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Full Length Research Paper

Larvicidal potential of *Mikania glomerata* SPRENGEL extract on *Ancylostoma caninum* larvae

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Parasitic diseases are seen as indicators of a country's socioeconomic development, constituting a major public health problem as they cause direct health problems related to the lack of piped water, no sewage system, and lack of orientation. Contamination by the geohelminth *Ancylostoma* spp, causes the Cutaneous larva migrans (CLM), also known as "sandworms", presenting skin lesions of linear and serpiginous character. The aim of this study was to evaluate the *in vitro* larvicidal potential of guaco extracts (*Mikania glomerata* SPRENGEL) at different concentrations on *A. caninum* larvae. Obtained results showed the larvicidal effect of the *M. glomerata* extract starting from a treatment of 10mg/ml of guaco extracts (p<0.01). The larvicidal activity was best demonstrated in the 25 mg/ml treatment, in which a decrease of 13.30% of L3 was observed compared to the control group, and in the 50 mg/ml treatment (61.66%) reduction of L3. By means of the results, the applicability of the plant extracts used is suggested in *A. caninum* larvae control. In addition, more research is suggested to assess their employability in different extract forms, 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, and research continuity regarding the use concentration of the plant *M. glomerata* SPRENGEL.

Key words: Larvicidal extract, *Mikania glomerata*, *Ancylostoma caninum* and cutaneous larva migrans.

INTRODUCTION

Currently, due to the presence of cases with resistance to anthelmintic drugs and the need for new approaches to nematode control consisting of great zoonotic potential, there has been a resurgence of research on substances with natural anthelmintic properties. Plants with "popular" use are most often the material studied and, this line of research has been encouraged mainly by the fact that such plants have been traditionally used by indigenous peoples, particularly in the tropics, against gastrointestinal nematodes of both humans and animals (Stepek et al., 2006). In this regard several studies using medicinal plants and its derivatives have shown ovicidal

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and larvicidal activity against various parasites (Lone et al., 2012; Bi and Goyal, 2012; Sousa et al., 2013).

The *Mikania glomerata* SPRENGEL., is a Brazilian native plant belonging to the Asteraceae family, made official in the 1st edition of the Brazilian Pharmacopoeia. Despite not having its chemical composition fully elucidated the *M. glomerata* Sprengel is one of the most studied species in the pharmacognostic aspect. For *M. glomerata* several pharmacological activity were observed, including antifungal action, antimicrobial, bronchodilator, anti-allergic, anti-inflammatory (Brandão et al., 2006; Amaral et al., 2009; Celeghini et al., 2001).

The soil and public parks is via transmission to parasitic zoonosis. One of the most frequent is the *Ancylostoma* spp, one geohelminth parasite that dogs and cats and, possibly affects human beings, causing larva migrans skin (CML) (Santarem et al., 2004). Faced with the gastrointestinal parasite *A. caninum* other plants were evaluated and the sensitivity of these parasites to the *Carica papaya* L. extract was observed, suggesting a potential use of the plant as an anthelmintic against *A. caninum* infection in mice (Lone et al., 2012; Bi and Goyal, 2012).

Thus, the potential of medicinal plants and their derivatives as ovicides and or larvicides is clear and there are a various different plant species which have not yet been evaluated as for this activity. *A. caninum* infection occurs by ingestion or skin penetration of infective larvae (L3), its penetration is mainly performed through the skin of the lower limbs. Through blood circulation, they reach the pulmonary capillaries, traverse the alveolar wall, ascend with mucous secretions from the bronchial tree to the larynx and pharynx and are swallowed, make it to the intestine, where the last changes and the final transformation in adult worms, male and female occurs, being necessary measures to prevent contamination (Rey, 2001).

