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Full Length Research Paper

In vitro comparative effect of extracts of the seeds of *Embelia rowlandii* (Myrsinaceae) on the eggs and L_1 and L_2 larval stages of the parasitic nematode *Heligmosomoides bakeri* (Nematoda; Heligmosomatidae)

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***In vitro* ovicidal and larvicidal activities of extracts of the seeds of *Embelia rowlandii* were investigated on unembryonated and embryonated eggs, first and second stage larvae of *Heligmosomoides bakeri*. Three different extracts were prepared with distilled water (DW) and 95% ethanol. The organic extract was first diluted with 3% Tween and then distilled water added to obtain five different concentrations (625, 1250, 2500, 3750 and 5000 µg/ml). These concentrations were put in contact with eggs and larvae of *H. bakeri*. One milliliter of each extract at different concentrations and controls were added to 1 ml solution containing 30 to 40 eggs or 10 to 15 larvae (L_1 and L_2 larvae) and distributed in different Petri dishes. Both eggs and larvae were incubated at 24°C and exposure time was 48 h for fresh (unembryonated) eggs, 6 h for embryonated eggs, 24 h for L_1 and L_2 larvae, respectively. Distilled water and 3% Tween were used as negative controls in the bioassay. These placebos neither affected egg development, hatching nor larval survival. A significant effect was obtained with cold water extract (CWE), hot water extract (HWE) and ethanolic extract (EtE) and differences were observed depending on the parasitic stage. CWE, HWE and EtE inhibited embryonic development (60.5, 82.5 and 46.9%) and hatching (85.8, 74.1 and 41.0%), respectively at 5000 µg/ml. All the three extracts killed both L_1 larvae (84.5, 86.0 and 61.2%) and L_2 larvae (65.2, 83.7 and 33.3%) respectively at 5000 µg/ml. These *in vitro* results suggest that extracts of *E. rowlandii* possess ovicidal and larvicidal properties.**

Key words: *Embelia rowlandii*, *Heligmosomoides bakeri*, plant extracts.

INTRODUCTION

Parasitic infection is one of the world's most devastating causes of death in livestock and human today. Intestinal helminthiasis is an important issue of public health in the tropical and subtropical zones. These infections have recently been neglected in favor of human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS), malaria and tuberculosis. Small ruminant livestock (sheep and goats) improves the life condition of populations in the rural farming environment where poverty is a great concern. In the traditional livestock system, animals are infected by diseases among which gastrointestinal nematode parasitism is highly prevalent and creates a huge

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economic loss. This loss results from the mortality of young animals and the decrease in production (Alawa et al., 2003; Githiori et al., 2003a, b; Krecek and Waller, 2006). In developed countries, gastrointestinal parasites are controlled by using commercial anthelmintics whereas in the developing countries, traditional methods of helminth control largely remain dependent on medicinal plants. The misuse and poor formulation of synthetic drugs for many decades have led to the development of anthelmintic resistant (AR) worm strains or population (Waller 1997; Lans and Brown, 1998; Law et al., 2003). Also, the use of disinfectants to control free living stages of the parasites is harmful to non target organisms and to the environment (Dunn and Greiner, 1997). However, new approaches to nematode parasite control are needed to counteract the problem of high cost and non availability of these drugs and anthelmintic resistant worm strains (Waller, 1997, 1999; Wolstenholm et al., 2004). This work aims at evaluating extracts of *Embelia rowlandii* known as *Embelia ribes burm* in India, reported to have anthelmintic activity, antihyperglycaemia and cures flatulence and colic disorders (Bhandari et al, 2002). It is used traditionally by the people of Aguambu in the Lebialem highlands, Southwest Region, Cameroon (Central Africa) as natural medicine for treating worms. This traditional treatment is based on eating the seeds each time one suffers from stomach ache. This plant commonly called Nphenyate by Aguambu and Bamumbu people has been classified as anthelmintic in Cameroon (Focho et al., 2009). *Heligmosomoides bakeri* (Syn. *Nematospiroides dubius* and *Heligmosomoides polygyrus,* Behnke and Harris, 2010) used in this experimental study is a useful organism and has been used for testing anthelmintics (Smyth, 1996). Studies carried out in this domain include those of Alawa et al. (2003), Wabo et al. (2005, 2006, 2010, 2011), Maciel et al. (2006) and Magdeleine-Marie et al. (2009), who assessed the *in vitro* anthelmintic activities of extracts of *Vernonia amygdalina* and *Annona senegalensis* in Nigeria, *Canthium mannii* in Cameroon, *Melia azedarach* in Brazil and *Cucurbita moschata* in Guadelope, respectively.

