Anthelmintic effect of *Stachytarpheta schottiana* in *Caenorhabditis elegans*

**Efeito anti-helmíntico de Stachytarpheta schottiana em Caenorhabditis elegans**

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ABSTRACT
Helminthiasis is considered a serious illness in tropical countries. The study of activity of natural products has proven to be an excellent alternative to parasite control. This work evaluated the anthelmintic activity of the crude extract of *Stachytarpheta schottiana* on viability, movements, reproduction behaviors and morphology of different stages of *Caenorhabditis elegans*. The extract did not decrease L1 hatching, and the survival of L1-L2 and adults in all tested concentrations. And it did not alter the movements of *C. elegans* adults and the egg-laying activity and brood size. Chemotaxis behavioral assays showed that *C. elegans* adults was not attracted or repelled by the extract. The morphological analysis of different forms showed several alterations as cuticular damages that promoted the detachment from the nematode body. The eggs were degraded or not present in adult bodies treated. The results obtained with the crude extract suggest that several secondary metabolites isolated from *S. schottiana* have an anthelmintic effect and for this confirmation, future analyses will be necessary.

Keywords: nematodes, *Caenorhabditis elegans*, *Stachytarpheta schottiana*, natural products, anthelmintic.

RESUMO
A helmintíase é considerada uma doença grave nos países tropicais. O estudo da atividade de produtos naturais tem se mostrado uma excelente alternativa no controle de parasitas. Este trabalho avaliou a atividade anti-helmíntica do extrato bruto de Stachytarpheta schottiana sobre a viabilidade, movimentos, comportamentos reprodutivos e morfologia de diferentes estágios de *Caenorhabditis elegans*. O extrato não diminuiu a eclosão de L1 e a sobrevivência de L1-L2 e adultos em todas as concentrações testadas. E não alterou os movimentos dos adultos de *C. elegans*, a atividade de postura e o tamanho da ninhada. Ensaios comportamentais de quimiotaxia mostraram que *C. elegans* não foi atraído ou repelido pelo extrato. A análise morfológica das diferentes formas mostrou diversas alterações como danos cuticulares que promoveram o desprendimento do corpo do nematóide. Os ovos estavam degradados ou não estavam presentes nos corpos adultos tratados. Os resultados obtidos com o extrato bruto sugerem que diversos metabólitos secundários isolados de *S. schottiana* possuem efeito anti-helmíntico e para esta confirmação serão necessárias análises futuras.

1 INTRODUCTION

Helminthiasis are considered neglected tropical diseases (NTDs) and affect hundreds of millions of individuals living in tropical countries and are of considerable relevance to public health. Among them, soil-transmitted helminthiasis are spread in worldwide scale and are caused by infection with the nematodes *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Ancylostoma duodenale* or *Necator americanus* (hookworms). In tropical countries, soil-transmitted helminthiasis are endemic and the parasite eggs excreted in the feces of infected individuals contaminated the soil. Humans become infected through ingestion of eggs or larvae and harbors the adult helminth in its intestine (WHO, 2017).

The human resistance to anthelmintics has not yet become a significant clinical problem due to long and complex life cycles of human helminths parasites. However, the possibility of pharmacological resistance of helminths has been monitored and studied (McKellar & Jackson, 2004; Jayawardene et al., 2021).

Plants provide a wide range of chemical substances during their metabolism. Some of these substances are known as active principles and are capable of promoting some type of biological response when administered in the animal body, including man, by different routes (Sousa et al., 1991).

Plants of the genus *Stachytarpheta* (Verbenaceae) have a pantropical distribution. The species *S. cayennensis* is used in folk medicine to treat diarrhea and dysentery, intestinal parasites and gastrointestinal disorders (Neiva et al., 2014). The only report of anthelmintic activity was from *S. jamaicensis* on *Strongyloides stercoralis* (Robinson et al., 1990).

*Stachytarpheta schottiana* is a shrub endemic of Southeast of Brazil and occurs in Atlantic Forest and restinga environment, including Parque Nacional da Restinga de Jurubatiba, that is a federal conservation unit, comprising the municipalities of Macaé, Quissamã and Campos dos Goytacazes (ICMBIO website, access in September 18th, 2023). Toledo e Silva et al. (2021) showed that *S. schottiana* presents secondary metabolites like iridoids, flavonoids, lignans and phenylethanoids. This plant is popularly known as “gervão da praia” and is use in folk medicine for the anthelmintic treatment in communities of Quissamã/RJ (Boscolo & Valle, 2008), however no antiparasitic activity was proven for this species.