New therapeutic approaches are also essential for the control of parasites responsible for zoonoses. Some alternative measures are needed to assist in soil decontamination. One alternative that has been evaluated is the use of herbal medicines and their derivatives (extracts, enriched fractions, essential oil, dye) in the control of gastrointestinal parasites. This measure, in addition to having been proven effective in several studies, has the advantage of being sustainable and not damaging the environment. Thus, the objective of this study was to evaluate the *in vitro* larvicidal potential of guaco extracts (*M. glomerata* SPRENGEL) at different concentrations on *A. caninum* larvae.

MATERIALS AND METHODS

Ethical aspects

This project was approved by the Ethics Committee on Animal Use (CEUA- UVV) University of Vila Velha (UVV-CEUA), which opinion is embodied No 292/2013.

Plant material

The crude extract of the plant *M. glomerata* SPRENGEL was provided by the Medicinal Plant Industry of the Department of Agriculture of the Federal University of Lavras - MG (DAG / UFLA). The plant was identified by Dr. Mara Ritter of the Institute of Biosciences, Federal University of Rio Grande do Sul, where the evidence samples (herbarium specimens) are deposited under registration number ICN 141992. The dried *M. glomerata* plant material (260 g) was submitted to percolation with 96° GL ethanol. The ethanolic extract was concentrated in a rotary evaporator at 50°C under reduced pressure, obtaining 55 g of *M. glomerata* ethanol extract.

Extract preparations

Extract concentrations were prepared as described in the 3rd edition of the Brazilian Homeopathic Pharmacopoeia. The crude extract was weighed on an analytical scale and added solvent ethanol 96.5 GL° and taken to ultrasonic ultrasound. The preparation was maintained protected from direct light and heat and hermetically sealed.

Obtaining of *Ancylostoma caninum* larvae

Fresh feces from dogs living in the city of Vitória - ES, Southeastern Brazil, were collected and from these fecal samples about 3 to 5 g of feces were taken for performance of the fecal flotation technique (Willis-Mollay technique) to analyze if there was presence *A. caninum* eggs. After identification of *A. caninum* eggs, fecal cultures were prepared with about 20 g of feces mixed with autoclaved industrial vermiculite and moistened with water, the larval cultures were incubated in a BOD chamber during a period of 7 days. After this period the 3rd stage larvae were extracted and identified by the Baermann technique and quantified in an optical microscope and 10x objective.

Experimental assay

The experimental trial aimed to analyze the larvicidal activity of the extracts at four different concentrations (1, 10, 25, and 50 mg/ml) on *A. caninum* infective larvae (L3). The testing was performed in monofactorial experiment with: 1. CW; 2.CE; 3. T1 mg/ml; 4. T10 mg/ml; 5. T25 mg/ml; 6. T50 mg/ml.

The larvicidal activities of the *M. glomerata* ethanol extract in concentrations, 1; 10; 25 and 50 mg/ml, and ethanol on *A. caninum* cultures and a control group without treatment (water), were evaluated. For this, Petri dishes of 9.0 cm in diameter with 6 ml of 2% agar medium were prepared with about 1000 *A. caninum* larvae in each plate, and added 1 mL of each concentration of the *M. glomerata* extract and control group (1 ml ethanol solvent used for preparation of extracts) and a group without treatment (1 ml of water). Each treatment consisted of three replicates. During the seven-day period the plates remained in the conservatory and every 24 h, 10 random fields of 4 mm were observed daily under a light microscope with 10x objective, and the number of larvae were counted in each field. At the end of seven days, *A. caninum* larvae were recovered from the content in the Petri dishes used in the experiment by the Baermann method (Lopes et al., 2015).

