissected, focusing mainly on...
the liver and kidneys because these are the most parasitized organs. A previous report indicated that the presence of parasites in the epaxial muscle, in the coelomic cavity, and on the serous surface of the viscera may vary (13). The parasites were collected in 1.5-mL Eppendorf vials with 0.9% (w/v) sterile sodium chloride solution. The parasites were then washed three times with sterile sodium chloride solution at 3,000 rpm. Between each wash, the vial was decanted and refilled with clean solution (15).

Vegetables. Fresh plants of epazote (D. ambrosioides), succulent (K. pinnata), and onion (A. cepa) were purchased in the municipality of Tulancingo de Bravo, Hidalgo (latitude: 20.0815, longitude: -98.3673; 20°08′53″ north, 98°22′2″ west). They were washed with water and disinfected by immersion in a 2.5% sodium hypochlorite (commercial bleach) solution for 10 minutes. They were then rinsed with distilled water, dried, and stored at 4°C.

Fruit. The xoconostle (Opuntia oligacantha) was collected from Ulapa Melchor Ocampo municipality, Tetepango, Hidalgo (altitude: 2040 m, latitude: 20°08′41.7″ north, longitude: 99°09′59.0″ west). It was washed with water and disinfected by immersion in a 2.5% (v/v) sodium hypochlorite (commercial bleach) solution for 5 minutes. It was then rinsed with distilled water (16) and stored at 4°C in a dark container until use.

Extraction of bioactive compounds. The extracts of D. ambrosioides leaves, A. cepa, and K. pinnata scales were obtained using the methodology of Soto and Rosales (17) with modifications. A 150-g sample was placed in a mortar for maceration, and 750 mL of distilled water was added. The mixture was then blended in an industrial blender for 10 minutes. The liquefied material was left to rest for 7 days, after which it was centrifuged at 10,000 rpm for 15 minutes at 2°C, and the supernatant was decanted. The filtered material was placed in a rotary evaporator at 40°C, with a pressure of 23 mbar and coolant water at 0°C. Subsequently, it was kept in a water bath at 40°C for 8 hours. Finally, it was stored in a deep freezer at −70°C until use.

The O. oligacantha extracts were obtained using the methodology of Medina-Pérez et al (16). Four extracts were prepared: whole fruit, peel, pulp, and seed. Samples of 1 g were added to 20 mL of methanol and 20 mL of distilled water and shaken for 30 minutes. The mixture was centrifuged at 10,000 rpm for 20 minutes, and the supernatant was placed in a 10-mL tube. It was subsequently heated at 65°C for 60 minutes in a water bath for complete elimination of methanol. The mixture was immediately cooled with cold water, and the extract was stored at −70°C.

Bioactive compounds. Total phenols: The methodology described by Singleton et al (18) was followed for extraction of total phenols using the Folin-Ciocalteau method. A total of 0.5 mL of each extract was placed in a test tube, followed by 2.5 mL of Folin-Ciocalteau reagent. The reaction mixture was allowed to rest for 7 minutes. Next, 2 mL of 7.5% sodium carbonate was added, and the mixture was incubated for 2 hours at room temperature. The absorbance was measured using a spectrophotometer at 760 nm, and the results were expressed as milligram equivalents of gallic acid.

Flavonoids: Flavonoids were obtained based on the methodology described by Tommasi et al. (19). In total, 250 µL of the extract to be evaluated was placed in 1.25 mL of distilled water and 75 µL of 5% sodium nitrite. The reaction mixture was allowed to stand for 5 minutes at room temperature in the dark. Next, 150 µL of 10% aluminum chloride was added, and the mixture was allowed to rest for 6 minutes. Finally, 500 µL of sodium hydroxide and 275 µL of distilled water were added. The absorbance was measured at 720 nm, and the results were expressed as milligram equivalents of quercetin.

Tannins: Tannins were determined using the methodology described by Price and Butler (20). A 200-µL aliquot of each aqueous extract was added to 600 µL of 0.1 M ferric chloride in 0.1 N hydrochloric acid. The extract was allowed to rest for 5 minutes, after which 600 µL of 0.008 M potassium ferricyanide was added. The mixture was then allowed to rest for 10 minutes to produce a reaction. The absorbance was read using a 720-nm spectrophotometer, and the results were expressed in mg equivalents of catechin.

