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Original Research Article

In vitro anthelminthic activities of *Cucumis melo* seeds against egg hatching, larval migration, and adult stage of *Haemonchus contortus*

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ABSTRACT

Haemonchosis is among the leading nematodosis of ruminants, particularly in sheep, with important economic losses. During the last decades, several approaches using anthelminthic drugs were conducted with a continuously decreasing success. Nowadays, it is well established that *Haemonchus contortus* is resistant to the three classes of anthelmintics in several countries. Currently, the approaches of grazing management and the use of alternative natural compounds are also implemented to control these infections. The present study aimed to evaluate the activity of the ethanolic extract of the seeds of *Cucumis melo* on *H. contortus*. For this purpose, the seeds were extracted in a 70% ethanol solution. The obtained powder was solubilized in phosphate buffer saline (PBS) at various concentrations (0 to 3.75 mg/mL) to which the assessed stages were submitted. The activity (mortality and egg hatching inhibition) was monitored at different time points (0, 12, 18, 24, and 48 h). The extract was active on diverse stages in a concentration-dependent manner. In adult females, a 100% mortality rate was reached after 24h with an LC50 of 0.5 ±0.09 mg/mL, which was significantly weaker than the levamisole control (0.33 ±0.02 mg/mL). The effect on larval migration was similar to the conventional drug effect, with IC50 values of 1.71 ± 0.10 mg/mL and 1.74 ± 0.25 mg/mL, respectively, for extract and levamisole. On larvae, the lethal activity was very similar in the presence of either levamisole or seeds extract, with an IC50 value of 0.86 ± 0.25 mg/mL and 1.19 ± 0.25 mg/mL, respectively. A qualitative phytochemical analysis revealed that the assessed extract contains alkaloids, tannins, phenolic compounds, flavonoids, glycosides, saponins, and triterpenes. The hydro-ethanolic extract of the seeds of *C. melo* did not induce any sign of toxicity in mice at the dose of 2000 mg/kg. Altogether, this extract is a good source of anthelminthic chemical compounds, especially those active on larval migration and egg hatching.

Key-Words: *Cucumis melo* seeds, Anthelminthic, *Haemonchus contortus*, levamisole.

RÉSUMÉ

L'haemonchose est l'une des principales nématodoses des ruminants, notamment les moutons à l'origine d'importantes pertes économiques. Au fil des décennies, diverses stratégies utilisant des médicaments anthelminthiques ont été mises en œuvre, avec un succès décroissant. Aujourd'hui, il est reconnu que *Haemonchus contortus* est résistant aux trois principales classes d'anthelminthiques dans plusieurs pays. Par conséquent, des approches de gestion des pâturages et l'utilisation de composés naturels alternatifs sont actuellement en développement pour lutter contre ces infections. Cette étude visait à évaluer l'activité de l'extrait éthanolique des graines de *Cucumis melo* contre *H. contortus*. Une extraction a été conduite sur des graines dans une solution d'éthanol à 70 %. La poudre obtenue a été dissoute dans du PBS (phosphate buffer saline) à différentes concentrations (0 à 3,75 mg/mL), puis soumise à des tests sur différents stades du parasite. L'activité de l'extrait, notamment la mortalité et l'inhibition de l'éclosion des œufs, a été évaluée à diverses tranches horaires (0, 12, 18, 24 et 48 heures). Les résultats montrent que l'extrait est actif selon la concentration et le stade du parasite. Chez les femelles adultes, un taux de mortalité de 100 % a été observé après 24 heures avec une CL_{50} de 0,5 ±0,09 mg/mL, inférieur à celle du lévamisole (0,33 ±0,02 mg/mL). Sur la migration larvaire, l'effet de l'extrait était comparable à celui du lévamisole, avec des valeurs IC50 de 1,71 ± 0,10

mg/mL et 1,74 ± 0,25 mg/mL respectivement. En ce qui concerne les larves, l'activité létale était similaire pour l'extrait et le lévamisole, avec des IC50 respectives de 0,86 ± 0,25 mg/mL et 1,19 ± 0,25 mg/mL. L'analyse phytochimique qualitative de cet extrait a révélé la présence d'alcaloïdes, de tanins et de composés phénoliques, de flavonoïdes, de glycosides, de saponines et de triterpènes. Aucun signe de toxicité n'a été observé chez les souris à la dose de 2000 mg/kg. En résumé, l'extrait hydro-éthanolique des graines de *C. melo* constitue une source prometteuse de composés chimiques à activité anthelminthique, notamment contre la migration larvaire et l'éclosion des œufs.