Statistical analysis

The data were interpreted statistically by analysis of variance at significance levels of 1 and 5% probability (Ayres et al., 2003). The

L_3 destruction efficiency compared to the control was evaluated by the Tukey test at 1% probability, with BioEstat 5.0. Later the larvicidal ability of the extracts was determined by the reduction percentage, using the following formula:

$$\text{Larval \% Reduction} = \frac{\text{Average larvae } (L_{3}) \text{ recovered from control - Average larvae } (L_{3}) \text{ recovered from treatments x100}}{\text{Average larvae } (L_{3}) \text{ recovered from control}}$$

RESULTS AND DISCUSSION

The pharmacognostic analysis of the crude extract of *M. glomerata* presented moisture content of (8.4 ± 0.2% g/g) and total ashes equal to (2.2 ± 0.2% g/g). The extractable matter demonstrated ethanol yield of (47.33 ± 2.1% g/g). The pharmacognostic analysis presented moisture and ash within the limits described in the Brazilian Pharmacopoeia. The levels of coumarin in *M. glomerata* samples were detected, but at concentrations below the quantitation limit for the established method.

The presence of coumarin in *M. glomerata* samples was reported in several articles, but there are also articles reporting its absence, since this compound could not be quantified in the samples (Bertolucci et al., 2013). However, there are other metabolites described as a majority in the plant under study, like diterpenes, in particular the class of kauranes, present in the species *M. glomerata* have other pharmacological actions, particularly antiparasitic activity, and therefore further investigation is needed (Gasparetto et al., 2010). The total action of an extract is the sum of the activities of its constituents' (Lone et al., 2012). What corroborates with the results of this essay, where *M. glomerata* extracts showed some action on the L3 different from the control.

During 7 days the plates remained incubated and counting of 10 random fields from each plate was performed. After the seven days of the experiment, the L_3 were recovered by the Baermnan method using the material in the Petri dishes. The means and standard deviations for each test and recovery of larvae are shown in Table 1. However, it can be observed that there was no difference between the treatments, but during the intervals of days studied differences were noted between the treated groups and the control groups (C.W. and C.E). For example, on Day 1 there were difference controls (C.W. and C.E) over the tested concentrations. Another difference was noted in example C.E., the relative concentration of 1 to 10 mg /ml. Some literatures suggest that L3 may eventually escape to the periphery of the plates on agar plates (Eren and Pramer, 1965).

Comparing the mortality rates of the treatments and control groups, it was observed that the negative control (C.W- control with water) and positive control (C.E- control with ethanol) were not able to significantly reduce the number of L_3. Difference was also noted ($p < 0.01$) between treatments. However, in the last two treatments T25 and T50 mg/ml reduction ($p<0.01$) of L_3 was observed, suggesting that in greater concentrations the plant extract began to exert activity on L_3. Contributing to this study, researchers recorded that the anthelmintic activity of *Origanum vulgare* (Lamiaceae) dye observing that the capacity reduction was also directly related to the concentration of the extract (Dias de Castro et al., 2013).

The T1 and T10 mg/ml showed differences ($p < 0.01$) compared with the C.W and C.E, but the average of the C.E (4.9 ± 3.06) was lower than in T1 mg/ml (12 63 ± 8.75) and T10 mg/ ml (12.26 ± 8.49), after the second day the T1 and T10 mg/ ml groups showed differences when compared to the control groups ($p < 0.01$). On the sixth day of the experiment the T10 mg/ml does not differ from T25 mg/ml, suggesting that in this time period the T10 mg/ml demonstrates its greatest effect. On the last day of the experiment the treatments showed a difference between concentrations T1 and T10 mg/ml ($p< 0.01$).

The extract's activity increased with interaction time and was higher in concentrations above T10 mg/ml. In a study with the aqueous extract *Morinda citrifolia* no effect was observed at lower concentrations used and after 48 h at a concentration of 26.96 $mg.ml^{-1}$, this effect not being statistically significant when compared to the negative control ($p>0.05$). However, in hours 72 and 96 at concentrations of 13.48 and 26.96 $mg.ml^{-1}$, there was a difference, considering the aqueous extract of *M. citrifolia*, the positive control and the negative control (water). Comparing the mortality rates of the treatment and the negative control, it is observed that in the last two periods of time, there is a greater discrepancy of the effectiveness of the *M. citrifolia* aqueous extract in relation to the test with water (Brito et al., 2009).