MATERIALS AND METHODS

E. rowlandii (Brown, 1810) is a climbing shrub and belongs to the family Myrsinaceae. Species identification was carried out at the National Herbarium of Cameroon (NHC) in Yaoundé. Seeds of *E. rowlandii* collected in "*Mberenka forest*" (Wabane Subdivision, Lebialem Division, Southwest Region of Cameroon) were spread on polyethylene bags for four hours every day for one week, ground using a grinding machine. The powder was sieved and preserved in air tight plastic bags at room temperature and relative humidity (RH) of about 67% for subsequent use.

Preparation of extracts

Three types of extracts were prepared to compare their effects and to increase the chances of detecting ovicidal and larvicidal activities. These extracts were cold water extract (CWE), hot water extract (HWE) and ethanolic extract (EtE) (Wabo et al., 2011a).

Ethanolic extracts

One hundred grams of seed powder of *E. rowlandii* were macerated in 1.5 L of 95% ethanol. The mixture was stirred daily at the same time to permit a better extraction of the active ingredients, and 72 h later, the ethanolic extract was obtained (Cuilei, 1982). This was followed by the dilution of 0.2 g of the concentrated extract (using a rotary evaporator) first with 0.2 ml of 3% tween-80, a diluent which helped to dissolve organic extracts and after 3 to 8 min, distilled water (DW) was added to obtain a volume of 20 ml. From this stock solution a series of dilutions were made to obtain five different concentrations (625, 1250, 2500, 3750 and 5000 µg/ml).

Cold and hot water extracts

One hundred grams of seed powder of *E. rowlandii* were macerated in 1.5 L of cold DW. The mixture was stirred daily and 48 h later the solution was filtered using a tea sieve and a filter paper. 200 ml of filtrate were distributed in four beakers, which were placed inside an oven heated at 50°C. After one week, a dry CWE was obtained. From this, 0.2 g was diluted with 20 ml of distilled water to obtain a stock solution from where a series of dilutions were done and five different concentrations were obtained as those of the organic extract.

For the HWE, a similar procedure to that of CWE was applied except for the fact that the DW was heated at 100°C and 100 g of the seed powder poured into the hot distilled water. The mixture was allowed to stand at room temperature for 3 h to allow the solution to cool.

Recovery of eggs and larvae

Freshly passed out feces of experimentally infected laboratory white mice (*Mus musculus)* were collected using a tea spoon. One gram of feces was homogenized in a mortar using a pestle with 60 ml of saturated NaCl solution. This mixture was successively filtered using a tea sieve and a 150 µm sieve. The filtrate was transferred into two test tubes and filled until the formation of an upper meniscus. A cover slip was used to cover each of the tubes and allowed to stand for 3 min. This was to enable the eggs of the parasite to move upwards, float and attach to the cover slip. The latter was removed and placed on a slide for observation under a microscope (40 X magnification) to confirm the presence of *H. bakeri* eggs. The cover slips and slide were rinsed into a beaker using sterile distilled water and the solution allowed to stand for 2 h to enable sedimentation of the eggs. Then the supernatant was siphoned using a 10 ml syringe and distilled water was added. This preparation was also allowed to stand for another 1 h. These manipulations were aimed at removing the salt solution.

The eggs obtained from above were allowed to stand at room temperature for three, four and five days, respectively with the aim of obtaining L_1 and L_2 larvae. These larvae were differentiated based on their morphological features and motility.

Evaluation of ovicidal and larvicidal properties

The ovicidal efficacy test of the different extracts was performed using two different procedures. To assess the effects on fresh eggs, 1 ml of suspension containing 30 to 40 eggs was distributed in each of the 18 Petri dishes (35x10 mm) and mixed with the same volume

