Activity of *Euterpe edulis* Martius, *Mikania glomerata* Spreng, and *Mikania laevigata* Schultz Bip. Extracts on Gastrointestinal Nematodes *Toxocara canis* and *Ancylostoma caninum*

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Background: Gastrointestinal parasitoses have high rates of morbidity and mortality. Each year about 3.5 billion people are affected by these diseases and 65,000 of them die, mostly in developing countries due to lack of basic sanitation, malnutrition, and poor access to medication. Thus, they constitute an important public health problem due to causing direct health problems related to lack of piped water, absence of sewage system, and lack of orientation.

Objectives: Two in vitro assays were performed to evaluate the larvicidal and/or ovicidal activity of ethanol extracts obtained from the plants *Euterpe edulis*, *Mikania laevigata*, and *Mikania glomerata* on the gastrointestinal nematodes *Toxocara canis* and *Ancylostoma caninum*.

Materials and Methods: In the first assay (A), *T. canis* eggs were exposed to three different concentrations (0.1 mg/mL, 1 mg/mL, and 10 mg/mL) of each extract, three different concentrations of albendazole (positive control), ethanol (solvent), and a negative control (no treatment), for 15 days at 26°C, under the shelter of light in order to evaluate the percentage of embryonated eggs in the presence of these extracts. In the second assay (B), the larvicidal activities of the species studied were evaluated in the different extract concentrations (0.1 mg/mL, 1 mg/mL, and 10 mg/mL), control, and solvent (ethanol), in coprocultures positive for *A. caninum* eggs.

Results: In assay A, the results demonstrated inhibitory embryogenesis activity on *T. canis* eggs; however, no difference ($P > 0.01$) was found between the activities of the extracts. In the control group, there was a difference ($P < 0.01$) in relation to the tested extracts, in which this difference was not concentration-dependent. In assay B, all extracts showed inhibitory ($P > 0.01$) hatchability activity of *A. caninum* eggs in the control group.

Conclusions: Through these results, the applicability of the used extracts in the control of eggs and/or larvae of *T. canis* and *A. caninum* is suggested. However, it is worth mentioning that further studies should be performed with the species *E. edulis*, *M. glomerata* and *M. laevigata*, using different extracts, new concentrations, and in vivo studies, in order to ensure further clarification on the agents responsible for the observed effects, degree of efficacy, and toxicity.

Keywords: Nematodes; *Ancylostoma*; *Toxocara canis*

1. Background

Gastrointestinal parasitoses have high rates of morbidity and mortality. Each year about 3.5 billion people are affected by these diseases and 65,000 of them die, mostly in developing countries due to lack of basic sanitation, malnutrition, and poor access to medication (1, 2). Thus, they constitute an important public health problem due to causing direct health problems related to lack of piped water, absence of sewage system, and lack of orientation (3, 4).

Although there are several control strategies available to combat gastrointestinal nematodes that affect humans and animals, synthetic drugs are the most used, having great positive impact on the conservation of human and

animal health (5). However, these drugs are not free of problems, such as drug resistance, and the amount of residual chemicals in the environment (5, 6). Therefore, the study for new control alternatives against these gastrointestinal parasites becomes imminent. Furthermore, the use of herbal extracts has shown great potential (7).

In this regard, various studies employing medicinal plants and their derivatives have shown ovicidal and larvicidal activities against several parasites (8-12). An in vitro study, conducted by Macedo et al. (8) with the essential oil of *Eucalyptus globulus* L. on hatching and development of *Haemonchus contortus* larvae, a gastrointestinal

nematode of ruminants, showed ovicidal and/or larvicidal activity and potential for use in its control. In another study, methanol extracts obtained from *Euphorbia helioscopia*, showed anthelmintic activity when tested *in vitro* and *in vivo* on *H. contortus*, suggesting a treatment alternative in cases of infections due to helminths in ruminants (9).