During the seven days of interaction statistical difference between treatment groups was noted, not being demonstrated only between T25 and T50 mg/ml, suggesting that these concentrations the larvicidal effect demonstrated action stability. This feature is also observed in other studies on anthelmintic activity (Lone et al., 2012; Dias de Castro et al., 2013; Brito et al., 2009; Santana et al., 2013).

During the experiment in some days the average of the treatments was higher than the control, as on day 2, where T 1 mg/ml showed (20.6 ± 6.66%) and C.W (5.4 ± 2.58%) which can be explained due to the count of 10 daily fields being random on the Petri dish, not being chosen a field with larva so as not to induce the experiment results. Another finding that contributes to this fact is the characteristic of L3 migration to the middle of

Table 1. Means and standard deviations for each test and recovery of larvae during 7 days.

Test	Day 1	Test	Day 2
	Mean (%) and standard deviation		Mean (%) and standard deviation
C.W	9^{A} ±4.17	C.W	5.4^{A} ±2.58
C.E	4.9^{A} ±3.0	C.E	4.6^{A} ±1.83
T 1 mg/mL	12.6^{ABA} ±8.75	T 1 mg/mL	20.6 ^{BA} ±6.66
T10 mg/mL	12.3^{ABCB}±8.49	T10 mg/mL	13.73^{BAC} ±7.31
T25 mg/mL	2.6^{BCB}±1.83	T25 mg/mL	4.46^{AABC}±3.06
T50 mg/mL	1.5^{BAABC}±1.01	T50 mg/mL	2.00^{AABC}±0.75
	Day 3		**Day 4**
C.W	2.4^{A} ±1.06	C.W	3.85^{A} ±1.70
C.E	3.0^{A} ±1.36	C.E	2.42^{A} ±1.55
T 1 mg/mL	14.0 ^{BA} ±6.40	T 1 mg/mL	7.57^{BA} ±5.73
T10 mg/mL	6.13^{BAC} ±2.38	T10 mg/mL	6.35^{AB} ±3.43
T25 mg/mL	3.0^{AABC}±1.19	T25 mg/mL	2.14^{ABC}±1.16
T50 mg/mL	1.2^{AABC}±0.41	T50 mg/mL	1.64^{ABC}±0.74
	Day 5		**Day 6**
C.W	1.76^{A} ±0.833	C.W	1.5^{A} ±0.52
C.E	2.38^{A} ±1.70	C.E	1.3^{A} ±0.48
T 1 mg/mL	8.0^{BA} ±3.5	T1 mg/mL	9.4^{BA} ±3.59
T10 mg/mL	6.23^{BAAC} ±3.70	T10 mg/mL	5.8^{BACA} ±3.96
T25 mg/mL	1.84^{AABC}±0.68	T25 mg/mL	3.1^{AABC}±1.72
T50 mg/mL	0.534^{AABA}±0.51	T50 mg/mL	0.1^{AABA}±0.31
	Day 7		**Recovery of larvae**
C.W	2.0^{A} ±0.47	C.W	5.28^{A} ±2.62
C.E	1.6^{A} ±0.6	C.E	3.85^{A} ±1.46
T1 mg/mL	5.3^{BA} ±2.,62	T1 mg/mL	7.71^{AB} ±2.87
T10 mg/mL	5.4^{BAA} ±4.45	T10 mg/mL	2.71^{AABA} ±1.11
T25 mg/mL	1.3^{AABC}±0.48	T25 mg/mL	0.85^{BACA}±0.37
T50 mg/mL	0.6^{AABC}±0.51	T50 mg/mL	1.85^{BACA}±0.37

Means followed by the same lower case letters (column) do not differ statistically (p>0.01). Tukey Test.

the dish or its extremities, thus demonstrating that the L3 are active, also observed in another study (Lopes et al., 2015). Therefore the completion of the L3 recovery after 7 days of interaction is necessary to verify reduction percentage in each group. After the seven days of the experiment, the L3 were recovered by the Baermnan method using the material in the Petri dishes. The means and standard deviations of each test are shown in Figure 1. At the end of the experiment, the T1 mg/ml average (7.71 ± 2.87%) was greater than the mean in the ethanol control group (C.E) (3.85± 1.46%) and control with water (C.W) (5.28± 2.62%), a fact also observed by Lopes et al. (2015), being common in experiments with larvae, as they migrate to the center or extremities of the dish, location with more moisture.