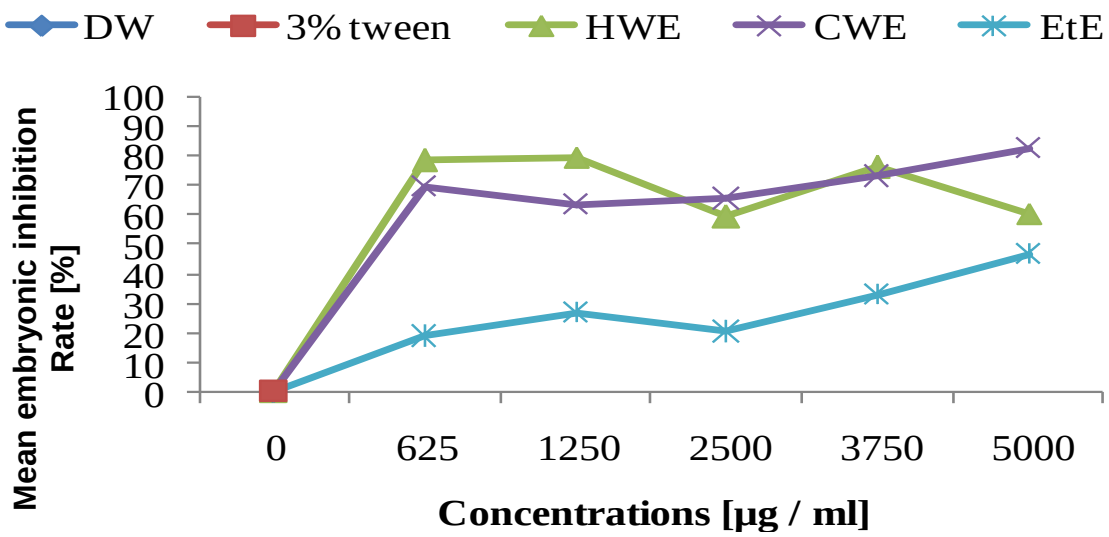

Figure 1. Effects of different concentrations (µg/ml) of cold water, hot water and ethanolic extracts of *Embelia rowlandii* on fresh eggs of *Heligmosomoides bakeri* after 48 h contact. CWE, Cold water extract; HWE, hot water extract; EtE, ethanolic extract; DW, distilled water.

of a specific concentration of given extract, giving the following final tested concentration 625, 1250, 2500, 3750 and 5000 µg/ml. The dishes were covered and the eggs incubated at room temperature for 24 h after which the number of embryonated eggs per Petri dish was counted using a microscope (at 4 X magnification).

To assess the effect of both extracts on embryonated eggs, the same numbers of fresh eggs were distributed in the same number of Petri dishes and allowed were allowed to stand at room temperature for about 24 h. When the L_1 larvae became visible and started moving actively within the eggshell (>90% in the control Petri dish), 1 ml of each concentration of extract was added to each Petri dish.

The dishes were then covered and incubated for a further 6 h at room temperature to allow hatching in the Petri dishes. When the eclodibility rates (E%) in distilled water and 3% dimethyl sulfoxide (DMSO) controls were higher than 90%, two to three drops of Lugol's iodine (5%) were added to each Petri dish to stop egg hatching (Pessoa et al., 2002). Then all the embryonated eggs and first stage larvae were counted (L_1) using a microscope (at 4 X magnification) and the percentage of eclodibility was calculated (Wabo et al., 2010, 2011a, b, c). All tests were repeated four times each including the control.

Data analysis

The 50% inhibitory concentration (IC_{50}) and the 50% larvicidal concentration (LC_{50}) were determined from the linear regression curve. Comparisons of different mortality rates were made using the Chi-square test and the results regarded as significant at $P< 0.05$.

RESULTS

Egg hatch assay

The effects of CWE, HWE and EtE extracts on the fresh eggs of *H. bakeri* are given in Figure 1. The mean inhibition rates were 0% for both distilled water and 3% Tween (negative controls). We observed that the CWE and HWE remained the most effective, irrespective of the concentration compared to the controls which exhibited no inhibitory effect on the fresh eggs, while the EtE remained the least effective. Both HWE and CWE showed significant ($P< 0.05$) concentration dependent effects, in other words, their effects increased as concentration increased from 3750 to 5000 µg/ml. Mean embryonic inhibition rates stood at 73.1 ± 0.85% and 82.5 ±1.18 % for CWE, 76.1 ± 1.09%, 60.5 ± 0.53% for HWE and from 32.9 ± 1.50 to 46.9 ±1.11% for EtE at 3750 and 5000 µg/ml, respectively (Figure 1).

At the end of this manipulation, unhatched eggs were considered dead. The transformation of mortality rates into probits and concentrations into Log10 gave a linear relation. From the linear equation the IC_{50} was calculated and were as follows: 29077.9, 314.5 and 14336.2 µg/ml for HWE, CWE and EtE, respectively.

The mean inhibition rates of embryonated eggs increased from 73.0 ± 0.76 to 85.8 ± 0.65% for CWE, 83.0 ±1.44 to 74.1 ± 1.13% for HWE and from 12.2 ± 0.19 to 41.0 ±1.15% for EtE, respectively as the concentrations increased from 3750 to 5000 µg/ml (Figure 2). Two slopes of the straight lines of the three different extracts were positive and one negative. Their inhibitory concentrations are as follows: 1469.1, 661.9 and 54750.8 µg/ml for HWE, CWE and EtE, respectively.