Antioxidant activity. 2,2-Diphenyl-1-picrylhydrazyl (DPPH·): The methodology of Brand-Williams et al. (21) was used to assess the antioxidant activity. A 0.5–mL aliquot of each extract was placed in 2.5 mL of DPPH·, and the change in absorbance at 515 nm was recorded every 5 minutes for a period of 30
minutes. The percentage of free radical inhibition was determined using the following equation:

\[
\text{Percentage of free radical inhibition} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100
\]

where:
\[
\begin{align*}
\text{Ac} &= \text{absorbance of DPPH\ before the reaction} \\
\text{As} &= \text{absorbance of the mixture after the reaction}
\end{align*}
\]

2,2′-Azino-bis(3-ethylbenzothiazolin) acid (ABTS⁺): This test was performed according to the methodology described by Re et al (22). A 100-μL aliquot of each extract was placed in 3 mL of ABTS⁺ reagent, and the mixture was allowed to react at room temperature for 6 minutes in the dark. The absorbance was then read at 734 nm. The percentage of free radical inhibition of ABTS⁺ was calculated using the following equation:

\[
\text{Percentage of free radical inhibition} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100
\]

where:
\[
\begin{align*}
\text{Ac} &= \text{absorbance of ABTS\ before the reaction} \\
\text{As} &= \text{absorbance of the mixture after the reaction}
\end{align*}
\]

Nematicidal activity of the extracts in vitro. Nine larvae per Petri dish were used to evaluate the extracts of *D. ambrosioides*, *A. cepa*, and *K. pinnata* at concentrations of 285, 570, and 855 mg/mL (27 larvae per extract for each concentration). For *O. oligacantha*, 27 larvae were used for each element (whole fruit, peel, pulp, and seed) at the same concentrations. The exposure to the extracts was observed at different post-exposure times: at 30 minutes and at 1, 2, 4, 6, 8, 12, 24, 48, and 72 hours post-exposure (hpe). To evaluate parasite mortality, tactile stimulation was performed to determine whether the parasites made wave movements. The time point at which they no longer exhibited movement was recorded (23).

Two control groups (27 larvae per group) were exposed to a stable phosphate-buffered saline solution. Three replicates were carried out for each concentration and control group, with each concentration tested separately.

Histopathology. After 72 hpe or upon the total mortality of a certain group of parasites exposed to the aqueous extracts, all the parasites were placed in 10% formaldehyde for 24 to 48 hours for fixation to avoid histological changes. Subsequently, all parasites were placed in histological cassettes, separated by the type of extract and concentration used. They were then dehydrated, embedded in paraffin, cut, and stained with hematoxylin–eosin. The sections were examined with a compound optical microscope, and the lesions were described and photographed (13).

**Statistical analysis.** Differences in mortality between the groups were calculated using Tukey’s multiple comparison test and the chi-square test (p≤0.05) (24).

**RESULTS**

**Bioactive compounds.** Significant differences were identified between the extracts (p<0.05). The highest concentrations of total polyphenols were obtained from *K. pinnata* and the peel and whole fruit of *O. oligacantha*. Similar significant differences (p<0.05) in the concentrations of flavonoids and tannins were also observed, with higher concentrations found in *K. pinnata* (Table 1).