In the T10 mg/ml (2.71 ± 1.11%); T25 mg/ml (0.85± 0.37%) and T50 mg/ml (1.85 ± 0.37%) groups the average of L3 recovered was lower than the average in the ethanol control group (CE) and control with water (CW), with a significant difference ($p < 0.01$). In a study with *Haemonchus contortus* larvae and eggs, the larvae and eggs were submitted to contact of four distinct extracts of hexane, chloroform, ethyl acetate, and methanol at five different concentrations (3.1, 6.2, 12.5, 25.0, and 50.0 mg/ml) from the plant *Spigelia anthelmia*. At a concentration of 50.0 mg/ml the ethyl acetate extract inhibited 100% of the eggs hatched and 81.2% of larval development. Similarly the methanol extract inhibited 97.4% of the hatching eggs and 84.4% of *H. contortus* larvae in development, while the other extracts showed lower percentages or even statistically identical to the control (Assis et al., 2003).

It is suggested that the larvicidal effect of *M. glomerata* extract was established starting at T10 mg/ml. Authors when studying the anthelmintic effect of *Euphorbia helioscopia* L., in the form of aqueous solution and methanolic extract observed that the *E. helioscopia* L. aqueous extract did not reduce egg count in the feces, *in vitro* studies showed increased nematode motility (98%) in higher concentrations of methanol extract (50 $mg.ml^{-1}$)