Mots-clés : *Cucumis melo* seeds, Anthelminthic, *Haemonchus contortus*, levamisole.

1. INTRODUCTION

Helminths are among the most frequent and ubiquitous animals, with several species living as parasites. They are involved in several parasitic diseases of animals and humans, inducing helminthiasis (Gaugler and Bilgrami, 2004). Human and livestock health and well-being are significantly limited by nematode infections, particularly in less developed countries, where they are responsible for chronic, acute, and even fatal parasitic diseases. Gastrointestinal helminth infections represent a major cause of economic loss in livestock breeding in the world (Sirbu et al. 2020).

Haemonchus contortus is among the most prevalent helminths of small ruminants, which are the most accessible and most frequent breeding animals for the local population in Cameroon (MINEPIA, 2021). *H. contortus* has a monoxenic short life cycle (about 20 days) with a parasitic and a free-living stage. The latest is characterized by larvae L1, L2, and L3 that can survive in unfavorable environmental conditions. Each of the progressive stages is separated by a molt, and the L3 stage has a particular cuticle that protects and allows it to infect pasture for several months until being swallowed by a favorable ruminant. In the host, the L3 molts to L4, and adults are localized *in the fine* in the abomasum, where they mate and produce eggs, feeding on the blood of their host. An individual can carry up to 5000 parasites, taking up 0.05 ml of blood daily (Urquhart et al. 1996). Losses due to haemonchosis (more than 90% of GI nematodes of ruminants) impede the productivity of meat, milk, and therefore deteriorate the financial health of a huge proportion of the population in Cameroon (Mbuh et al. 2008; Sassa et al. 2014). Furthermore, goat and sheep breeding is done for subsistence and mostly in rural areas, in conditions favorable to infection by GI nematodes. The scarcity of grazing fields in the southern part of the country and the quality of pastures in some of the northern areas are the main issues encountered by breeders. They usually overuse these fields or keep their animals in limited breeding spaces, which favors the infection (Mbuh et al. 2008). The control of that gastrointestinal nematode is based on the use of synthetic drugs on a large scale when possible. The economic and geographic difficulties in accessing that medication facilitate the mismanagement of its use. Herdsmen and breeders, to a greater extent, are not able to sustain appropriately the indications of posology in treating their herds. This inappropriate posology is a bad practice that reduces either the active principle or the exposure of the parasite to the drug and strongly contributes to the emergence of helminth resistance to anthelminthics. Thus, the unsuitable administration of these treatments, in addition to the lack of alternative molecules, induced the emergence of resistance of the parasite against most of the synthetic molecules (Hoste et al. 2006).

To provide alternative molecules, plant products represent a credible source of solutions. *Cucumis melo* is an herbaceous plant of the family of Cucurbitaceae used for the management of various ailments such as kidney stones, leprosy, anemia, jaundice, obesity, and several abdominal disorders (Asif et al. 2014). Extracts of *C. melo* have shown anti-ulcer (Gill et al. 2011), anticancer (Chen et al. 2012), antidiabetic (Chen and Kang, 2013), antimicrobial, and anthelmintic properties (Shubha et al. 2018). Very few studies assessing the anthelminthic activity of *C. melo* have been conducted, particularly on GI nematodes. Nevertheless, that genus was shown to be active against *Meloidogyne incognita* (Liu et al. 2016). This research aims to evaluate an alternative anthelminthic against haemonchosis and GI nematodes with the hydroethanolic extract of the seeds of *Cucumis melo*. We assessed the anthelminthic potential of the seeds of this plant and its safety on Swiss albinos mouse model.

