In vitro anthelmintic activity of *Euphorbia hirta* L. aqueous extracts on small ruminant’s gastrointestinal parasites evaluation.

Amadou DICKO (✉ dicko.amadou36@yahoo.fr)  
Institut de l'Environnement et de Recherches Agricoles  
https://orcid.org/0000-0001-7439-7053  

Almamy KONATE  
Institut de l'Environnement et de Recherches Agricoles  

Sami Eric KAM  
Université Nazi BONI  

Hadidajatou BELEM  
Université Nazi BONI  

Basile TINDANO  
Université Joseph KI-ZERBO  

Abdoul Aziz Almoustapha CISSE  
Etudiant stagiaire -IPR/IFRA de Katibougou (Mali)  

Gaoussou KEITA  
IPR/IFRA de Katibougou  

Roland Nâg-Tiéro Meda  
Université Nazi BONI  

Adama Kaboré1  
Institut de l'Environnement et de Recherches Agricoles  

Amadou Traoré  
Institut de l'Environnement et de Recherches Agricoles  

Balé BAYALA  
Université Joseph KI-ZERBO  

Hamidou Hamadou Tamboura  
Institut de l'Environnement et de Recherches Agricoles

Research Article

**Keywords:** Anthelmintic plant, Euphorbia hirta, Gastrointestinal nematodes, Haemonchus contortus, In vitro tests

**Posted Date:** October 31st, 2023
Abstract

Purpose

With the aim of proposing an effective and accessible alternative for small ruminant gastrointestinal parasitosis control, the *in vitro* anthelmintic activity of *Euphorbia hirta* on *Haemonchus contortus* biology was evaluated.

Methods

Aqueous extracts were used to determine phytochemistry and for the biological tests of adult worm mortality as well as egg hatching and L3 larval migration inhibition. Aqueous extract concentrations of 6.25, 12.5, 25, 50 and 100 mg/mL were used for the adult worm mortality test, while concentrations of 0.31, 0.62, 1.25, 2.5 and 5 mg/mL were used for the egg hatching and L3 larval migration inhibition tests.

Results

The results show a total polyphenol content of 17.5 mgEAG/100 mg, total flavonoids of 0.24 mgEQ/100 mg and condensed tannins of 0.17 mgEAT/100 mg. Adult worm mortality was concentration dependent, reaching 100% at 50 and 100 mg/mL concentrations, from the sixth hour, with a lethal concentration 50 ($LC_{50}$) of 21.09 mg/mL. The 50% inhibitory concentration of egg hatching ($IC_{50}$) was 1.7 mg/mL, while the $IC_{50}$ of larval migration recorded was 0.78 mg/mL.

Conclusion

In view of the results obtained, the *in vitro* anthelminthic activity of the aqueous extract of *E hirta* was confirmed. However, further biological studies will be needed to validate these results, with a view to their wider use.

Introduction

Gastrointestinal parasites remain one of the major constraints to small ruminant farming development. These parasitic worms affecting pasture-raised animals cause countless economic losses for livestock farmers (Charlier et al., 2014). Several gastrointestinal parasites species are known, but the most widespread and dangerous remains the species *Haemonchus contortus*. Indeed, *H. contortus* is a hematophagous parasite that adapts to several types of climate and causes severe anemia that can lead to small ruminant death (Higuera-Piedrahita et al., 2021).

Management of these parasites is usually based on the use of anthelmintic molecules. However, the massive and sometimes inappropriate use of these chemical molecules has led to the emergence of
resistant nematode strains in many livestock farms around the world (Hoste et al., 2005; Gonzalez-Cruz et al., 2018). In addition, consumer concern over the possible presence of these chemical molecule residues in food product, and the environmental risks inherent in their use are increasingly expressed (Bernier, 2019).

In the face of this problem, the search for alternative solutions to the use of chemotherapy is becoming essential to improve the productivity of small ruminants. Among the alternative solutions developed is the use of bioactive plants. Indeed, certain herbaceous plant species, such as *Euphorbia hirta*, could provide a sustainable solution for effective control that is easily accessible to livestock farmers.

*E. hirta* is an herbaceous plant commonly known in the African local language as "Daba dablé" in Bambanakan, Dioula; "Wallé-bisum" in Mooré; and "Dabbirteeki" in Fulani, belonging to the Euphorbiaceae family. Many studies have demonstrated the chemical and pharmacological properties of *E. hirta*. This herbaceous plant possesses several chemical compounds such as alkanes, terpene compounds, tannins, polyphenols, organic acids, and flavonoids (Kumar et al., 2010; Tuhin et al., 2017).