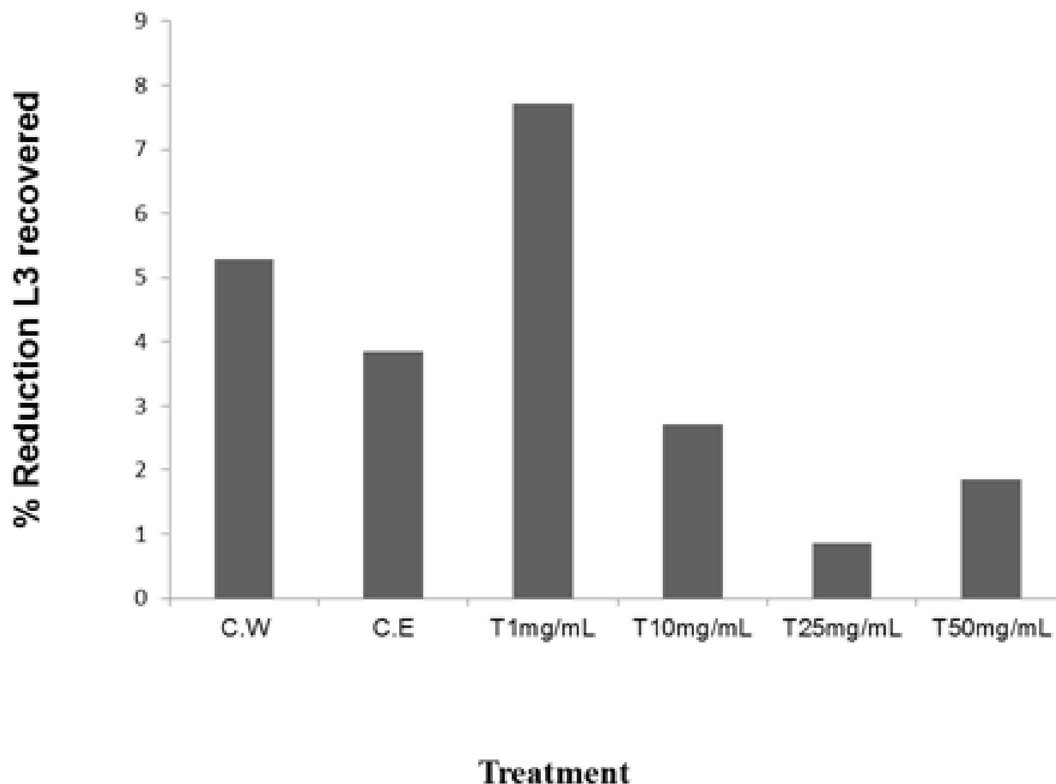

Figure 1. Averages of *Ancylostoma caninum* infective larvae (L3) recovered after treatment with the ethanolic extract of *Mikania glomerata* (0.1 mg/ml, 10 mg/ml, 25 mg/ml, and 50 mg/ml), and the negative water control group and ethanol control after 7 days of interaction. C.W (water control); C.E (ethanol control), T (treatment). ($P < 0.01$) - Tukey test.

instead of aqueous extracts at the same concentration. In that study it was recorded that the methanol extracts showed good anthelmintic activity *in vitro* and *in vivo* and this may be due to the presence of a higher concentration of an alcohol soluble active molecule in the extract (Lone et al., 2012).

Of all the plants that have been studied, the anthelmintic activity was confirmed by *in vitro* or *in vivo* studies and, depending on the plant species or investigated parasite, this activity was or was not confirmed (Sousa et al., 2013; Camurça-Vasconcelos et al., 2005). It is therefore necessary when evaluating the anthelmintic activity of plant extracts to consider at least important factors, including: type of extract, plant part used, concentration / dose, route of administration, bioassay used, infected animal species and parasite species. These factors can interfere with the test and promote a false negative. Therefore, positive results from *in vitro* tests, alone, as well as performed in the present study are not enough to validate researched activity (Camurça-Vasconcelos et al., 2005).

The results showed that the ethanol extract of *M. glomerata* SPRENGEL at different concentrations (1, 10, 25 and 50 mg/ml), exhibited larvicidal activity against gastrointestinal nematode *A. caninum*, the causative agent of CLM, therefore helping families with low conditions, and decreasing treatment costs. Further studies are needed for *in vivo* assays, to improve the methodology and for further clarification of the agents responsible for the observed effects. The use of *in vitro* assays for anthelmintic research from herbal extracts has several advantages, such as ease of implementation, low cost and speed, also serving as an early indication of the activity being investigated and allowing to select the most promising extracts, reducing costs, avoiding loss of time and the indiscriminate use of mice (Stepek et al., 2006; Camurça-Vasconcelos et al., 2005). Thus, this is the first report of *M. glomerata* activity on L3 of *A. caninum*, which no doubt can lead to larger studies to combat other zoonotic geohelminths.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Amaral MPH, Vieira FP, Leite MN, Amaral LH., Pinheiro LC, Fonseca BG, Pereira MCS, Varejão EV (2009). Determinação do teor de cumarina no xarope de guaco armazenado em diferentes temperaturas. Rev. Bras. Farmacogn. 19(2b):607-611.

Assis LM, Bevilaqua CML, Morais SM (2003). Ovicidal and larvicidal activity in vitro of *Spigelia anthelmia* Linn extracts on *Haemonchus contortus*. Vet. Parasitol. 117(1-2):43-9.

Ayres M, Ayres JM, Ayres DL, Santos AS (2003). Aplicações estatísticas nas áreas de ciências biomédicas.Belém: Sociedade civil maniraua.

Bertolucci SKV, Pereira ABD, Pinto JEBP, Oliveira AB, Braga FC (2013). Isolation and hplc quantitation of kaurane-type diterpenes and cinnamic acid derivatives of long-term stored leaves of *Mikania laevigata* and *Mikania glomerata*. An. Acad. Bras. Cienc. 85(73):486.