Larval development

All the three extracts showed a significant increase ($P<0.05$) in mortalities with increase in concentration. The mean L_1 larval mortality rates increased from 58.6 ± 2.31

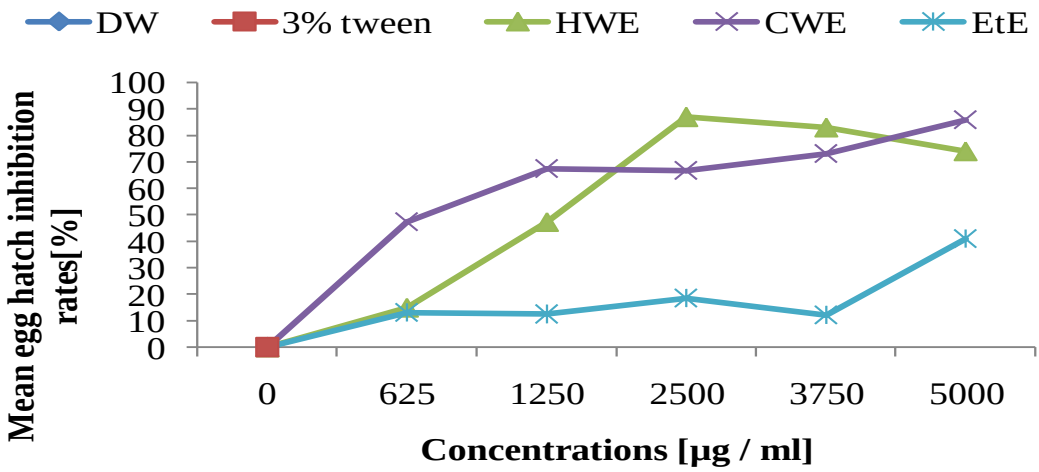

Figure 2. Effects of concentrations (µg/ml) of cold water, hot water and ethanolic extracts of *Embelia rowlandii* on embryonated eggs of *Heligmosomoides bakeri* after 6 h contact. CWE, Cold water extract; HWE, hot water extract; EtE, ethanolic extract; DW, distilled water.

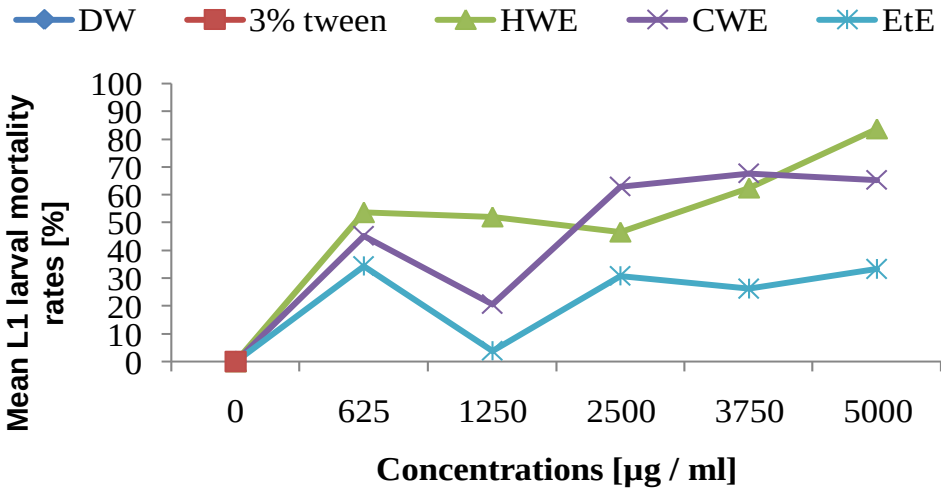

Figure 3. Effects of different concentrations (µg/ml) of cold water, hot water and ethanolic extracts of *Embelia rowlandii* on the L_1 larvae of *Heligmosomoides bakeri* after 24 h contact. CWE, Cold water extract; HWE, hot water extract; EtE, ethanolic extract; DW, distilled water.

to 86.0 ± 2.50% for HWE; 62.4 ± 0.59 to 84.5 ± 3.40% for CWE and 36.2 ± 1.62 to 61.2 ± 2.20% for EtE, respectively, as concentration increased from 3750 to 5000 µg/ml (Figure 3). Their LC_{50} were; 1974.9, 1593.6 and 3669.9 for HWE; CWE and EtE, respectively.

The effects of different extracts of *E. rowlandii* are shown in Figure 4. The larvicidal activity of HWE and CWE on L_2 larvae were effective as concentrations increased from 3750 to 5000 µg/ml. The values moved from 62.4 ± 0.66 to 83.7 ± 0.97% for HWE; 67.6 ± 1.86 to 65.2 ± 1.55% for CWE and 26.2 ± 0.79 to 33.3 ± 0.31% for EtE, respectively. HWE remained the most effective extract showing an increasing trend as concentration increased. This extract induced a significant increase ($P<0.05$) of L_2 mortality rate. All the regression coefficients of the three straight line equations are positive and their calculated LC_{50} values were as follow: 889.0, 1871.4 and 10596.4 µg/ml for HWE, CWE and EtE, respectively.