Many plant extracts containing flavonoids have anthelmintic activity and affect the morphology of nematodes, cestodes and trematodes (Gonçalves et al., 2023). Thus, the aim of this work was to evaluate the anthelmintic activity of the crude extract of *Stachytarpheta schottiana*, using the *Caenorhabditis elegans* as an experimental model.
2 MATERIAL AND METHODS

2.1 OBTAINING THE STACHYTARPHEA SCHOTTIANA CRUDE EXTRACT

Stachytarpha schottiana was collected in the Restinga de Jurubatiba National Park and its crude extract was produced by maceration with ethanol for 72 hours, repeating the procedure 3 times, until complete extraction. Details about the collection and the extract chemical composition have been previously described (Toledo e Silva et al., 2021).

2.2 MAINTENANCE OF CAENORHABDITIS ELEGANS

The N2 strain of C. elegans was maintained in NG medium (3g/L of NaCl; 17 g/L of agar; 2.5 g/L of peptone; 1 mM CaCl₂; 5 mg/L of cholesterol in ethanol; 1 mM MgSO₄ and 25 mM KPO₄) at 20°C (Brenner, 1974; Sant'anna et al., 2013), using Escherichia coli OP50 strain as a food source. Helminths and bacteria were kindly donated by the Caenorhabditis Genetics Center (CGC-University of Minnesota). The C. elegans were transferred to a new culture dish every 7 days. The bacteria Escherichia coli was maintained in LB medium (5g of Tryptone, 2.5g of Yeast extract, 2.5g of NaCl, 7.5g of Agar and 500ml of distilled water) and transferred to a new culture dish every 7 days. For the experiments, E. coli was maintained in L-Broth medium (5g of Tryptone, 2.5 g of Yeast Extract, 2.5g of NaCl and 500ml of distilled water).

2.3 ANTHELMINTIC EFFECT OF STACHYTARPHEA SCHOTTIANA

2.3.1 Obtaining the Different Forms of Caenorhabditis Elegans

First, the culture has been synchronized. For this, the culture plate containing the helminths was washed with M9 medium (3 g of KH₂PO₄, 6 g of Na₂HPO₄, 5 g of NaCl, 1 ml of 1 M MgSO₄ and 1 L of H₂O). Then, this liquid was transferred to a sterile tube containing a lysis solution (0.5 mL of 5M NaOH and 1 mL of 6% NaOCL) to maintain only eggs. This solution promotes the lysis of adult and larval helminths. The eggs obtained were transferred to a NG culture medium with food source. According to the life cycle of C. elegans, the different forms were used. For the experiments with eggs, the eggs obtained were used. For the experiments with L1-L2, the eggs were maintained in NGM for 15 hours. For the experiments with adult worms, the eggs were maintained in NGM for 72 hours to obtain the adults.

2.3.2 Assays With Eggs, Larvae and Adults of Caenorhabditis Elegans

The eggs, L1-L2 or adults were incubated in medium S. (S.basal medium (5.85 g of NaCl, 1 g of K₂HPO₄, 6 g of KH₂PO₄, 5mg/ml of cholesterol in ethanol and 1mL of H₂O), 10 mL 1 M potassium citrate, pH 6.0, 10 mL trace metal solution (1.86 g EDTA, 0.69 g
FeSO$_4$.7H$_2$O, 0.2 g MnCl$_2$. 4H$_2$O, 0.29 g ZnSO$_4$.7H$_2$O, 0.025 g CuSO$_4$.5 H$_2$O and 1L H$_2$O), 3 ml 1M CaCl$_2$, 3 ml MgSO$_4$. The E. coli was added to the incubation medium as a food source, having previously been cultivated in a liquid medium. About 30 eggs, L1-L2 or adults were incubated in different conditions: without drugs (control), 1% ethanol (extract dissolution vehicle), crude extract of S. schottiana at concentrations of 500, 750, 1000 and 1250 μg/mL. The eggs were incubated for 24 hours and the effect of the extract on eggs was read as the hatching percentage of L1. The L1-L2 were incubated for 24 hours and the adults for 72 hours. The survival of L3-L4 and the adults were monitored in these intervals (Sant’anna et al., 2013).