Additionally, *E hirta* is traditionally used to treat respiratory conditions and has multiple pharmacological properties, including antidiarrheal, antimicrobial, anti-inflammatory, antiviral, and antifungal properties (Singh et al., 2004). Thus, the particularity of this plant due to its chemical and medicinal properties and especially its ease of access has led us to take a particular interest in considering a better use of these properties in the treatment of small ruminant gastrointestinal parasitosis.

This study aimed to evaluate the *in vitro* anthelmintic activity of *E. hirta* aqueous extracts.

**Materials and methods**

**Plant material**

Whole plant samples of *E. hirta* were collected early in the morning in the urban communities of Dori and Ouagadougou. Plant samples were washed with water, then dried and ground into powder. The plant species were identified by a botanist, and voucher specimen number 8760 was preserved at the Herbarium of the National Center of Technological and Scientific Research (CNRST) in Burkina Faso.

**Animal material**

Adult worms, infesting L3 larvae and eggs of *H. contortus* were used as animal material for this study.

**Study area presentation.**

*E. hirta* samples were collected in the urban communes of Dori and Ouagadougou. The commune of Dori is the Séno province and the Sahel region capital in northern Burkina Faso. Ouagadougou is the chief town of Kadiogo Province in central region and the political capital of Burkina Faso.
Biological tests were carried out in the Animal Biology and Heath Laboratory of the Animal Production Department at the Center of Environmental, Agricultural and Training (CREAF) of Kamboinsin in Ouagadougou (Fig. 1).

**Methods.**

**Preparation of aqueous extraction**

One hundred grams (100 g) of *E. hirta* whole plant powder was added to 1 L of distilled water for 24 hours. The macerate was filtered three (3) times and freeze-dried (marque ALPHA 1–2 LD).

**Phytochemical studies**

**Total polyphenol content**

The method described by Meda et al. (2010) was used to determine the total polyphenol content. One hundred and twenty-five microliters (125 µL) of plant aqueous extract (at 0.1 mg/mL) was mixed with 625 µL of Folin-Ciocalteu reagent (0.2 N). After 5 min incubation in the dark, 500 µL sodium carbonate (Na2CO3, 75 g/L) was added to the mixture. The resulting solution was incubated for an additional 2 h in the dark before determination of the total polyphenol content at 760 nm against a gallic acid calibration curve (y = 4668e-3 * x-0.034, r2 = 0.9991). Each test was repeated 3 times, and the results were expressed as mg gallic acid equivalent per 100 mg extract (mg GAE/100 mg extract).

**Total flavonoid content**

Six hundred and twenty-five microliters of methanolic solution of each extract (at 0.1 mg/mL) was mixed with 625 µL of aluminum trichloride (AlCl3, 2%). After 10 min of incubation in the dark, the flavonoid content was determined at 415 nm, using a quercetin calibration curve (Y = 1.259e-2 * x; r2 = 0.9990). Each test was repeated 3 times and the results were expressed in milligram of quercetin equivalent per 100 mg of extract (mg EQ/100 mg of extract). (Meda et al., 2010).

**Condensed Tannin Content**

A sulfuric vanillin solution was prepared by dissolving 1 g of vanillin in 100 mL of sulfuric acid (70%).

Half a milliliter (0.5 mL) of plant sample diluted at 1/100 in ethanol was then mixed with 1 mL of the sulfuric vanillin solution. After 15 min of mixture incubation in the dark in 30°C water bath, the condensed tannin content of the extracts was measured at 500 nm against a tannic acid calibration curve. The tests were repeated 3 times, and the results were expressed in milligrams tannic acid equivalent per gram of extract (mg ATE/g extract). (Agbangnan et al., 2012).
Biological tests

Adult worm mortality tests

The test was performed according to the modified method of Akouedegni et al. (2019).

_H. contortus_ adult worms were collected with forceps from freshly slaughtered sheep, incised longitudinally and emptied of their contents. The harvested worms were placed in a Petri dish (80 x 15 cm) containing a physiological solution: phosphate-buffered saline (PBS 1x).

Aqueous extract concentrations were prepared by diluting 1 g of extract in 10 mL of PBS1x to obtain a concentration of 100 mg/mL. This was followed by cascade dilution to produce 4 other aqueous extract concentrations: 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL. A negative control, PBS1x, and a positive control, levamisole 2.5 mg/mL, were used.

The test consisted of contacting five perennial adult worms of both sexes with 1.5 mL of each _E. hirta_ extract concentration test in 24-well culture microplates. After the worms were placed in contact with the extract concentrations, the whole set was incubated for 20 h at 27°C. Observations were made at 1 h, 2 h, 4 h, 6 h and 20 h. The number of dead adult worms was assessed 20 hours later. When a worm remains in continuous immobility for 30 seconds, with no return of vitality 30 minutes after immersion in PBS, it is declared dead.