DISCUSSION

The mean embryonic inhibition rates on fresh eggs of *H. bakeri* obtained in the controls are 0.0%. These values indicated that they had no inhibitory effect on the fresh eggs of *H. bakeri*. These results are similar to those obtained by Wabo et al. (2006) on the fresh eggs of

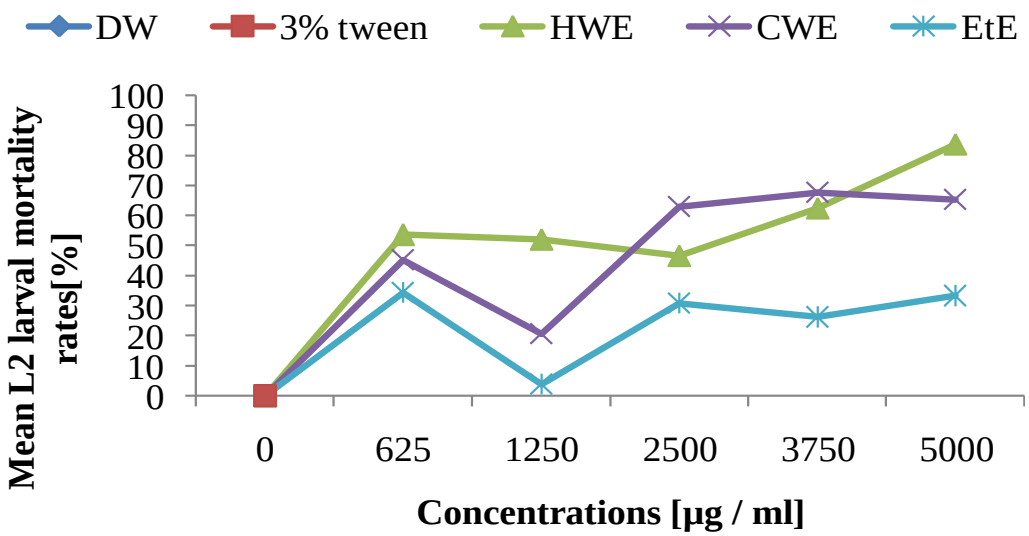

Figure 4. Effects of different concentrations (µg/ml) of cold water, hot water and ethanolic extracts of *Embelia rowlandii* on the L_2 larvae of *Heligmosomoides bakeri* after 24 h contact. CWE, Cold water extract; HWE, hot water extract; EtE, ethanolic extract; DW, distilled water.

Ancylostoma caninum (1.2%) and Wabo et al. (2011d) on the fresh eggs of *H. bakeri* (3.1%). The variation in mean embryonic inhibition rates observed inside the Petri dishes treated with the different plant extracts could be due to their ovicidal properties. The values obtained from embryonation tests with the various concentrations of different extracts were significantly different ($P< 0.05$) from the negative controls. Generally, extracts of this plant presented an important inhibitory effect on the embryonic development of fresh eggs of *H. bakeri*. Similar findings were shown by Wabo et al. (2010) with extracts of *C. mannii* on the fresh eggs of *H. bakeri*. The extracts of this plant had concentration dependent effects with concentrations greater than or equal to 3750 µg/ml. This observation was similar to the findings of Assisa et al. (2003) on the ovicidal effects of *Spigellia anthelmia* on *Haemonchus contortus* eggs. In this study, the three plant extracts inhibited embryonation of fresh eggs. This activity was more effective with the CWE (IC_{50} = 314.5 µg/ml).

These activities may be due to the fact that active ingredients such as saponin, tannin, glycosides, polyphenols, flavonoides and terpenoides present in the different extracts penetrate the egg shells and stopped the segmentation of blastomeres (Alvarez et al., 2001; Wabo et al., 2011a). The three extracts of the plant were dose dependent with concentrations greater than or equal to 3750 µg/ml. A similar finding was obtained by Wabo et al. (2011c) with extracts of *Ageratum conyzoides* on embryonated eggs of *H. bakeri*. The same finding was shown by Eguale et al. (2011) with extracts of *A. schimperiana* on *H. contortus* eggs. The CWE, HWE and EtE extracts of *E. rowlandii* inhibited the egg hatching mechanism with the highest effects exhibited by the CWE (661.9 µg/ml). This value was similar to the results obtained by Jabbar et al. (2007) in one hand, during their *in vitro* study with the aqueous and methanolic plant extracts of *Chenopodium album* and *Caesalpinia crista* and on the other hand by Adama et al. (2009) during their *in vitro* study with the aqueous extracts of *Anogeissus leiocarpus* and *Daniellia oliveri* on the eggs of *H. contortus*. The egg hatching could have been inhibited by saponins in the extracts, as these molecules are known to stop nematodes from egg hatching (Camurca-Vasconcelos et al., 2007; Eguale et al., 2011). The fact that the CWE was more effective had earlier been highlighted by Marie-Magdeleine et al. (2009) with the aqueous extracts of the seeds of *Curcuibita moschata* on embryonated eggs of *H. contortus*. The fact that the larvae remained inside the embryonated eggs treated with various extracts may be justified as follows: i) either the active ingredients of the extract crossed the egg shell through to the cuticle of the larvae into its circulatory system and killed the larvae or ii) the larvae during the normal process of the hatching mechanism, absorbed water from the environment which contained the test product remained in the eggs and died or could die by the effect of the active substances. The different solvents used for each plant extract might have played a role in the difference in activity observed. In fact, both water and alcohol are polar solvents which allow the extraction of different polar compounds found in plants (Ciulei, 1982). Aqueous extracts contain heterosides and iridoids which are water hydro-soluble compounds (Balansard et al., 1991) where as ethanolic extracts in addition to hydro-soluble compounds, also have lipid substances, alkaloids and polyphenols, terpenoids and tannins (Ciulei, 1982).