2.3.3 Reproduction and Locomotion Behavior Assays

Reproduction of hermaphrodites was assessed by the number of eggs laid and the larval stages in culture for 72 hours. For experiments to determine the brood size, about 10 synchronized adults were transferred to treatment plates and incubated at 20 °C for 1, 24 or 72 hours with 1% ethanol or 1250 μg/mL of extract of S. schottiana, and the number of all stages beyond the egg was counted. For experiments to assess egg laying, about 5 synchronized adults were transferred to treatment plates and incubated at 19 °C for 1, 24 or 72 hours with 1% ethanol or 1250 μg/mL of extract of S. schottiana, and the number of eggs was counted.

To assay locomotion behavior, the head thrash was determined in conditions without and with E. coli. About 10 synchronized adults were transferred to treatment plates and incubated at 20 °C for 1, 24 or 72 hours with 1% ethanol or 1250 μg/mL of extract of S. schottiana, and the number of head thrashes was determined after 1 minute. The head thrash was defined as a change in the direction of bending at the mid body.

2.3.4 Chemotaxis Assay

Chemotaxis assays were performed using 5 cm plates with NG medium divide into 4 equal quadrants, as described (Margie et al. 2013). In brief, a circle of radius 0.5 cm was made around the origin and a point in each quadrant was made. The points were identified as "T" for the test or a "C" for control. The extract was the test and 1% ethanol was the control. In E. coli assays, test LB medium alone was the control. In assays with extract, 1% ethanol was the control and 1250 μg/mL of extract of S. schottiana was the test. In all conditions, 0.5 M azide was added. Then about 50 synchronized adults were placed in the origin. After 1 hour or 24 hours, the plate was placed in a 4°C incubator and removed when it could be counted. The number of adults in each quadrant was counted and the Chemotaxis Index was calculated:
number of adults in test quadrants minus number of adults in control quadrants divided by total adults added in plate. The assay was made in triplicate.

2.3.5 IC50 Calculation and Statistical Analysis

Percent survival and hatching of L1 were plotted as a function of crude extract concentrations using a non-linear analysis with the GraphPad Prism 9 program (GraphPad Software, Inc., USA). Statistical comparisons were performed by analysis of variance (One-way ANOVA, Newman-Keuls posttest) and significance was accepted if p<0.05. To calculate the IC50, the same program was used. For brood size, egg laying and head thrashes analysis, the values were expressed in mean ± standard error of the mean (SEM) and Two-Way ANOVA, Tukey’s multiple comparisons test was used. For chemotaxis index, the values were expressed in mean ± standard error of the mean (SEM) and the unpaired t test was used.

2.4 MORPHOLOGICAL EVALUATION OF THE EFFECT OF STACHYTARPHETA SCHOTTIANA EXTRACT ON CAENORHABDITIS ELEGANS BY OPTICAL MICROSCOPY

The different stages of C. elegans obtained from tests with different concentrations of the extract were fixed in AFA (glacial acetic acid, 37% formaldehyde and 70% ethanol), washed in 0.1M sodium cacodylate buffer (pH 7.2) and observed in Olympus BX51 optical microscope equipped with an Olympus DP71 camera (Sant’anna et al., 2013, 2016).

3 RESULTS

The effect of the crude extract on eggs of C. elegans was determined by the percentage of eggs hatched in each experimental condition. The extract did not decrease L1 hatching from treated eggs at any of the tested concentrations (Fig. 1A). The IC50 obtained was 2162 µg/mL. Also, the extract did not decrease the survival of L1-L2 (Fig. 1B) and adults (Fig. 1C) in any tested concentrations, with IC50 of 16445 µg/mL and 10937 µg/mL, respectively.