2. MATERIAL AND METHODS

2.1. Plant collection and extraction

The fruits of *Cucumis melo* were collected from local farms in November 2024 at Dang (Adamawa, Cameroon) and identified by Dr Fawa, Botanist at the University of Ngaoundere (Cameroon). A specimen was registered at

the national herbarium, Cameroon, under the reference number 8073/SRF/Cam. The extraction was conducted following the method described by Ndjonka *et al*. (2011) with a few modifications. Concretely, the seeds of *Cucumis melo* were extracted from the fruit, washed with tap water, and left to dry in the shade. The resulting dry seeds were ground in a wooden mortar and subsequently sieved in a 0.4 mm mesh sieve. Fifty (50) g of the seed powder was macerated in 500 mL of hydroethanolic (70%) solution for 48 hours at room temperature under continuous stirring. The mixture was centrifuged (Eppendorf centrifuge 5804) at 3500 rpm for 10 min, and the supernatant was collected and filtered with a Whatman paper N°4. The filtrate was evaporated in an oven at 40°C until a dry plant matter was obtained. The extracted product was kept at 4°C until use.

2.2. Collection of parasite stages and culture

Isolation and culture of parasitic stages were conducted according to the method described by Dedehou *et al*. (2014) with slight modifications. Briefly, *Haemonchus contortus* adults were isolated from the abomasum collected from the goat abattoir of Ngaoundere. The emptied, freshly collected abomasum was cleaned with tap water and opened longitudinally. From the cleaned abomasa, *H. contortus* adults were then collected with forceps, identified, and cleaned thrice in a sterile saline solution (PBS). The healthiest and vigorous individuals were selected under a microscope and immediately used for bioassays.

The larvae were obtained by coproculture in dried heat-sterilized bovine feces (at 90°C). These feces were ground and distributed in 60 mL Petri dishes for culture. Gravid adult females were then collected following the previously described procedure, cleaned, ground roughly, and suspended in tap water. The prepared feces in Petri dishes were watered with the worm solution and left for 10 days at room temperature, with a periodic check from the 7th day. Once the presence of larvae (L1) was noticed, they were concentrated using the Baerman funnel method and numbered. Infectious larvae (L3) were prepared chemically to evaluate their sensitivity to the extract (De Jesús-Martínez et al. 2018). Precisely, they were collected and submitted to a treatment with 1.5 % sodium hypochlorite for 15 min to induce an "artificial molt" and remove their cuticle. The solution containing these larvae was subsequently centrifuged at 1000 rpm for 10 min, and the pellet containing larvae was then rinsed with distilled water. This procedure was repeated three times, and the larvae were kept in distilled water for anthelminthic assays.

2.3. *In vitro* biological assays

The anthelminthic activity of the extract was assessed in 24-well culture plates for all the studied stages (female adults, L3, and eggs). The culture worms + PBS-extract solutions were incubated at 37°C and worm mortality and room temperature for inhibition of egg hatching. The concentration range of 0, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL was used for adult females; 0, 0.75, 1.5, 2.25, 3, and 3.75 mg /mL for L3, and mortality was checked by observation under the microscope every 6 h, and using the MTT/formazan test after 48 (Ndjonka et al. 2011). An immotile worm after 20 seconds of observation, after an initial shaking, was considered dead. The mortality rate (MR) of L3 and adults was calculated according to the formula described by Hubert and Kerboeuf (1992).

$$\textbf{Motality Rate} = (\textbf{number of immotile individuals} \div \textbf{number of individuals in the culture}) \textbf{x100})$$

The evaluation of the inhibitory effect of *Cucumis melo* hydro-ethanolic extract on egg hatching was performed following the protocol of Cole *et al*. (2006) with a few modifications. Seventy eggs were added to each of the wells and incubated with various concentrations of the products. A range of concentrations of 0, 0.75, 1.5, 2.25, 3, and 3.75 mg /mL of plant extract and reference product was used for assessing inhibition and mortality of L3 larvae. The assays were conducted during 24 and 48 h for mortality evaluation. The controls were levamisole (positive) and PBS (negative), and all the tests were performed in triplicate. The hatching inhibition rate (HIR) of the egg was calculated using D'Angelo *et al*. (2014) as follows:

$$\textbf{HIR}\ (\%) = [(\textbf{70} - \textbf{number of L1} \div \textbf{number of deposited eggs})\textbf{x100}]$$

2.4. Qualitative analysis of phytochemical constituents

The qualitative phytochemical content of the seeds of *Cucumis melo* was evaluated following standard protocols from an initial aqueous solution extract of 0.1 mg/mL.