Sensitivity of the gastrointestinal parasites *Toxocara canis*, and *Ancylostoma caninum* to other plant species was also observed. A study involving *Carica papaya* L. extract, suggested a potential role for this plant as an anthelmintic against infections caused by *A. caninum* in mice (10, 11). Another, *in vitro* study showed larvicidal activity of leaf extracts from *C. ambrosioides* L., against *T. canis* infective larvae (13).

2. Objectives

Thus, the aim of this study was to evaluate the ovicidal and/or larvicidal activity of the plants *Euterpe edulis* Martius, *Mikania glomerata* Spreng, and *Mikania laevigata* Schultz Bip. on the gastrointestinal nematodes *T. canis* and *A. caninum*, and investigate the larvicidal and/or ovicidal activity shown by these medicinal plants.

3. Materials and Methods

3.1. Ethical Aspects

The project was approved by the Ethics Committee on Animal Use (CEUA- UVV) of the University Vila Velha, Brazil (UVV-CEUA), according to the opinion consubstantiated No. 292/2013.

3.2. Obtaining Plant Material and Preparation of *Mikania glomerata* Spreng and *Mikania laevigata* Schultz Bip. ex Baker Extracts

The plants *Mikania glomerata* Spreng. and *Mikania laevigata* Schultz Bip. ex Baker were provided by the Sector of Medicinal Plants - Department of Agriculture, from the Federal University of Lavras (DAG/UFLA). Exsiccates from *M. glomerata* Spreng., and *Mikania laevigata* Schultz Bip. ex Baker were deposited in the herbarium of the Institute of Biosciences, UFRGS (Universidade Federal do Rio Grande do Sul), under the registration ICN141992 and ICN 141990.

The dried plant material from *M. glomerata* (260 g) and *M. laevigata* (100 g) was subjected to percolation with ethanol at 96 °GL. The ethanolic extract was concentrated in a rotary evaporator at 50 °C under reduced pressure, until residue was obtained.

3.3. *Euterpe edulis* Martius

The fruits of palmito jucara (1 kg) were provided by INCAPER (Espírito Santo Research Institute, Technical Assistance and Rural Extension), which is in accordance

with the Normative Instruction 003/2013 (Espírito Santo, 2013). The sample was kept in a transparent plastic bag, with a hermetic closure, and kept in a domestic freezer at -18 °C until use.

The fibrous layer of the fruit, as well as the thin oily coverage, and the mesocarp were removed, and the ethanol extract of *E. edulis* was prepared using homogenization for 30 seconds, and 10 g of this material without the seed in 70 mL of aqueous ethanol 80%. The mixture was subjected to an ultrasonic bath for 20 minutes.

3.4. Obtaining *Toxocara canis* Eggs

Pregnant, *T. canis*, adult females were dissected and uterine fragments containing eggs were removed. Subsequently, the eggs were centrifuged in distilled water. The content in the centrifuge tube was homogenized, and from it, three aliquots of 10 µL were removed. Eggs were counted using a stereomicroscope at × 10 magnification. The total amount of these eggs was estimated according to Araujo et al.(14).

3.5. Obtaining *Ancylostoma caninum* Larvae

About 150 g of fresh feces from dogs naturally infected with *A. caninum* were collected and used in the preparation of coprocultures. To do so, confirmation of contamination by *A. caninum* was required by means of a fecal flotation technique, in accordance with the technique of Willis-Mollay (15), and observation under an optical microscope, initially under a × 10 objective, and later confirmed under a 40x objective. The average egg count per gram of feces (EPG) was 125 eggs/3g.

3.6. Experimental Assays

Two *in vitro* experimental assays, denominated A and B, were performed. In assay A, the percentage of embryonated *T. canis* eggs when using extracts from *E. edulis*, *M. glomerata*, and *M. laevigata* at different concentrations was evaluated, as described by Reis et al. (13). In assay B, the larvicidal activity of the species in different concentrations within coprocultures containing *Ancylostoma caninum* was evaluated.