In addition to evaluating the effect on survival, the effect of the extract was tested in behavioral assays. The head thrashes assays were performed in the presence and absence of E. coli, since the adults could be feeding on the extract in the medium. In both assays, the movement of adults decreased over time, being lower at 72 hours compared to the initial times. In the absence of E. coli, there was no difference between groups (Fig. 2A) and in presence of E. coli, S. schottiana was different from the control in the time of 10 minutes (Fig. 2B).
In reproduction behavioral assays, *S. schottiana* did not alter the brood size (Fig. 3B) but the number of larvae does not increase over time. *S. schottiana* seemed to decrease the number of eggs in the medium, but this result was not statistically significant compared to the control (Fig. 3A).

Chemotaxis behavioral assays are widely used to test environmental pollutants, like chemical substances, and to recognize pathogens bacteria (Queirós et al., 2021). In this study, this assay was performed to verify whether *C. elegans* was attracted or repelled by the extract. To test *S. schottiana*, 1% ethanol was used as control. There is no difference between the extract and *E. coli* 1 hour (Fig. 4A) or 24 hours (Fig. 4B) after the incubation, showing that *C. elegans* is not attracted or repelled by the extract.

Although it does not alter the survival and behavioral parameters, *S. schottiana* causes important alterations in treated larvae and adults. Larvae hatched from untreated and treated eggs with different concentrations were analyzed by optical microscopy. The larvae from the control and 1% ethanol group were intact, with the anterior end and oral opening with preserved morphology, followed by the bulbopharyngeal esophagus, intestine, and posterior end without any alteration and intact cuticle.

The larvae obtained from eggs treated with the extract of *S. schottiana* at concentrations of 500, 750, 1000 and 1250 μg/mL show morphological changes: cuticular changes such as detachment from the body wall (Fig. 5B, 5C), damage to structures of the digestive system (Fig. 5A, 5B), such as the esophagus.

The L3-L4 larvae of the control group and 1% ethanol were presented in their intact form, with the anterior end and oral opening with preserved morphology, bulbopharyngeal esophagus followed by the intestine and posterior end without any alteration and intact cuticle (Fig. 5D, 5E).

At a concentration of 500 μg/mL, it is not possible to observe morphological changes, however at concentrations of 750, 1000 and 1250 μg/mL of the extract, cuticular changes are observed in the region of the oral opening (Fig. 5F) and in organs of the digestive system, with the most pronounced effect at the highest tested concentration of 1250 μg/mL.

The adults in the control group and 1% ethanol were intact, with the anterior end and oral opening with preserved morphology (Fig. 5G, 5H). The cuticle along the entire body was intact. The bulbopharyngeal esophagus (Fig. 5H) and intestine were normal and well delimited. The well-defined eggs inside the nematode demonstrate the normal and adequate development of the adult form (Fig. 5I). The posterior end is unchanged with the development and presence of the reproductive tract.
Under the conditions of treatment with the extract of *S. schottiana*, morphological changes were observed in adult *C. elegans* at all tested concentrations. At a concentration of 500, 750, 1000 and 1250μg/mL of the extract, it is already possible to observe a cuticular alteration, with its detachment from the nematode body (Fig. 5K, 5L). At 750, 1000 and 1250μg/mL the eggs are degraded or not present (Figure 5J). At 1250μg/mL, there is also damage inside the adult, such as a change in the adult's esophagus (data not shown).

Figure 1: Anthelmintic effect of crude extract of *Stachytarpheta schottiana*. (A) Percentage of L1 hatching from untreated *Caenorhabditis elegans* eggs and those treated with crude extract of *Stachytarpheta schottiana*. Result of 24 hours of incubation in different concentrations. Values are the mean ± Standard Error of the Mean (SEM) of three triplicate experiments. P>0.05; One-way ANOVA, Newman-Keuls post-test. (B) Percentage of L1-L2 survival of *C. elegans* untreated and treated with crude extract of *S. schottiana*. Result of 24 hours of incubation in different concentrations. Values are the mean ± Standard Error of the Mean (SEM) of four triplicate experiments. P>0.05; One-way ANOVA, Newman-Keuls post-test. (C) Percentage of survival of adult forms of *C. elegans* untreated and treated with crude extract of *S.schottiana*. Results of 72 hours of incubation in different concentrations. Values are the mean ± Standard Error of the Mean (SEM) of three triplicate experiments. P>0.05; One-way ANOVA, Newman-Keuls post-test.