**Table 1.** Bioactive compounds present in aqueous extracts of *Dysphania ambrosioides*, *Allium cepa*, and *Kalanchoe pinnata* as well as seed, pulp, peel, and whole fruit of *Opuntia oligacantha*.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Polyphenols (mg GAE/g)</th>
<th>Flavonoids (mg QE/g)</th>
<th>Tannins (mg CE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. ambrosioides</em></td>
<td>0.4431 ±0.0081a</td>
<td>0.7806 ±0.003a</td>
<td>ND</td>
</tr>
<tr>
<td><em>A. cepa</em></td>
<td>0.0366 ±0.0006a</td>
<td>0.0233 ±0.006a</td>
<td>ND</td>
</tr>
<tr>
<td><em>K. pinnata</em></td>
<td>23.3671 ±0.0127c</td>
<td>10.8197 ±0.2556c</td>
<td>6.5486 ±0.0056c</td>
</tr>
<tr>
<td><em>O. oligacantha</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>1.9808 ±0.0876a</td>
<td>0.4313 ±0.0872a</td>
<td>0.0098 ±0.0007b</td>
</tr>
<tr>
<td>Pulp</td>
<td>15.8623 ±0.8621b</td>
<td>1.5733 ±0.0987b</td>
<td>0.7176 ±0.0865b</td>
</tr>
<tr>
<td>Peel</td>
<td>18.0154 ±0.4461bc</td>
<td>4.8960 ±0.9876b</td>
<td>0.3168 ±0.0986b</td>
</tr>
<tr>
<td>Complete fruit</td>
<td>17.7619 ±0.6652bc</td>
<td>5.3190 ±0.4720c</td>
<td>0.8709 ±0.0865c</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. Different letters in each column indicate significant differences (p<0.05) between each extract. mg GAE/g, milligram gallic acid equivalents per gram of sample; mg QE/g, milligram quercetin equivalents per gram of sample; mg CE/g, milligram catechin equivalents per gram of sample; ND, not detectable.
Antioxidant activity. The antioxidant capacity by DPPH· was directly proportional to the content of bioactive compounds. Kalanchoe pinnata and O. oligacantha showed a higher percentage of inhibition with significant differences (p<0.05) compared to D. ambrosioides and A. cepa. In the determination of antioxidant activity with ABTS, the extracts with the highest percentage of inhibition were the pulp and the whole fruit of xoconostle, presenting significant differences (p<0.05) from the other extracts (Table 2). In both cases, xoconostle exhibited a higher percentage of inhibition for DPPH· and ABTS⁺.

Table 2. Antioxidant activity (DPPH· and ABTS⁺) of bioactive compounds present in the aqueous extracts of Dysphania ambrosioides, Allium cepa, and Kalanchoe pinnata as well as seed, pulp, and whole fruit of Opuntia oligacantha.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Percentage inhibition by DPPH·</th>
<th>Percentage inhibition by ABTS⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. ambrosioides</td>
<td>12.0476 ± 0.0064²</td>
<td>14.7661 ± 0.0044²</td>
</tr>
<tr>
<td>A. cepa</td>
<td>10.7143 ± 0.0050ᵃ</td>
<td>14.8095 ± 0.0037ᵃ</td>
</tr>
<tr>
<td>K. pinnata</td>
<td>59.8714 ± 0.485ᵇ</td>
<td>59.9381 ± 0.0012ᵇ</td>
</tr>
<tr>
<td>O. oligacantha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>59.0714 ± 0.08765ᵇ</td>
<td>52.3815 ± 0.4154ᵇ</td>
</tr>
<tr>
<td>Pulp</td>
<td>64.0333 ± 0.1752ᵃ</td>
<td>70 ± 0.1862ᶜ</td>
</tr>
<tr>
<td>Peel</td>
<td>62.5865 ± 1.9862ᵃ</td>
<td>59.4286 ± 1.1920ᵇ</td>
</tr>
<tr>
<td>Complete fruit</td>
<td>62.5033 ± 1.9732ᵇ</td>
<td>67.1861 ± 2.2515ᵇ</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. Different letters in each column indicate significant differences (p<0.05) between each extract.

Mortality. In total, 243 live parasites were collected and exposed to the aqueous extracts. The mortality rate was determined by the number of nematodes with no mobility upon stimulation. Parasites exposed to D. ambrosioides showed 33% mortality at 48 hpe. The K. pinnata extract caused 100% mortality at 48 hpe. For O. oligacantha, the whole fruit, peel, and pulp caused 66% mortality at 72 hpe, while the seed caused 33% mortality at 72 hpe (Table 3). Exposure to the A. cepa extract and the buffer solution resulted in no parasite mortality. Using the results of accumulated larva mortality, the chi square test and Tukey’s test were performed to identify significant differences between the groups. The analysis revealed three significantly different groups, where the mortality caused by the K. pinnata extract and the whole fruit of O. oligacantha showed no significant differences (p<0.5).