3.6.1. Assay A

In this assay, 100 *T. canis* eggs were transferred into test tubes containing 5 mL of ethanolic extracts at concentrations 0.1 mg/mL, 1 mg/mL, and 10 mg/mL of the medicinal plants in study (*E. edulis*, *M. glomerata*, and *M. laevigata*). The control group contained 100 eggs in 5 mL of ethanol (the same diluent used for preparation of the extracts) in the same container. A positive control was also performed using 100 eggs in test tubes containing albendazole at 0.01 mg/mL, 0.05 mg/mL, and 0.1 mg/mL (13). There was also one group containing 100 eggs, which received no treatment. All tubes were properly identified and enclosed, and incubated at 26 °C, in the dark, for 15 days.

Each treatment consisted of six replicates. After each study period, the total number of embryonated and/or non-embryonated eggs, present in each tube of the treated and control groups, was calculated according to the methodology described by Braga et al. (16).

3.6.1.1. Statistical Analysis

The average number of recovered fertilized eggs was calculated. Data were statistically interpreted by analysis of variance at significance levels of 1 and 5% probability (17). The efficiency of embryonation compared to the control was assessed by Tukey's test at 1% probability using the 5.0 BioEstat program. Subsequently, the average percentage reduction of embryonated eggs was calculated according to the following Equation 1:

$$(1) \frac{\bar{x}_{\text{embryonated eggs from control}} - \bar{x}_{\text{embryonated eggs from treatment}}}{\bar{x}_{\text{embryonated eggs from control}}} \times 100$$

3.6.2. Assay B

Approximately, 3 g of positive feces were mixed with fragmented (autoclaved) industrial vermiculite and moistened in each cup. Subsequently, 10 mL of ethanolic extracts from *E. edulis*, *M. glomerata*, and *M. laevigata* were added at concentrations of 0.1 mg/mL, 1 mg/mL, and 10 mg/mL and taken to BOD for 8 days at 26°C, forming the treated group. Each treatment consisted of three replicates. Two control groups were performed: one consisting of the coprocultures without extracts and another containing only the solvent used (ethanol). At the end of this period, third stage larvae (L_3) were obtained by the Baermann method. These larvae were identified and quantified according to the criteria described by Urquhart et al. (18), in optical microscope and 10 × objective.

3.6.2.1. Statistical Analysis

The average number of recovered *A. caninum* L_3 was calculated. Data were statistically interpreted by analysis of variance at significance levels of 1 and 5% probability (17). Destruction efficiency of L_3 in relation to the control was assessed by Tukey's test at 1% probability, using the 5.0 BioEstat program. Subsequently the average percentage reduction of L_3 was calculated according to the following Equation 2:

$$(2) \frac{\bar{x}_{\text{Recovered } L_3 \text{ from control}} - \bar{x}_{\text{Recovered } L_3 \text{ from treatment}}}{\bar{x}_{\text{Recovered } L_3 \text{ from control}}} \times 100$$

4. Results

4.1. Assay A

The embryogenesis of *T. canis* eggs occurred properly during assay A, as shown in the control, and the results obtained after 15 days of interaction between eggs and ethanol extracts are shown in Table 1. All investigated species (*M. glomerata*, *M. laevigata*, and *E. edulis*) showed inhibitory activity in the percentage of embryonated *T. canis* eggs in relation to the control group. Table 2 shows the results of the positive control (Albendazole), and the negative control with the solvent used in the extracts (ethanol), under the same analysis conditions of the assay A.