Figure 2: Effects of extract of *Stachytarpheta schottiana* on head thrashes of *Caenorhabditis elegans* adult. (A) Number of head thrashes per minute without *E. coli* and (B) with *E. coli* in control and 1% ethanol or 1250μg/mL of *S. schottiana* in time of incubation (0 minute), 10 minutes, 1 hour, 24 hours and 72 hours after incubation with the compounds. Values are the mean ± Standard Error of the Mean (SEM) of three triplicate experiments. P>0.05; Two-Way ANOVA, Tukey's multiple comparisons test.

Source: Authors
Figure 3: Effects of extract of *Stachytarpheta schottiana* on reproduction of *Caenorhabditis elegans* adult. (A) Egg-laying activity without treatment (control) and after treatment with 1% ethanol or 1250μg/mL of *S. schottiana* in 1 hour, 24 hours and 72 hours. (B) Effect of the same treatments on brood size of nematodes. Values are the mean ± Standard Error of the Mean (SEM) of three triplicate experiments. *P*>0.05; Two-Way ANOVA, Tukey’s multiple comparisons test.

Source: Authors

Figure 4: Effect of *Stachytarpheta schottiana* on chemotaxis index. (A) Chemotaxis index in control (*E. coli*) and after treatment with 1250μg/mL of *S. schottiana* per 1 hour. (B) Chemotaxis index in control (*E. coli*) and treatment with 1250μg/mL of *S. schottiana* per 24 hours. Values are the mean ± Standard Error of the Mean (SEM) of two triplicate experiments. *P*>0.05; Unpaired t test.

Source: Authors
Figure 5: Effect of *Stachytarpheta schottiana* in morphology of *Caenorhabditis elegans*. (A) Light microscopy of L1 obtained from eggs treated with 500μg/mL of *S. schottiana*, showing the oral opening (OO) and esophagus (E) and cuticular alterations (arrow) (Bars 50 μm). (B, C) Light microscopy of L1 obtained from eggs treated with 1250μg/mL of *S. schottiana*, showing the esophagus (E) and cuticular alterations (arrows) (B - Bars 50 μm; C - Bars 100 μm). (D) Light microscopy of L3-L4 obtained from L1-L2 untreated, showing the oral opening (OO) (Bars 50 μm). (E) Light microscopy of L3-L4 obtained from L1-L2 treated with 1% ethanol, showing the oral opening (OO) and esophagus (E) (Bars 100 μm). (F) Light microscopy of L3-L4 obtained from L1-L2 treated with 1000μg/mL of *S. schottiana*, showing cuticular alterations (A) (Bars 50 μm). (G, H) Light microscopy of adults untreated with normal morphology and anterior region with the oral opening (OO) and esophagus (E) (G - Bars 500 μm; H - Bars 200 μm). (I) Light microscopy of adults treated with 1% ethanol with normal morphology and mid region of the body showing the vulva (V) and normal eggs (Egg) (Bars 500 μm). (J) Light microscopy of adults treated with 750μg/mL of *S. schottiana* showing the altered eggs (Egg) (Bars 500 μm). (K) Light microscopy of adults treated with 1000μg/mL of *S. schottiana* showing cuticular alterations (A) (Bars 500 μm). (L) Light microscopy of adults treated with 1250μg/mL of *S. schottiana* showing cuticular alterations (A) (Bars 200 μm).

Source: Authors
4 DISCUSSION

The root parts of *S. schottiana* are used in traditional medicine for the anthelmintic treatment (Boscolo & Valle, 2008). Other species of *Stachytarpheta* showed biological effects against many different organisms.

*Stachytarpheta cayennensis* was used in traditional medicine as anti-inflammatory, analgesic, antipyretic, hepatoprotective, laxative and in the treatment of gastric disorders (Mathias & Emily, 1993; Mesia-Vela *et al*., 2004), also in the treatment of skin lesions (Caribe & Campos, 1991), including in ulcerated lesions caused by *Leishmania* sp. (Moreira *et al*., 2002). In fact, the extract obtained from leaves of *S. cayennensis* demonstrated anti-*Leishmania* activity *in vitro*, with IC₅₀ of 73.7 μg/mL for *L. braziliensis* and 382.5 μg/mL for *L. amazonensis* (Moreira *et al*., 2007).