The mortality rate (MR%) was calculated using the following formula:

\[
\text{MR} \; (\%) = \frac{\text{DAWN}}{\text{IAWN}} \times 100
\]

**DAWN**: Adult Worm Number of Death

**IAWN**: Incubated Adult Worm Number.

Egg hatching inhibition tests.

Eggs were obtained using the modified method of Hussain et al. (2011).

The females were sorted and lightly crushed in a mortar using a porcelain pestle to obtain the eggs. The crushed eggs were filtered through sieves of decreasing mesh size (100, 50 and 38 µm) and the egg solution was readjusted to 100 eggs per mL.

Five aqueous extract concentrations were prepared. Zero point one (0.1) gram extracts were diluted in 10 mL of PBS 1x to obtain a stock solution of 10 mg/mL. Next, a cascade dilution was performed to obtain five other concentrations to be used for testing: 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.62 mg/mL, 0.31 mg/mL. A negative control, PBS1x, and a positive control, levamisole 2.5 mg/mL, were used.
The test was carried out according to the modified method of Coles et al. (1992). One milliliter of egg suspension at 100 eggs per mL was placed in contact with 1 mL of each of the test concentrations in Petri dishes (60 x 15 Cm) and incubated for 48 hours at 27°C. After 48 hours, two drops of Lugol’s solution were placed in each Petri dish to halt egg development. Next, 40 µL were placed between the slide and coverslip, and the number of L1 larvae and unhatched eggs were assessed under a light microscope (10x). The test was repeated three times, with three replicates of each concentration in each run. The Percentage inhibition of egg hatching (EHI%) was calculated using the following formula:

\[
EHI \, (%) = (1 - \frac{EHTC}{EHNC}) \times 100
\]

**EHTC**: Number of eggs hatched in the tested concentration  
**EHNC**: Number of eggs hatched in the negative control

**L3 larval migration inhibition tests.**

L3 larvae were obtained using the modified method of Olounladé et al. (2011) after culturing fresh eggs of *H. contortus* for 14 days at 31°C and then harvested using the Baermann device based on the positive hygrotopism of the larvae. The larval solution was readjusted to 1000 larvae/mL.

The test was carried out using the modified method of Hernandez-Villegas et al. (2011).

Five concentrations of aqueous extracts were made as previously described for the egg hatch inhibition test. A negative control, PBS1x, and a positive control, levamisole 2.5 mg/mL, were used.

One milliliter of larvae solution at 1000 larvae/mL was placed in contact with 4 mL of each test concentration in Petri dishes (60 x 15 Cm) for 3 hours at 27°C. After 3 hours, the larvae were rinsed by centrifugation at 2000 rpm for 10 minutes and then allowed to migrate through a 20 µm diameter membrane for 3 hours. The number of migrated larvae was assessed under a light microscope, and the percentage inhibition of larval migration (IML%) was calculated using the modified formula of Rabel et al. (1994):

\[
IML \, (%) = \left(\frac{X1 - X2}{X1}\right) \times 100
\]

**X1**: Number of larvae that migrated in the negative control  
**X2**: Number of larvae that migrated into the test concentration

**Data analysis**

Excel software 2016 was used for data entry and calculation of means, standard deviations and percentages. The data collected for each test were subjected to a one-factor analysis of variance (ANOVA) followed by multiple comparison of means using Tukey’s method at the 5% significance level using R software version 4.2.1, the Rcmdr version 2.8-0 package and the R studio version 4.2.1 interface.
Prism software version 5.00.288 was used to produce the histograms and calculate the lethal and inhibitory concentrations.

**Results**

**Phenolic compound content**

*E. hirta* aqueous extract contains a wide range of phenolic compounds. Total polyphenols (TP) are well represented in the aqueous extracts, while total flavonoids (TF) and condensed tannins (CT) are present at slightly lower levels (Table 1).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total Polyphenols (mgEAG/100 mg)</th>
<th>Total Flavonoids (mgEQ/100 mg)</th>
<th>Condensed Tannins (mgEAT/100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>17.5 ± 0.49</td>
<td>0.24 ± 0.0</td>
<td>0.17 ± 0.01</td>
</tr>
</tbody>
</table>

**Adult worm mortality assay**

A high and significant mortality (p < 0.05) compared to the negative control was obtained with the aqueous extracts. A mortality rate of 100% was noted at a concentration of 50 mg/mL from the sixth hour (Table 2).