2.4.1. Polyphenols and tannins

The polyphenols and tannins were assessed following the method described by N'guessan et al. (2009) using ferric chloride. Concretely, 1 mL aqueous solution of the extract was introduced into a glass tube to which a

few drops (3 to 4) of 10% methanolic solution of ferric chloride (FeCl3) were gently added. A green color indicated the presence of polyphenols and tannins in the tested extract.

2.4.2. Flavonoids

Flavonoid's presence was checked using the method of Wilstater described by Fankam *et al* (2011). One (1) mL of extract was placed in a glass tube, then 3 chips of magnesium and 1 mL of hydrochloric acid were added consecutively. The mixture was mixed thoroughly, and the displayed color was registered. Red, orange, and purple colors indicated, respectively, flavonols, flavones, and flavonones.

2.4.3. Alkaloids

The alkaloids' presence was assessed using the technique of Dragendorff described by Bidie *et al*. (2011). To 1 mL of extract solution, 1 mL of 5 % chlohydric acid, and 3 drops of Dragendorff reagent were subsequently and successively added. An orange or white coloration indicated the presence of alkaloids.

2.4.4. Triterpenes and steroids

Triterpenes and steroids were detected according to the method of Liebermann Burchard as described by Fankam *et al*. (2011). To each sample, 1 mL of chloroform was added, and the mixture was vigorously mixed and filtered. Two drops of acetic anhydride and a few drops of sulfuric acid were added to the filtrate, and the whole was mixed thoroughly. A greenish coloration with a red ring characterizes steroids and triterpenes.

2.4.5. Saponins

The presence of saponins was assessed following the method of Yadav *et al*. (2014) with a few modifications. To 1 mL of extract solution, 4 mL of distilled water was added, and the whole was vigorously shaken. The apparition and abundance of foam indicated the presence and abundance of saponins.

2.4.6. Glycosides

The presence of glycosides was determined according to the method described by Yadav *et al*. (2014). One (1) milliliter of chloroform and acetic acid was added successively to 1 mL of extract solution; the subsequent blue color indicates the presence of glycosides. To 1 mL of extract solution, 2 mL of HCl and ammonia were added, and a purple coloration indicated the presence of anthocyanes (Yadav et al. 2014).

2.5. Toxicity studies on mice

An assessment of acute toxicity was performed to evaluate the safety of *C. melo* ethanolic extract *in vivo*. The animals were obtained from LANAVET (veterinary laboratory of Cameroon) and acclimated for about a week at room temperature (22±3 °C) with a relative humidity of 30% and artificial lighting. All experimental procedures performed on animals were approved by the regional delegation of Livestock, Fisheries and Animal Industries at Ngaoundere (N°075/16/L/RA/DREPIA). Six (06) Swiss albino mice (*Mus musculus*) weighing 28 ± 0.5 g on average were divided into two groups of three mice each. The first one, negative control, received by gavage through a gastric tube 1 mL of distilled water, and the second received the extract dissolved in distilled water at the dose of 2000 mg/Kg. The manifestation of toxicity was monitored by registering the mice's behavior and death. Behavioral parameters such as grooming, convulsion, piloerection, stool aspect, and mortality were monitored on an hourly basis for the first 12 hours post administration and daily for 14 days.

2.6. Data analysis

The data were presented as mean and standard deviation; the LC_{50} and IC_{50} were evaluated using log probit calculation. GraphPad Prism Version 5 and SPSS software were used for data handling and graph drawing. The analysis was performed by ANOVA with Tukey post-test or Student T test, with a difference threshold of 5% considered significant.

3. RESULTS

3.1. Anthelmintic activity of the extract on the female adult stage

Haemonchus contortus females were sensitive to *Cucumis melo* hydroethanolic extract of seeds from 12 to 24 h in a concentration-dependent manner. Figure 1 below presents their survival in PBS in the presence of various concentrations of the extract ranging from 0 to 1.5 mg/mL. The mortality rate increases with the concentration of the extract at most of the time points (6, 12, 18, and 24 h), with a 100 % mortality rate at 1.5 mg/mL. At 6

h, no mortality was observed at any concentration. It was demonstrated in a preliminary study that in an extract-free culture medium (PBS), the female can live up to 24 h (data not shown).