*S. cayennensis* has also been reported for the treatment of intestinal parasites and gastrointestinal disorders in traditional medicine in São Luís, Maranhão, Brazil (Neiva *et al*., 2014). In this study, the activity against trophozoites of *Giardia lamblia* was demonstrated, with IC₅₀ of 120.93 ± 2.54 μg/mL. *Stachytarpheta jamaicensis* also demonstrated giardicidal activity *in vitro* (Ordóñez *et al*., 2001). Although, *S. jamaicensis* was the only species that had its anthelmintic effect investigated. The extract obtained from green leaves was capable of inactivating the filariform larvae of *Strongyloides stercoralis* 81.5 hours after treatment (Robinson *et al*., 1990).

The only report of biological activity of crude extract of *S. schottiana* was in *Mycobacterium bovis*. Araujo *et al*. (2021) showed that crude extract inhibits *M. bovis* with MIC₅₀ of 78.77±1.32 mg/mL.

The concentrations used in the present work are higher than those reported in the literature for protozoa and bacteria, since we are using a pluricellular organism as a model. Moreira *et al*. (2007) related the cytotoxicity of *S. cayennensis* extract against macrophages and demonstrated that IC₅₀ was 1441.4 μg/mL, a concentration 1.15 higher than the highest concentration used in this work.

To assess anthelmintic activity in *C. elegans* the worm motility is considered the gold standard for measuring drug effectiveness (Liu *et al*., 2020). The worm motility is a parameter for worm survival. McGraw *et al*. (2000) tested two species of Verbenaceae family in *C. elegans* adults survival and showed that ethanolic extract of leaves of *Clerodendrum glabrum* and hexane, ethanolic and water extract of leaves and twigs of *Lippia javanica* were active in concentration of 2 mg/mL, but no IC₅₀ was calculated. Acetone leaf extract of *Lantana rugosa* (Verbenaceae) killed 30% of adult *C. elegans* at concentrations of 1 and 2 mg/mL, and
presented lethality in brine shrimp with LC$_{50}$ of 0.69 mg/mL (McGaw & Eloff, 2005). In this work, the concentrations tested were 500, 750, 1000 and 1250 μg/mL and the IC$_{50}$ obtained for adults was 10937 μg/mL, showing that is necessary a high concentration of the extract of S. schottiana to kill the C. elegans adults.

The alteration of different stages of C. elegans is used to verify the anthelmintic activity of compounds. Parameters such as egg hatch test, larval development test, larval mortality/motility test and larval migration test were used (Liu et al., 2020). Extracts of leaves and stems of Clerodendrum myricoides (Verbenaceae) obtained with petroleum ether, dichloromethane and ethanol presented minimal lethal concentration between 0.26 and 2.08 mg/mL in larvae of C. elegans. In this work, the IC$_{50}$ obtained for larvae L1-L2 was 16445 μg/mL, a value greater than that found for adults.

In this work, the same concentrations of the extract were tested in the egg hatch and the IC$_{50}$ obtained was 2162 μg/mL, showing that a high concentration of the extract is necessary to inhibit the egg hatch. In some cases, the substances tested have an effect on all forms of C. elegans. Sant’anna et al. (2013, 2016) showed that albendazole and dinitroanilines herbicides (oryzalin and trifluralin) decreased the percentage of egg hatched and the survival of L1-L2, L3-L4 and adults of C. elegans.

The behavioral endpoints were also used as parameters of effect of substances on C. elegans, such as locomotor, feeding and reproduction behavior. Head thrashes were diminished in C. elegans treated with Genkwa Flos (the dried flower bud from Daphne genkwa) (Qiao et al., 2014) and Leucaena leucocephala extract (Widaad et al., 2022). In this work, the head thrashes were measured in the absence and presence of E. coli. In absence of E. coli, the C. elegans adult remained active as well as in control. In presence of E. coli, S. schottiana decreased the head thrashes 10 minutes after the initial exposure, showing that the extract cannot alter the movements of C. elegans adult over the hours. The same pattern occurs in reproduction assays. S. schottiana does not alter the number of eggs released and the brood size. However, despite not altering the brood size in relation to the controls, the number of larvae in the S. schottiana group remains stable, showing that there is no increase in the number of larvae in the medium, unlike what occurs with the control. Extract of Ceiba speciosa (Malvaceae) does not alter the survival of C. elegans adult and the brood size (Santos et al., 2020). These authors show that C. elegans adults were ingesting the extract and feeding normally (Santos et al., 2020). C. elegans adults treated with S. schottiana extract remained active, even in absence of E. coli. Likely they were ingesting the extract, which explains why the survival has not changed.
To test whether the *S. schottiana* extract attracts or repels the adult *C. elegans*, the chemotaxis assay was performed. The chemotaxis index with *S. schottiana* was not different from the chemotaxis index with *E. coli*, showing that extract neither attracts or repels *C. elegans*. There are few studies relating the effect of compounds on the chemotaxis behavior of *C. elegans* with their anthelmintic effect. Sobkowiak *et al.* (2018) tested synthetic and plant-derived nematicides and showed that the compounds had no effect on behavioral nematode chemotaxis.