<table>
<thead>
<tr>
<th>Dose</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>PBS</td>
<td>0&lt;sup&gt;a&lt;/sup&gt; ± 0</td>
</tr>
<tr>
<td>6.25 mg/mL</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; ± 0</td>
</tr>
<tr>
<td>12.5 mg/mL</td>
<td>0&lt;sup&gt;ab&lt;/sup&gt; ± 0</td>
</tr>
<tr>
<td>25 mg/mL</td>
<td>0&lt;sup&gt;b&lt;/sup&gt; ± 0</td>
</tr>
<tr>
<td>50 mg/mL</td>
<td>0&lt;sup&gt;ab&lt;/sup&gt; ± 0</td>
</tr>
<tr>
<td>100 mg/mL</td>
<td>48.88&lt;sup&gt;d&lt;/sup&gt; ± 0</td>
</tr>
<tr>
<td>Levamisole (2.5 mg/mL)</td>
<td>20&lt;sup&gt;c&lt;/sup&gt; ± 0</td>
</tr>
</tbody>
</table>

p value 0.00212
Egg Hatching Inhibition Assay

Aqueous extracts of *E. hirta* produced a high and significant (p < 0.05) egg hatching inhibition compared with negative controls. A hatching inhibition rate of 76.67% at 5 mg/mL was recorded (Table 3).

<table>
<thead>
<tr>
<th>Dose</th>
<th>Egg hatch inhibition rate (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
<td>R2</td>
<td>R3</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>0a ± 0</td>
<td>0a ± 0</td>
<td>0a ± 0</td>
<td>0a ± 0</td>
<td></td>
</tr>
<tr>
<td>0.31 mg/mL</td>
<td>4b ± 1</td>
<td>3b ± 1</td>
<td>3b ± 1</td>
<td>3,33b ± 0,58</td>
<td></td>
</tr>
<tr>
<td>0.62 mg/mL</td>
<td>30c ± 6,66</td>
<td>10c ± 3,29</td>
<td>20c ± 5</td>
<td>20c ± 4</td>
<td></td>
</tr>
<tr>
<td>1.25 mg/mL</td>
<td>50d ± 10</td>
<td>50d ± 14,06</td>
<td>30d ± 8,03</td>
<td>43,33d ± 4,54</td>
<td></td>
</tr>
<tr>
<td>2.5 mg/mL</td>
<td>70e ± 4,81</td>
<td>60e ± 12,05</td>
<td>50e ± 6,67</td>
<td>60e ± 10</td>
<td></td>
</tr>
<tr>
<td>5 mg/mL</td>
<td>80f ± 17,34</td>
<td>80f ± 19,25</td>
<td>70f ± 12,79</td>
<td>76,67f ± 5,77</td>
<td></td>
</tr>
<tr>
<td>Levamisole 2.5 mg/mL</td>
<td>80f ± 19,24</td>
<td>80f ± 19,25</td>
<td>70f ± 4,81</td>
<td>76,67f ± 5,77</td>
<td></td>
</tr>
</tbody>
</table>

*p value = 0.003411*

Inhibition of L3 larval migration assay.

A high and significant (p < 0.05) L3 larval migration inhibition was obtained. *E. hirta* aqueous extract showed concentration dependent migration inhibition that reached 94% at a concentration of 5 mg/mL (Fig. 2).

Discussion

Parasitic diseases caused by gastrointestinal nematodes severely limit sheep production. The emergence of parasite strains resistant to the synthetic anthelmintics usually used to treat these parasites means that we need to find alternative, sustainable solutions to improve the productivity of sheep farms.

Quantification of the secondary metabolites responsible for the medicinal plant properties in aqueous extracts of *E. hirta* shows a wide range of levels of total polyphenols, total flavonoids and condensed tannins. In contrast to our present study, numerous studies have qualitatively shown the presence of these secondary metabolites within different plant extracts (Wadré et al., 2015; Hougnimassoun et al., 2017). However, Azando et al. (2022) obtained a slightly higher condensed tannin content in *Newbouldia laewis* and *Zanthoxylum zanthoxyloides* extracts compared to our present study. This difference could be
explained by the nature of the plant, our study plant being an herbaceous plant, and by the packaging and assay methods used.