The controls (distilled water and 3% Tween) did not kill the rhabditoid and non-sheathed larvae. This confirms the fact that, the dead larvae found inside the treated Petri dishes were due to active ingredients of the plant extracts, thus indicating their larvicidal properties.

The results obtained showed high larval mortality with concentrations greater than or equal to 3750 µg/ml. Also there was an increase in the mean larval mortality rates with increase in concentration. These observations are similar to the findings of Soetan et al. (2011) during their trial on the anthelmintic effects of aqueous extracts of the seeds and leaves of the African locust bean (*Parkia biglobosa*) on bovine nematode eggs. Throughout this study, we observed that L_1 larvae were more vulnerable to the different plant extracts than L_2 larvae. This finding was contrary to earlier reports by Soulsby (1982). The larvicidal properties of these extracts may be due to the penetration of active compounds across the cuticle of the larvae on one hand or the absorption of the substance by the larvae through the mouth on the other hand. Enriquez (1993) mentioned that active compounds penetrate the cuticle of nematodes and prevent the absorption of glucose or block the post-synaptic receptors, thus, paralyzing the larvae. These active compounds can also stimulate the secretion of glutamate and gamma amino-butyric acid (GABA) which may block the transmission of nervous impulses or decoupling the phosphorylation oxidative reactions, which led to energy exhaustion of the larvae thus leading to death (Wabo et al., 2011a). Adama et al. (2009) mentioned that active compounds found with food can cross the intestinal lining of larvae and gain access to the circulatory system of the organism. Also, active compounds like tannin may bind to the cuticle of the nematode, destabilize the membrane and increase cell permeability by combining with membrane-associated sterols (Price et al., 1987; Gee and Johnson, 1988) which lead to death. Extracts of this plant possessed larvicidal properties with the most effective LC_{50} values (1593.6 and 889.0 µg/ml) obtained with CWE for L_1 larvae and HWE for L_2 larvae, respectively.

Ovicidal and larvicidal properties of our plant extracts may be due to the presence of secondary metabolites like (alkaloids, saponin, polyphenols, carotenoids, tannin, coumarine, cardenolides, triterpenes, saponoside, embeline, sesquiterpenlactones) which enter the phytochemical composition of extracts of *E*. rowlandii (Stahl and Anderberg, 2004). Other researchers including Hounzangbe-Adote et al. (2004), Anja et al. (2005), Monglo et al. (2006), Athanasiadou et al. (2007), Vagionas et al. (2007), Ajayi et al. (2008), Adama et al. (2009), Kabore et al. (2009), Marie-Magdeleine et al. (2009), Katiki et al. (2011), Eguale et al. (2011) and Wabo et al. (2011a, b, c) have shown that carotenoids, triterpenes, saponin steroids coumarines, tannins and other chemical compounds of plants like glycosides, enzymes, anthraquinones, essential oils, lipid, protein, fibers if present in a plant implies that plant has anthelmintic properties.

From the above results, we concluded that, aqueous and ethanolic extracts of *E. rowlandii* proved to have ovicidal and larvicidal properties. They were able to inhibit embryonic development and egg hatching as well as larval survival of *H. bakeri.* Further experiments incorporating *in vivo* studies are required to find out and establish the effectiveness and pharmacological rationale for the use of *E. rowlandii* extracts as anthelmintic drugs. Furthermore, toxicological studies should also be carried out on this plant.

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REFERENCES

Adama K, Belem AMG, Tamboura HH, Traore A, Sawadogo L (2009). *In vitro* antelmintic effect of two medicinal plants (*Anogeissus leiocarpus* and *Daniellia oliveri*) on *Haemonchus contortus*, an abomasal nematode of sheep in Burkina Faso. Afr. J. Biotechnol. 8(18):4690-4695.