Table 3. Mortality of nematodes of the Anisakidae family exposed to aqueous extracts of epazote (Dysphania ambrosioides), onion (Allium cepa), succulent (Kalanchoe pinnata), and xoconostle (Opuntia oligacantha).

<table>
<thead>
<tr>
<th>Vegetable/fruit Concentration¹</th>
<th>%Mortality</th>
<th>Exposure time</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. ambrosioides 855 mg/mL</td>
<td>33³abc</td>
<td>72 hours</td>
</tr>
<tr>
<td>A. cepa 855 mg/mL</td>
<td>0³</td>
<td>72 hours</td>
</tr>
<tr>
<td>K. pinnata 855 mg/mL</td>
<td>100⁸</td>
<td>48 hours</td>
</tr>
<tr>
<td>O. oligacantha Seed 570 mg/mL</td>
<td>22³bc</td>
<td>72 hours</td>
</tr>
<tr>
<td></td>
<td>855 mg/mL</td>
<td>33³abc</td>
</tr>
<tr>
<td>Pulp 285 mg/mL</td>
<td>33³abc</td>
<td>72 hours</td>
</tr>
<tr>
<td>Peel 570 mg/mL</td>
<td>22³bc</td>
<td>72 hours</td>
</tr>
<tr>
<td>Complete fruit 855 mg/mL</td>
<td>66³</td>
<td>72 hours</td>
</tr>
<tr>
<td></td>
<td>855 mg/mL</td>
<td>66³</td>
</tr>
</tbody>
</table>

¹285 mg/mL (low concentration), 570 mg/mL (medium concentration), 855 mg/mL (high concentration)

Differences in each column indicate significant differences (p<0.05).

Histopathology. The parasites exposed to the phosphate-buffered saline solution did not exhibit morphological changes (Figure 1(a1–3)). The A. cepa and D. ambrosioides extracts did not result in apparent pathological changes.

Kalanchoe pinnata extract: Moderate to severe multifocal intestinal epithelial degeneration was observed (Figure 1(b1)). Focal and multifocal vacuoles of varying severity were present between the cuticle and the muscle (Figure 1(b2)). Accentuated undulations and degeneration of the intestinal epithelium were also observed (Figure 1(b3)).

Opuntia oligacantha extract. (a) Complete fruit: Vacuoles were present in the gonads (Figure 1(c1)), and liquefaction of the intestinal epithelium and cellular debris in the intestine were observed. The intestinal tract was segmented (Figure 1(c2)). In the cuticle, accentuated
undulations were observed near the region where the lesions occurred in the intestinal tract (Figure 1(c3)). (b) Peel and pulp: Multifocal areas of liquefaction and degeneration of the intestinal epithelium were present. (c) Seed: The lesions were characterized by edema and vacuolization of the intestinal epithelium (Figure 1(d1)). Marked undulation of the cuticle was observed in the areas close to the intestinal lesions. Vacuolization and liquefaction of the intestinal epithelium; degeneration, vacuolization, and edema in the pharynx and esophagus; and vacuolization of the oral apparatus were present (Figure 1(d3)).

**DISCUSSION**

Parasites of the Anisakidae family are ichthyozoanotic agents, causing public health problems through various clinical symptoms when consumed in raw or semi-raw marine products (1). The use of plant extracts as nematicidal substances against this family has been previously carried out using different types of plants, including the tea tree (*Melaleuca alternifolia*) (25), basil (*Nepeta cataria*) (23), and oregano (*Origanum compactum*) (15), among others. This research has shown that the use of plant extracts is a viable option for controlling parasitosis. The quantification of phenolic compounds in plant extracts provides useful information for developing pesticides, medicines, and other bioactive substances (26). This approach aids in identifying new plant elements that can help control microorganisms, which can pose problems for specific hosts.