Bi S, Goyal PK (2012). Anthelmintic effect of Natural Plant (*Carica papaya*) extract against the Gastrointestinal nematode, *Ancylostoma caninum* in Mice. ISCA J. Biol. Sci. 1(1):2-6.

Brito DRB, Rozeviter MF, Fernandes MZLCM, Ferreira MDS, Rolim FRL, Silva Filho ML (2009). Atividade anti-helmíntica dos extratos aquoso e etanólico do fruto da *Morinda citrifolia* sobre *Ascaridia galli*. Rev. Bras. Parasitol. Vet. 18(4):32-36.

Camurça-vasconcelos ALF, Morais SM, Santos LFL, Rocha MFG, Bevilaqua, CML (2005). Validação de plantas medicinais com atividade anti-helmintica. Rev. Bras. Plant Med. 7(3):97-106.

Celeghini RMS, Vilegas JHY, Lanças FM (2001). Extraction and Quantitative HPLC Analysis of Coumarin in Hydroalcoholic Extracts of *Mikania glomerata* Spreng. (“guaco”) Leaves. J. Braz. Chem. Soc. 12(6):706-709.

Dias de Castro LL, Madrid IM, Aguiar CLG, Castro LM, Cleff MB, Berne MEA, Leite FPL (2005). *Origanum vulgare* (Lamiaceae) ovicidal potential on gastrointestinal nematodes of cattle Farmacopeia Brasileira 4th ed. Rio de Janeiro: Atheneu. Cienc. Anim. Bras. Goiânia 14(4):508-513.

Eren J, Pramer D (1965). The most probable number of nematode-trapping fungi in soil. Soil Sci. 99:285.

Gasparetto JC, Campos FR, Budel JM, Pontarolo R (2010). *Mikania glomerata* Spreng. e *M. laevigata* Sch. Bip. ex Baker, Asteraceae: estudos agronômicos, genéticos, morfoanatômicos, químicos, farmacológicos, toxicológicos e uso nos programas de fitoterapia do Brasil. Rev. Bras. Farmacogn. 20(4):627-640.

Lone BA, Chishtia MZ, Bhatd FA, Takb H, Bandha SA (2012). *In vitro* and *in vivo* anthelmintic activity of *Euphorbia helioscopia* L. Vet. Parasitol. 189(2-4):317-321.

Lopes ACG, Hiura E, Soares FEF, Fonseca LA, Sena CC, Ferraz CM, Lacerda L, Senna T, Aguiar AR, Araújo AL, Araújo JV, Braga FR (2015). Predatory Activity of the Fungus *Pleurotus eryngii* on *Ancylostoma caninum* Infective Larvae. SOJ Vet. Sci. 1(1):104.

Santana LCLR, Silva AO, Brito MRM, David JPL, David JM, Galvão KCS, Moraes J, Freitas RM (2013). Avaliação do potencial antioxidante, atividade antimicrobiana e antihelmíntica do extrato etanólico padronizado das folhas de *Mikania glomerata* Sprengel. Rev. Bras. Farmacogn. 94(2):120-129.

Santarém VA, Giuffrida R, Zanin GA (2004). Cutaneous larva migrans: reports of pediatric cases and contamination by *Ancylostoma* spp larvae in public parks in Taciba, São Paulo State. Rev. Soc. Bras. Med. Trop. 37(2):179-181.

Sousa RG, Falcão HS, Barbosa Filho JM, Melo MFFD, Batista IM (2013). Atividade anti-helmíntica de plantas nativas do continente americano: uma revisão. Rev. Bras. Plant Med. 15(2):287-292.

Stepek G, Buttle DJ, Duce IR, Behnke JM (2006). Human gastrointestinal nematode infections: Are new control methods required? Int. J. Exp. Pathol. 87(5):325-341.