The vermicidal activity of *E. hirta* aqueous extracts was high and significant compared with PBS taken as a negative control. Dose-dependent vermicidal activity was observed, with total mortality of *H. contortus* adult worms at the 6th hour, starting at a concentration of 50 mg/mL. In contrast to our present study, *Hedera helix* aqueous extracts obtained a lower mortality rate of *H. contortus* adult worms (Eguale et al., 2007). The difference in results found may be due to the content of secondary metabolites in the plants, as well as the nature of the plants and the packaging used. However, aqueous extracts of *Artemisia absinthium* obtained a high mortality rate of *Haemonchus contortus* adult worms at the 8th hour after exposure to 25 mg/mL concentration, similar to our present study (Tariq et al., 2009). The similarity of the results obtained is inherent in the anthelmintic activity. The presence of secondary metabolites in *E. hirta* aqueous extracts would explain its vermicidal activity. Indeed, Githiori et al. (2006) report that the presence of secondary metabolites such as saponins, alkaloids, condensed tannins and polyphenols are responsible for the plants’ anthelmintic properties.

The ovicidal activity of *E. hirta* aqueous extracts was high compared with PBS taken as a negative control. Costa et al., (2008) obtained similar results to our present study, with high inhibition of *H. contortus* egg hatching using extracts from *Azadirachta indica* leaves in Brazil. Different *Phytolacca icosandra* extracts produced high *H. contortus* egg hatching inhibition in a dose-dependent manner identical to the results of our present study (Hernández-Villegas et al., 2011). Similarly, the *Melia azedarach* aqueous and hydroalcoholic leaf extracts significantly inhibited the hatching of *H. contortus* eggs, corroborating the results of our study (Kamaraj et al., 2010). The ovicidal activity similarity observed is due to the presence of secondary metabolites in the different extracts tested, as emphasized by many authors (Bizimenyera et al., 2006).

The L3 larval migration inhibition by *E. hirta* aqueous extracts was high and significant compared with that of the negative control. This result shows that *E. hirta* aqueous extracts could prevent or reduce the settlement of infective larvae in the mucosal wall of the digestive tract. Our results are identical to those obtained using *Phytolacca icosandra* extracts with high inhibition of *H. contortus* L3 larvae migration (Hernández-Villegas et al., 2011). Additionally, the hydroalcoholic grape pomace fractions resulted in total *H. contortus* L3 larval migration inhibition (Soares et al., 2018). The different extracts used all possess secondary metabolites that would be at the origin of the anthelmintic activity of plants, which would explain the similarity of the results obtained.

The anthelmintic activity of *E. hirta* aqueous extracts is related to the presence of total polyphenols, total flavonoids and condensed tannins, as emphasized by some authors (Azando et al., 2022). These secondary metabolites act specifically on egg cell membranes and larvae cuticle collagen proteins to modify membrane permeability and reduce membrane cholesterol levels. These modifications will allow the passage of these metabolites inside eggs and larvae, inhibiting blastomere segmentation in the case of eggs damaging the cuticle and digestive system of larvae or binding to glycoproteins on the nematode
cuticle, leading to worm death (Williams et al., 2014; Teufact et al., 2017; Ondua et al., 2021). Further studies will enable us to validate the results obtained in vitro and confirm the E. hirta anthelmintic activity.

**Conclusion**

The present study demonstrates the in vitro anthelmintic efficacy of E. hirta aqueous extracts. Phytochemical assays indicate the presence, at variable levels, of the secondary metabolites responsible for the plants' anthelmintic activity in the aqueous extracts tested. Biological tests show high adult worm mortality, as well as strong inhibition of egg hatching and L3 larval migration of H. contortus. These results confirm some of the data in the literature attributing anthelmintic activity to E. hirta, justifying its use in traditional veterinary medicine.

These results are promising, but it would be essential to carry out additional biological tests, in particular safety tests, as well as in vivo station tests, to validate the results obtained in this study.

**Declarations**

Competing interests: The authors declare no competing interests.

**Authors' Contributions**

**Amadou Dicko, Almamy Konaté, Sami Eric Kam, Hadidjatou Belem, Basile Tindano; Abdoul Aziz Almoustapha Cissé**: Have contributed to the realization of the biological tests in laboratory. Collected and analyzed data. Writing of the article.

**Gaoussou Keïta; Roland Nâg-Tiéro Meda; Adama Kaboré, Amadou Traoré, Balé Bayala, Hamidou Hamadou Tamboura**: Have contributed to the realization of the biological tests by validating the protocols. Followed the work in the laboratory. Amendment and validation of the article.

All authors reviewed the manuscript.

**Funding**

No funding was received for conducting this study

**Conflict of interest declaration**

No funding was received for conducting this study.

**Ethical standards**

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on the care and use of laboratory animals.
References


**Figures**

**Figure 1**

**Study area.**
Figure 2

Effects of *E. hirta* aqueous extracts on the L3 larvae migration of *H. contortus*.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.docx