Ajayi IA, Ademola IO, Okotie SV (2008). *In vitro* larvicidal effects of *Aframomum danielli* seed extracts against gastrointestinal nematodes of sheep. Afr. J. Tadit. Complement. Altern. Med. 5(3):244-246.

Alvarez LI, Mottier ML, Sanchez SF, Lanusse CE (2001). *Ex vivo* diffusion of albendazole and its sulfoxide metabolite into *Ascaris suum* and *Fasciola hepatica*. Parasitol. Res. 87:929-934.

Annonyme (2006). *Heligmosomoides polygyrus* once known as *Nematospiroides dubius*. http:// Poly.blog.Spot.com//. Consulté le 30 Septembre 2011.

Assisa LM, Bevilaqua CML, Morais SM, Vieira LS, Costa CTC, Souza JAL (2003). Ovicidal and larvicidal activity *in vitro* of *Spigelia anthelmia* Linn. extracts on *Haemonchus contortus*. Vet. Parasitol. 117:43-49.

Athanasiadou S, Githiori J, Kyriazakis I (2007). Medicinal plants for helminth parasite control: Fact and fiction. The animal consortium 1(9):1392-1400.

Behnke J, Harris P (2009) Heligmosomoides *bakeri* or *Heligmosomoides polygyrus?* Am. Soc. Trop. Med. Hyg. 80(4):684-685.

Ciulei I (1982). Methodology for the analysis of vegetable drugs. Practical manual on the industrial utilization of medicinal and aromatic plants. Bucarest, Romania, p. 67.

Dunn AR, Greiner C (1997). Managing Hookworms in the Landscape. Institute of Food and Agricultural Science (IFAS), University of Florida.

Eguale T, Dereje T, Mirutse G (2011). *In vitro* anthelmintic activity of crude extracts of five medicinal plants against egg-hatching and larval development of *Haemonchus contortus*. J Ethnopharm. In press.

Enriquez FJ, Scarpino V, Cypen RH, Wasson DL (1988). *In vitro* and *in vivo* egg production by *Nematospiroides dubuls* during primary and challenge infection in resistant and susceptible strain of mice. J. Parasitol. 74(2):262-266.

Focho DA, Ndam WT, Fonge BA (2009). Medicinal plants of Aguambu-Bamumbu in the Lebialem highlands , Southwest Region of