Despite not altering survival and behavior parameters, *S. schottiana* extract alters the morphology of treated *C. elegans*. L1 obtained from treated eggs, L3-L4 and adults presented cuticular alterations and alterations on digestive tract. In adults, the eggs in utero were degraded or absent. Extract of *Rumex crispus* (Poligonaceae) also changed the morphology of internal structures of *C. elegans* adult and causes a deformity in eggs treated (Idris *et al.*, 2022). By scanning electron microscopy, they also demonstrated damages in cuticles in adults treated (Idris *et al.*, 2022). The dinitroanilines herbicides (oryzalin and trifluralin) also caused morphological alterations in larvae and adults of *C. elegans*, with cuticular alterations and structural damage in several organs, especially in the uterus (Sant’anna *et al.*, 2016).

The results of the present work showed that the extract of *S. schottiana* did not alter the survival of L1-L2 and adult, not alter the egg hatched, head thrashes, egg-laying to the medium, brood size and chemotaxis index. However, *S. schottiana* caused morphological alterations in all *C. elegans* treated. A limitation in the use of *C. elegans* for drug discovery is the thickness of its cuticle. As the cuticle is very thick, it forms a barrier decreasing the absorption of the drug (Giunti *et al.*, 2020). Burns *et al.* (2010) demonstrated that the internal concentration of the drug is much smaller than the concentration applied in the medium. Therefore, the concentration of the drug must be much higher to achieve a biological effect. On the other hand, even if it does not have an immediate effect on *C. elegans*, a compound may have an effect on a parasitic nematode, which has a thinner cuticle (Giunti *et al.*, 2020).

Many plants used in folk medicine require scientific proof (Vieira, 1999; Jayawardene *et al.*, 2021). The process of extraction is an important stage and can affect the result. The polarity and quality of solvents alter the profile of secondary metabolites obtained and consequently alter the efficacy of the extracts (Wang, 2011; Akkari *et al.*, 2016). Toledo e Silva *et al.* (2021) showed that *S. schottiana* collected in Restinga de Jurubatiba National Park had 25 secondary metabolites, such as iridoids, flavonoids, lignans and phenylethanoids. Flavonoids are polyphenolic compounds that have anthelmintic effects against many different helminths (Gonçalves *et al.*, 2023). Extracts from *Rumex crispus* (Idris *et al.*, 2022) and
Genkwa Flos (Qiao et al., 2014) present flavonoids as secondary metabolites and decrease survival and lead to morphological changes in *C. elegans*. Two phenylethanoids were the major compounds in *S. schottiana* (Toledo e Silva et al., 2021), verbascoside and isoverbascoside. Diet with herbs (which contained several metabolites, including verbascoside) decreased egg shedding and parasite load of *Haemonchus contortus* in sheep (Váradyová et al., 2018) and extracts from the plants *Lantana canescens* and *Handroanthus serratifolius* (that have many secondary metabolites, including verbascoside and isoverbascoside) decreased the hatching of *Haemonchus placei* larvae (Borges et al., 2019). As the crude extract of *S. schottiana* caused important morphological changes in *C. elegans*, these effects may have been caused by its secondary metabolites.

In conclusion, the crude extract had no effect on survival and behavior of *C. elegans*, but showed important morphological effects, especially in adults. Therefore, the possibility that several secondary metabolites isolated from *S. schottiana* have an anthelmintic effect cannot be discarded.

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