The present study showed that the highest amount of phenolic compounds in the *K. pinnata* extract was similar to that obtained by Castro-Parra et al (27), which was 22.98±1.10 mg GAE/g of an aqueous extract. For *O. oligacantha*, the peel (18.0 mg GAE/g), whole fruit (17.7 mg GAE/g), and pulp (15.8 mg GAE/g) also presented high values. However, a study by Fernández-Luqueño et al (28) revealed a higher content of 278±2.2 mg GAE/100 g of phenolic compounds in the extract of the whole fruit of *O. oligacantha*. The difference between the values may be related to the type of extract and the method of preparation; aqueous extracts typically yield lower concentrations than those obtained from methanol, which is toxic (17).

The flavonoid content in *K. pinnata* was 10.8 mg QE/g. This is higher than the concentration reported by Báez et al (29), who obtained 5.07±0.05 mg QE/g using a different species from same genus. The extracts of the whole fruit of *O. oligacantha* presented a concentration of 5.31 mg QE/g. This is similar to the results obtained by Fernández-Luqueño et al (28), who obtained a concentration of 4.77±0.10 mg QE/100 g of the whole fruit. These variations are related to different plant species and phenolic compounds (30,31,32).

The concentration of tannins in *K. pinnata* was higher (6.54 mg CE/g) than that in the other extracts. High concentrations of tannins have previously been reported to be part of the bioactive compounds of this species (33). By
contrast, the concentration of tannins from *O. oligacantha* ranged from 0.00 to 0.87 mg CE/g. This is lower than that previously reported by Medina-Pérez et al (34), who obtained 71.067 mg CE/100 g. The presence of phenolic compounds favors the capture of the DPPH radical, making it important to evaluate the antioxidant activity of the extracts using both DPPH and ABTS assays (35). The antioxidant activity by DPPH was higher in the extracts of *K. pinnata* and *O. oligacantha* than in the plant extracts of *D. ambrosioides* and *A. cepa*.

Osorio-Esquível et al (36) obtained a mean DPPH inhibition percentage of 51.70±0.55% in the whole fruit of *O. oligacantha*; this is similar to the values obtained in the present study. Báez et al (29) assessed the antioxidant activity of ethanolic extracts of *K. daigremontiana* belonging to the same genus and reported an inhibition percentage of 56.87%, a result similar to those obtained in the present study. Hernández–Fuentes et al (30) analyzed the antioxidant activity of different varieties of xoconostle fruits, identifying *O. oligacantha* as the species with the highest antioxidant activity. These results are also consistent with the present study.

Phenolic compounds and their antioxidant capacity are strongly and positively correlated with the number of hydroxyl groups attached to the aromatic rings. The ortho and para positions of these hydroxyl groups improve the antioxidant and antiradical activity of phenolic compounds (31). The high antioxidant activity demonstrated by the extracts of *K. pinnata* and *O. oligacantha* (pulp and whole fruit) is due to their high content of antioxidant substances (36), elements that are possibly related to nematode mortality.

The main bioactive compounds in *O. oligacantha* are ferulic acid, quercetin, 4-hydroxybenzoic acid, apigenin, caffeic acid, and kaempferol (16,32), while *K. pinnata* contains phenolic compounds and flavonoids such as rutin, quercetin, luteolin, kaempferol, and tannins (33), among others. These phenolic compounds and flavonoids can act at the level of the central nervous system of the class Insecta and the phylum Nematoda, and tannins can have toxic effects (17). The aqueous extracts of *K. pinnata* and *O. oligacantha* (whole fruit, peel, and pulp) caused high mortality rates in nematodes of the Anisakidae family (100% at 48 hpe and 66% at 72 hpe, respectively) at a concentration of 855 mg/mL. At a concentration of 285 mg/mL, none of the plant extracts caused mortality except for the pulp of *O. oligacantha*, which caused 33% mortality at 72 hpe. Additionally, *D. ambrosioides* caused 33% mortality at 48 hpe at the highest concentration.

The use of the monoterpenoid, also known as geraniol, has been studied as a nematicidal agent. At a concentration of 250 µg/mL, geraniol caused the death of 10% of the larvae of the Anisakidae family (*Contraacema* spp.) at 2 hpe. At 48 hpe, 90% of the larvae were dead (37). Other studies have investigated the nematicidal capacity of peppermint (*Mentha piperita*). In these studies, *Anisakis* spp. larvae were exposed to essential oils from peppermint, which resulted in 83% mortality at 8 hpe at a concentration of 187.5 µL and up to 100% mortality at 4 hpe at a concentration of 250 µL (38).