Cameroon. Afr. J. Pharm. Pharmocol. 3(1):001-013.
Gee JM, Johnson IT (1988). Interaction between haemolytic saponins, bile salt and small intestinal mucosa in rates. J. Nutr.118:1391-1397.
Githiori JB (2004). Evaluation of anthelmintic properties of ethnoveterinary plants preparation used as livestock dewormers by pastoralist and small holder farmer in Kenya. Doctoral dissertation, Department of Biomedical Sciences and Veterinary. Public Health, SLU. Acta Universitatis Agricultural Suliae, p. 76.
Githiori JB, Hoglund J, Waller PJ, Baker RL (2003a). The anthelmintic efficacy of the plant, *Albizia anthelmintica,* against the nematode parasites *Haemonchus contortus* of sheep and *Heligmosomoides bakeri* of mice. Vet. Parasitol. 166(1):23-34.
Githiori JB, Hoglund J, Waller PJ, Baker RL (2003b). Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and small holder farmer in Kenya against *Heligmosomoides bakeri* infections in mice. Vet. Parasitol. 118(3/4):215-226.
Hounzangbe-Adote MS, Paolini V, Fouraste I, Mountairou K, Hoste H (2004). *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus.* Res. Vet. Sci. 78(2):155-160.
Jabbar A, Iqbal Z, Nisar KM (2006). *In vtro* anthelmintic activityof *Trachyspermum ammi* seeds. Pharmacon. Mag. 6:126-129.
Jabbar A, Zaman MA, Iqbal Z, Yaseen M, Asim S (2007). Anthelmintic activity of *Chenopodium album* (L) and *Caesalpinia crista* (L) against trichostrongylid nematodes of sheep. J. Ethnopharmacol. 114:86-91.
Katiki LM , Chagas ACS, Bizzo HR, Ferreira JFS, Amarante AFT (2011). Antehlmintic activity of *Cymbopogon martinii*, *Cymbopogon schoenanthus* and *Mentha piperita* essential oils evaluated in four different *in vitro* tests. Vet. Parasitol. 183:103-106.
Lans C, Brown G (1998). Ethnoveterinary medicines used for ruminants in Trinidad and Tobago. Prev. Vet. Med. 33(3):149-163.
Marie-Magdeline C, Hoste H, Mahieu M, Varo Archimede H (2009). *In vitro* effects of *Cacurbita moschata* seed extracts on *Haemonchus contortus*. Vet. Parasitol. 161:99-105.
Monglo D, Njongmeta LM, Musongong G, Ngassoum M, Nukenine EN (2006). Evaluation of antelmintic potentials of ethanolic plant extracts from Northern Cameroon against eggs and infective larvae of *Haemonchus contortus*. J. Bio. Sci. 6:426-433.
Pessoa LM, Morais SM, Bevilagua CML, Lucino JHS (2002). Anthelmintic activity of essential oil of *Ocimum gratissimum L.* and euguenol against *Haemonchus contortus*. Vet. Parasitol. 24(22):1-5.
Price KR, Johnson INT, Fenwick GR (1987). The chemistry and *biological* significance of saponin in food and feed stuffs : Res. Food Sci. 26:27-135.
Smyth JD (1994). Animal Parasitology. 3rd Edition Cambridge University Press. Great Britain. p. 549.
Soetan KO, Lassisi OT, Agboluaje AK (2011). Comparative assessment of *in vitro* anthelmintic effects of the aqueous extracts of the seeds and leaves of the African locust bean (*Parkia biglobosa*) on bovine nematode eggs. J. Cell Anim. Biol. 5(6):109-112.
Soulby FJL (1982). Arthropods and protozoa of domesticated animals. 7th edition, Bailler. Tindal. London. p. 809.
Wabo Poné J (2007). Effets des extraits de *Canthium mannii*, Hiern, 1877 (Rubiacées) sur deux nématodes parasites strongylides : *Ancylostoma caninum* Ercolani, 1859 et *Heligmosomoides polygyrus* Dujardin, 1845. Unpublished PhD Dissertation, Département de Biologie et Physiologie Animales, Faculté des Sciences, Université de Yaoundé I, Cameroun. p. 155.
Wabo Poné J, Bilong Bilong CF, Mpoame M (2010). *In vitro* nematicidal activity of extracts of *Canthium mannii* (Rubiaceae) on different life cycle stages of *Heligmosomoides polygyrus* (Nematoda, Heligmosomatidae). J. Helmenthiol. 84:156-165.
Wabo Poné J, Bilong Bilong CF, Mpoame M, Fusi NC, Coles CG (2006). *In vitro* activity of ethanol, cold and hot water extracts of stem bark of *Canthium mannii (Rubiaceae) on Ancylostoma caninum* eggs*.* East Central Afr. J. Pharm. Sci. 9(1):14-18.
Wabo Poné J, Fossi TO, Yondo J, Marie CK, Mpoame M, Bilong Bilong CF (2011c). The *in vitro* effects of aqueous and ethanolic extracts of the leaves of *Ageratum conyzoides* (Asteraceae) on three life cycle stages of the parasitic nematode *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). Vet. Med. Inter. doi: 104061 / 2011 140293.-450.
Wabo Poné J, Kenne TF, Mpaome M, Bilong Bilong CF(2011d). *In vitro* anthelmintic activity of acetone extracts from leaves of three forage legumes (*Calliandra calotyrsus, Gliricidia sepium and Leucaena diversifolia*) on *Haemonchus contortus*. Asia Pac. J. Trop. Med. 125-128.
Wabo Poné J, Mpoame M, Bilong Bilong CF (2009). *In vivo* evaluation of potential nematicidal properties of ethanol extract of *Canthium mannii* (Rubiaceae) on *Heligmosomoides polygyrus* parasite of rodent. Vet. Parasitol. 166:103-107.
Wabo Pone J, Mpoame M, Bilong Bilong CF, Kerboeuf D (2005). Etude comparée *in vitro* de l'activité anthelmintique de la poudre d'écorce de *Canthium mannii* (Rubiaceae) et du Mebendazole. *Revue de Médecine Vétérinaire* 156(12):633-636.
Wabo Poné J, Ngankam Ntemah JD, Bilong Bilong CF, Mpoame M (2011b). A comparative study of the ovicidal and larvicidal activities of aqueous and ethanolic extracts of pawpaw seeds *Carica papaya* (Caricaceae) on *Heligmosomoides bakeri.* Asian Pac. J. Trop. Med. p. 447.
Wabo Poné J, Yondo J, Fossi TO, Marie CK, Bilong Bilong CF, Mpoame M (2011a). The *in vitro* effects of *Chenopodium ambrosioides* (Chenopodiaceae) extracts on the parasitic nematode *Heligmosomoides bakeri.* J. Pharmacogn. Phytother. 3(4):56-62.
Waller PJ (1997). Sustainable helminth control of ruminants in developing countries. Vet. Parasitol. 71:195-207.
Waller PJ (1999). International approaches to the concept of integrated control of nematode parasites of livestock. Inter. J. Parasitol. 29:155-164.
Wolstenholm AJ, Fairweather I, Prichard R, Von Samson-Himmelst-jerna G, Sangster NC (2004). Drug resistance in veterinary helminthes. Trends Parasitol. 20:469-476.