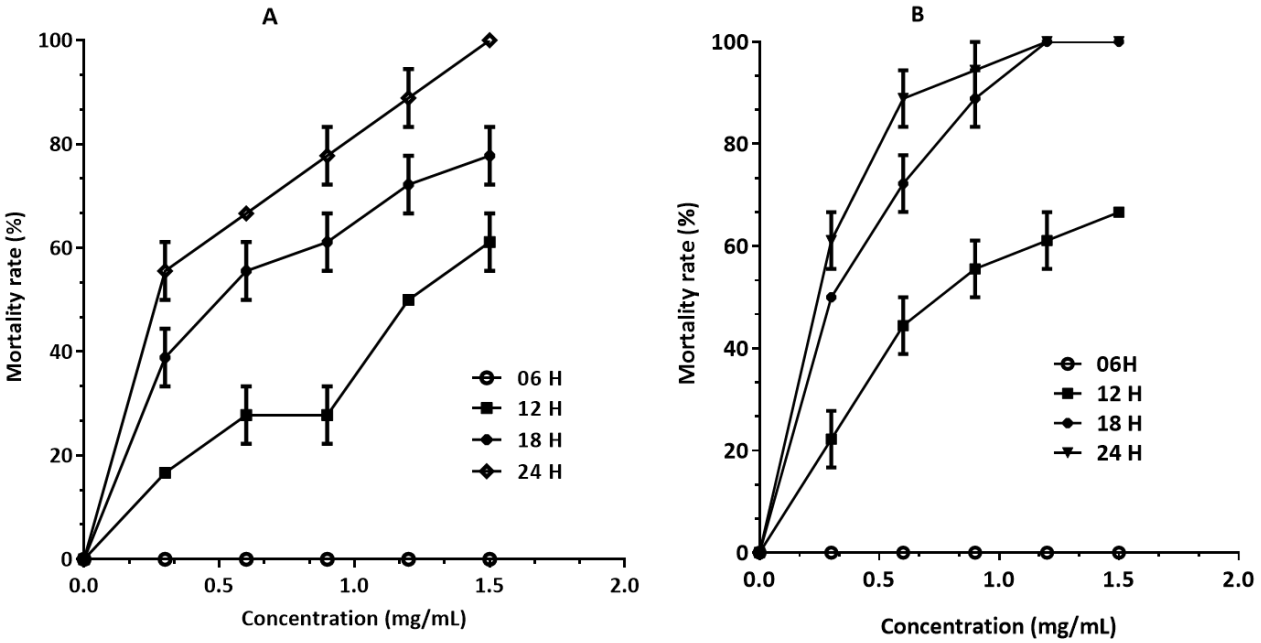

Figure 1: Activity of hydroethanolic extract of *Cucumis melo* seeds (A) and levamisole (B) on the survival of *Haemonchus contortus* females. Error bars represent standard deviation.

Hydroethanolic extract of seeds of *C. melo* was significantly less lethal for 50% of the tested worm population than levamisole, the reference medication, at all the time points (12, 18, and 24h). The calculated LC_{50} values were 1.30 ± 0.16 mg/mL, 0.50 ± 0.09 mg/mL, and 0.50 ± 0.09 mg/mL for the extract; and 0.79 ± 0.01 mg/mL, 0.33 ± 0.02 mg/mL, and 0.33 ± 0.02 mg/mL for levamisole after 12, 18, and 24h, respectively. From these data, an increase in efficiency of the extract was noticed from 12 h of incubation (Table 1).

Table 1: Lethal effect of Cucumis melo extract on *Haemonchus contortus* females

Parasitic stage		12 h	18 h	24 h
Females	Extract	1.30 ± 0.16	0.50 ± 0.09	0.50 ± 0.09
	levamisole	0.79 ± 0.01**	0.33 ± 0.02*	0.33 ± 0.02*

**= for p ≤0.01, *= for p <0.05

3.2. Inhibition of egg hatching and anti-larval activity

Hydroethanolic extract of seeds of *C. melo* demonstrated an anthelminthic activity against larval migration after 24 h incubation in coproculture. Figure 2 below shows the variation of the mortality rate as a function of the concentration of extract. The positive control (levamisole) as well as the extract showed a maximal mortality rate after 24 h at a concentration of 3 and 4 mg/mL, respectively. Both products presented a rapid increase in mortality rate at weak concentrations (≥1 mg/mL) and lower, constant, and continuous mortality for higher concentrations (above 1 mg/mL). The anthelminthic activity on L3 larvae was significantly higher for levamisole compared to the extract, with respective LC_{50} values of 1.19 ± 0.25 mg/mL and 0.86 ± 0.25 mg/mL.