Table 1. Averages and Standard Deviations of the Percentage of Embryonated *Toxocara canis* Eggs After Prior Contact With the Groups Treated With Ethanol Extracts From *Mikania laevigata*, *Mikania glomerata*, *Euterpe edulis*, and the Control Group After 15 Days of Interaction

Species Extract Concentration	Average, % ^a	Embryonation, %
<i>Mikania laevigata</i>		
0.1, mg/mL	43.3 ± 22.5	56.7
1, mg/mL	59.1 ± 11.1	40.9
10, mg/mL	31.6 ± 15.7	68.4
Control	100	100
<i>Mikania glomerata</i>		
0.1, mg/mL	40.8 ± 22.5	59.2
1, mg/mL	42.5 ± 11.1	57.5
10, mg/mL	31.6 ± 15.7	68.4
Control	100	100
<i>Euterpe edulis</i>		
0.1, mg/mL	57 ± 22.5	43
1, mg/mL	40.8 ± 11.1	59.2
10, mg/mL	35 ± 15.7	65
Control	100	100

^a Different values in this column do not differ, $P > 0.05$ and $P > 0.01$ - Tukey.

Table 2. Averages and Standard Deviations of the Percentages of Embryonated *Toxocara canis* Eggs After Previous Contact With the Groups Treated With Albendazole and Ethanol After 15 Days of Interaction.

Treatment	Average, % ^a	Embryonation, %
Albendazol, mg/mL		
0.01	43.3 ± 22.5	56.7
0.05	59.1 ± 10.8	40.9
0.1	68.3 ± 15.7	31.7
Ethanol	31.6 ± 11.7	68.4
Control	100	100

^a Different values in this column do not differ, $P > 0.05$ and $P > 0.01$ - Tukey.

4.2. Assay B

In assay B, as shown in Table 3, all ethanol extracts tested (*M. glomerata*, *M. laevigata*, and *E. edulis*) in coprocultures were able to inhibit the hatching of *A. caninum* eggs after 8 days of interaction; although no significant difference ($P > 0.01$) was observed compared to the exposure concentration of the extract, showing only difference compared to the control.

Table 3. Averages and Standard Deviations of the Percentages of *Ancylostoma caninum* Infective Larvae (L_3) Recovered from Coprocultures in the Groups Treated With Ethanol Extracts From *Mikania laevigata*, *Mikania glomerata*, *Euterpe edulis* and the Control Group After 8 Days of Interaction

Species Extract Concentration	Average, % ^a	Embryonation, %
<i>Mikania laevigata</i>		
0.1, mg/mL	4.9 ± 2.9	57.3
1, mg/mL	3.0 ± 1.7	73.9
10, mg/mL	2.7 ± 2.7	78.2
Control	11.5 ± 3.1	0
<i>Mikania glomerata</i>		
0.1, mg/mL	4.5 ± 3.5	56.9
1, mg/mL	3.1 ± 4.7	69.5
10, mg/mL	2.7 ± 2.0	74.1
Control	10.4 ± 4.3	0
<i>Euterpe edulis</i>		
0.1, mg/mL	2.4 ± 1.4	74.7
1, mg/mL	5.75 ± 5.2	39.4
10, mg/mL	2.0 ± 1.7	78.9
Control	9.5 ± 4.7	0

^a Different values in this column do not differ, $P > 0.05$ and $P > 0.01$ - Tukey.

5. Discussion

There are few studies that assess, or demonstrate, plant in vitro antiparasitic activity on *T. canis* eggs. Most research is directed towards the evaluation of *T. canis* larvae (13, 19) or towards the activity of nematophagous fungi, among which *Pochonia chlamydosporia*, which mostly produce proteases capable of destroying the eggs of gastrointestinal nematodes (16).

Regarding the ethanol extracts tested, there is no significant difference between the concentrations investigated (0.1 mg/mL, 1 mg/mL, and 10 mg/mL), unlike that found by Lone et al. (9) in their in vitro study with extracts from *Euphorbia helioscopia* on *H. contortus*, where the extract concentration was more effective in inhibiting parasite motility as it was increased, 12.5, 25, and 50 mg/mL.