In the present investigation, the parasite genus was not determined. However, the mortality rates and histopathological lesions identified were consistent across the group and replicates exposed to the aqueous extracts. A higher mortality rate was observed for *K. pinnata* and *O. oligacantha* than for *D. ambrosioides* and *A. cepa*.

In previous studies investigating the nematicidal activity of extracts, the concentration, exposure time, presentation of the extract (aqueous and/or oil), and genus and species of the Anisakidae family were shown to influence the mortality rates generated by exposure (15). This is consistent with the present study. The different compounds at different concentrations caused various mortality rates among the nematodes. When used as an aqueous extract, it is possible that higher concentrations of *O. oligacantha* (whole fruit, peel, and pulp) may increase the mortality rate; however, additional studies are necessary to determine this.

The general morphology of the Anisakidae family is characterized by an elongated and cylindrical body with pointed ends. The anterior end features three ventrolateral lips with a cuticular tooth, and short undulations are present at both ends, located between the cuticle and the muscles of the parasite (1) (Figure 1(a3)). However, the appearance of marked bumps (Figure 1(b2), (c3), and (d3)) indicates that exposure to the aqueous extracts causes histological deformation of the parasite. This deformation alters the cuticle and muscles (Figure 1(b2)) by increasing the space between these two layers, affecting nutrient absorption and movement.
Parasites exposed to different concentrations of aqueous extracts of *K. pinnata*, as well as the seed and whole fruit of *O. oligacantha*, exhibited swelling, edema, vacuolization between the hypodermis and the cuticle, destruction of the intestinal tract, marked undulations, and vacuolization of internal tissues. Additionally, edematization and alteration of the intestinal epithelium were observed. The histological damage caused by exposure to these plant extracts can be attributed to the interaction of bioactive components with the parasite cuticle. The interaction of glycoproteins in the cuticle with antioxidants leads to oxidation and accumulation of proteins on the surface of the parasite, which disrupts its osmosis. This results in swelling, altered metabolism, impaired nutrient absorption, and finally death (8).

Other reported morphological alterations include the formation of spines, roughness, or marked deformations in the cuticle in parasites that do not possess structures such as spines, veils, and rugae, which can alter their morphology (39). However, in the present study, no additional structures or deformation favoring the appearance of foreign morphological elements were observed. Some antioxidants can cause lesions in the mouth, gastrointestinal tract, and anus of exposed parasites. The severity of these lesions can vary, and they may not necessarily lead to the death of the parasites, as seen in the case of Castile chamomile (*Matricaria chamomilla*) (39).

Although the mechanisms by which antioxidants affect nematodes are not completely understood, it has been hypothesized that antioxidants are capable of damaging the nervous system of parasites (present in the form of a ring), leading to paralysis. Additionally, they may interfere with glucose absorption by blocking postsynaptic receptors (8). The results obtained in this study demonstrate that the lesions primarily occurred in the intestinal epithelium, with observations of degeneration, liquefaction, segmentation, and vacuolization, as well as vacuolization of the cells that make up the gonad. These lesions are important because of the damage they cause to the digestive and reproductive systems of the parasite.

No previous reports or investigations have referred to the use of *O. oligacantha* as a nematicidal agent against larvae of the Anisakidae family. This is the first report to demonstrate the nematicidal capacity and histological alterations caused by exposure of parasites to aqueous extracts of *K. pinnata* and *O. oligacantha*.

In conclusion, the aqueous extracts of the *K. pinnata* plant and the *O. oligacantha* fruit exhibit nematicidal activity. This activity is induced by their bioactive compounds and antioxidant properties, which cause both histological alterations and death in parasites.

**Conflict of Interest**

The authors report no conflicts of interest.

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**Authors’ contributions**

Conceptualization (RGCM, APZV). Investigation (RGCM, APZV, FRGA). Methodology (RGCM, LCP). Writing - original draft (APZV, FRGA). Writing - review and editing (APZV).

**REFERENCES**