In similar conditions, *in* vitro, the hatching of eggs submitted to increasing concentrations of *C. melo* hydroethanolic extract of seeds was influenced negatively. Hence, the extracts inhibited egg hatching the increasing concentrations after 48h of incubation. Figure 3 presents the variation of egg hatch inhibition according to the concentration increase of levamisole and the extract of seeds. Both products present a very close tendency and inhibit 100 % of eggs at a concentration of 4 mg/mL after 48 h. They inhibited in similar ways eggs from hatching with EHI_{50} values of 1.71 ± 0.10 mg/mL and 1.74 ± 0.25 mg/mL, respectively, for extract and levamisole after 48h.

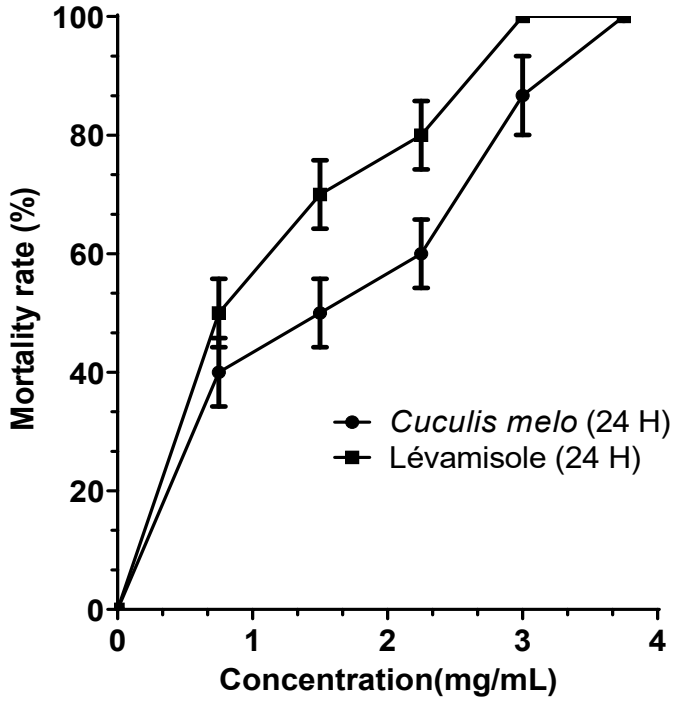

Figure 2: Activity of hydroethanolic extract of Cucumis melo seeds on the viability of Haemonchus contortus L3 larvae.*H. contortus* eggs in nature hatch into larvae in suitable conditions of temperature and moisture. Error bars represent standard deviation.