In general, a plant extract contains low concentrations of active compounds, but in large numbers (20). Thus,

the use of in vitro assays with medicinal plant extracts, in addition to the benefits of ease of use, low cost, and speed, serve as an early indication of the activity being investigated and allow the selection of the most promising extracts, decrease spending, avoid loss of time and the indiscriminate use of laboratory animals (21).

According to Gasparetto et al. (22) diterpenes, especially the class of kauranes, present in the species *M. glomerata* and *M. laevigata*, antiparasitic activity among other pharmacological actions, corroborating with the results found here, in which extracts from *M. laevigata* and *M. glomerata* showed embryonation rates lower than the control, i.e. there were substances capable of preventing embryogenesis of *T. canis* eggs in these extracts. Vieira et al. (23) also endorse this information when reporting the antiparasitic activity of a cauranic diterpene, on trypomastigote forms of *Trypanosoma cruzi*, in an in vitro study.

The species *E. edulis* has not yet been explored regarding its antiparasitic activity; however, studies have demonstrated the antiparasitic activity of flavonoids, substance found in *E. edulis*. Quercetin, a flavonoid revealed in an in vitro study by Weiss et al. (24), was able to inhibit the synthesis of hsp90, hsp70, hsp27, and even suppress the induction and development of bradyzoite in *Toxoplasma gondii*. Tasdemir et al. (25) have also demonstrated that quercetin and their derivatives exhibit promising in vitro activities against the genera *Leishmania* and *Trypanosoma*. Molan et al. (26) demonstrated the in vitro anthelmintic activity of various flavonoids and their derivatives, including epicatechin and catechin, also present in *E. edulis*, on *T. colubriformis* eggs and larvae, nematode affecting ruminants, in a concentration-dependent relation; contradicting the findings in this assay with *E. edulis*, where the percentage difference ($P > 0.01$) of embryonated *T. canis* eggs although higher when compared to control, did not increase depending on the concentration.

According to the data obtained by statistical analysis, assay B, obtained satisfactory results in all tested ethanol extracts and their concentrations from *M. laevigata*, *M. glomerata*, and *E. edulis* (0.1 mg/mL, 1 mg/mL, and 10 mg/mL), since the *A. caninum* eggs hatched resulting in a considerable number when compared with the control group, which did not contain any concentration of the extract. It's worth mentioning that a solvent control (ethanol) was also conducted and it also did not interfere with the assay's results.

In a study conducted by Assis et al. (27) *H. contortus* larvae and eggs were subjected to four different extracts: hexane, chloroform, ethyl acetate, and methanol at five different concentrations (3.1, 6.2, 12.5, 25.0, and 50.0 mg/mL) obtained from the plant *Spigelia anthelmia*. At the concentration of 50.0 mg/mL, the ethyl acetate extract inhibited 100% of egg hatching, and 81.2% of larval development. Similarly, the methanol extract inhibited 97.4% of hatching and 84.4% of *H. contortus* larvae in development, while the other extracts showed lower or statistically

igual percentages to the control, such as the chloroform extract at a concentration of 50.0 mg/mL.

The results showed that the ethanol extracts from *M. laevigata*, *M. glomerata*, and *E. edulis* in the tested concentrations (0.1 mg/mL, 1 mg/mL, and 10 mg/mL), showed ovicidal and/or larvicide activity on the gastrointestinal nematodes *T. canis* and *A. caninum*. There was no difference between extracts from *M. laevigata*, *M. glomerata* and *E. edulis* in relation to ovicidal and/or larvicide activity on the gastrointestinal nematodes investigated ($P > 0.01$). Tested concentrations of these extracts (0.1 mg/mL, 1 mg/mL, and 10 mg/mL) did not differ ($P > 0.01$) from each other, regarding the ovicidal and/or larvicidal activity on *T. canis* and *A. caninum*. However, further in vivo studies are needed for the improvement of the methodology and for further clarification of the agents responsible for the observed effects, action mechanisms.

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