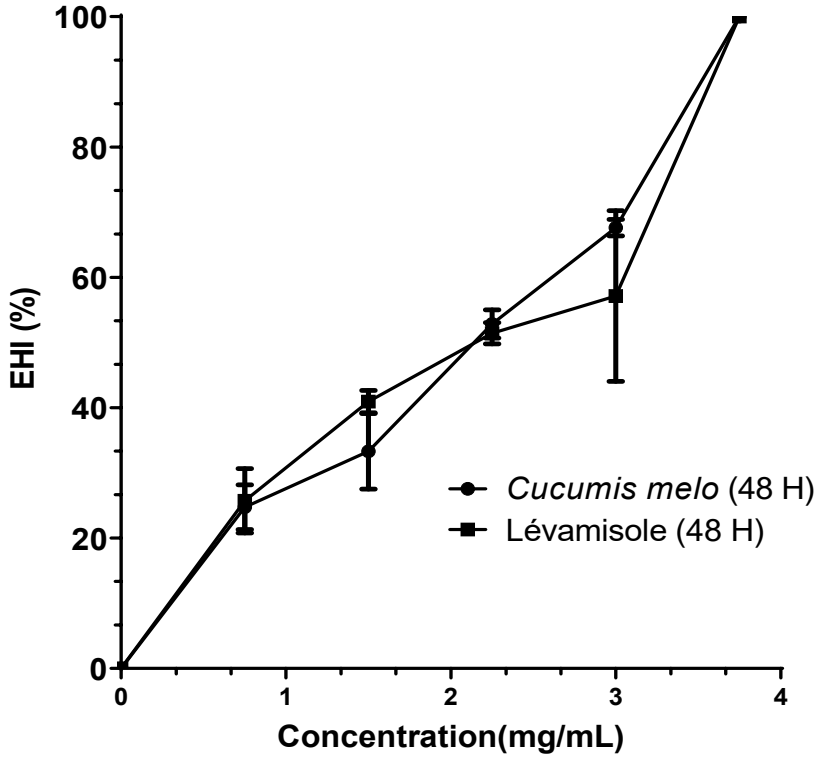

Figure 3: Activity of hydroethanolic extract of Cucumis melo seeds and levamisole on Haemonchus contortus eggs hatching (EHI: egg hatching inhibition). Error bars represent standard deviation.

3.3 Analysis of the extract content

A qualitative phytochemical analysis revealed a rich diversity of phytochemicals in the hydroethanolic extract of seeds of *C. melo*. Table 2 presents the presence and absence of the main groups of phytochemical compounds. Several compounds, such as alkaloids, phenolic compounds and tannins, flavonoids, glycosides, saponins, and triterpenes were detected. In the meantime, anthocyanins and steroids were absent from the extract.

Table 2: Qualitative phytochemical analysis of hydroethanolic Cucumis melo seeds extract

Phytochemical compounds	Presence
Alkaloids	+
Tannins and phenolic compounds	+
Flavonoids	+
Glycosides	+
Saponines	+
Anthocyanes	-
Steroids	-
Triterpenes	+

+ For present, - for absent.

3.4 Acute toxicity on mice

The orally administered extract at the dose of 2000 mg/kg did not induce behavioral modification or mortality after 14 days of observation.

4. DISCUSSION

The present study reveals the efficiency of the hydro-ethanolic extract of seeds of *Cucumis melo* on *Haemonchus contortus* adult and larval stages. The extract was shown to be significantly less active than levamisole, although lethal for females of *H. contortus*. All the assessed stages were sensitive to the extract with various susceptibilities. The observed activities could be linked to the numerous chemical compounds such as tannins and polyphenols, saponins, alkaloids, steroids, flavonoids, glycosides, anthocyanidins, etc. (Table 2) as reported by other authors (Kalmobé et al. 2017; Nwosu et al. 2022). These compounds may exist in higher proportions compared to the other and therefore justify the observed activity as reported by Menga *et al*. (2017), who evaluated the activity of *Anacardium occidentale* on *Onchocerca ochengi* and *Caenorhabditis elegans*. The resulting activities on diverse stages should arise from the individual pharmacological activity of these bioactive molecules. Tannins and polyphenols are reported to be highly active as anthelminthic compounds and are used in an integrated control of gastrointestinal nematodes of ruminants in grazing management (Hoste et al. 2022, 2006; Karonen et al. 2020; Morais-Costa et al. 2015). In addition, the other compounds, such as flavonoids and their derivatives and saponins, were reported to be active on *Schistosoma* sp, *Trypanosoma* sp, and *Leishmania* sp (Simoben et al. 2018, Hamad 2023, Wahid et al. 2023).

Anthelminthic molecules are known to act on helminths in various ways, altering their physiology the preventing them from moving or feeding. On vegetative stages, namely adults, the activity of the hydro-ethanolic extract of *C. melo* was moderate and globally less strong compared to that of levamisole. The observed weak activity against female adults could be explained by the cumulative activities of the compounds present in the studied hydro-ethanolic extract. Some of the compounds may act as an antagonist of the anthelminthic ones and lead to a resulting weak effect on the worm. Few studies evaluated the anthelminthic activity of *C. melo* (Vishwakarma et al. 2017), but several plants of the same family (Cucurbitaceae), such as *Cucurbita pepo ovifera,* showed anthelminthic activity against nematodes in mice (Beshay et al. 2019; Saleh et al. 2024), in small ruminants, and *in vitro* against filariae (Kalmobe et al. 2017) in addition to its diverse other properties (Jorge et al. 2022). Several authors revealed the same chemical compounds as presented by Vidya *et al. (2022),* demonstrating *C. melo's* potential in cancer treatment (Muhamad et al. 2021; Vidya et al. 2022). The high tannin content of *C. melo* could explain its activity on *H. contortus* as reported by previous studies, which highlighted

the tannins' activity on gastrointestinal nematodes (Quijada et al. 2015; Tibe et al. 2013). Elsewhere, the levamisole is a pure compound compared to the active principles of the extracts that may be less abundant to induce significant mortality in the worms (Nwosu et al. 2022).

The hydro-ethanolic extract of seeds of *C. melo* was active against the migration of *H. contortus* larvae in similar ways as levamisole, showing comparable IC_{50} values (1.71 ± 0.10 mg/mL and 1.74 ± 0.25 mg/mL, respectively) after 24h incubation. The inhibition of larval migration and development is a common characteristic of tannin-rich plants, which may disturb molting and development of larvae (Morais-Costa et al. 2016, 2015; Naumann et al. 2014).

Similarly, *C. melo* extract inhibited egg hatching to levamisole (Figure 3) with EHI_{50} values of 1.71 ± 0.10 mg/mL et 1.74 ± 0.25 mg/mL, respectively, after 48h (p=0.85). The extract presented excellent potential in inhibiting *H. contortus* eggs, as noticed on tannin-rich plants by some authors on the same parasite (Hoste et al. 2022; Silva Soares et al. 2018). The revealed phytochemical content was similar to the content of the methanol extract of the fruits of *Dennetia tripetala* (Nwosu *et al*. 2022). Several phytochemicals found in *C. melo,* such as flavonoids (Toklo et al. 2021), are known to be active against *H. contortus*. HPLC analysis of phytochemical content of ethanolic extract of the seeds of *C. melo* revealed about 10 different phytochemicals (Wahid et al. 2023), among which several flavonoids known for their anthelminthic properties. Rutin was shown to be active against *Schistosoma mansoni* in mice, preventing the production of eggs and therefore the induced pathology in the liver (Hamad 2023). Quercetin and Kaempferol may also induce the anthelminthic activity of *C. melo* as reported by other authors on *Caenorhabditis elegans* (Machado et al. 2015) and *H. contortus* (Pavičić et al. 2023). The synergistic activity of these flavonoids could be in origin of the anthelminthic activity of *C. melo* on *H. contortus*. Complementary and more specific information may be collected by assessing the activity of fractions of *C. melo* hydro-ethanolic extract of the seeds and subsequently performing *in vivo* studies.

The acute toxicity assays revealed an acceptable safety of the hydro-ethanolic extract of the seeds of *C. melo* at the dose of 2000 mg/kg. The hydro-ethanolic extract of the seeds showed a comparable safety in the rodent model to numerous plant extracts efficient against *H. contortus*. The observed effect could arise from the extraction with a hydro-ethanol solution that might have extracted less toxic chemical compounds (Nwosu et al. 2022).

5. CONCLUSION

This study demonstrated anthelminthic activity of the ethanolic extract of seeds of *Cucumis melo* on adult and larvae of *Haemonchus contortus* and its inhibitory effect on egg hatching. The results showed a considerable inhibition of egg hatching and a significant toxicity on L3 larvae, similar to the levamisole control. Nevertheless, this extract showed moderate activity in female adults compared to the positive control. The assessment of its acute toxicity on mice revealed an acceptable safety at the dose of 2000 mg/kg. Therefore, the seeds of *Cucumis melo* have good potential as an alternative source of natural anthelminthic against *H. contortus,* particularly against juvenile stages and eggs. These results constitute preliminary data for further fractionation to isolate the specific compounds responsible for the anthelminthic effect. In addition, these findings do not consolidate the current use of *C. melo* for its deworming properties in livestock but rather encourage its use to prevent pasture infection.

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CONFLICT OF INTEREST. The authors declare no conflict of interest.

AUTHOR CONTRIBUTION. DB mentored the study and wrote the manuscript draft, MMDG designed the study and ran the experiments, AM, AAH mentored the study, and ND provided equipment and reviewed the manuscript.

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