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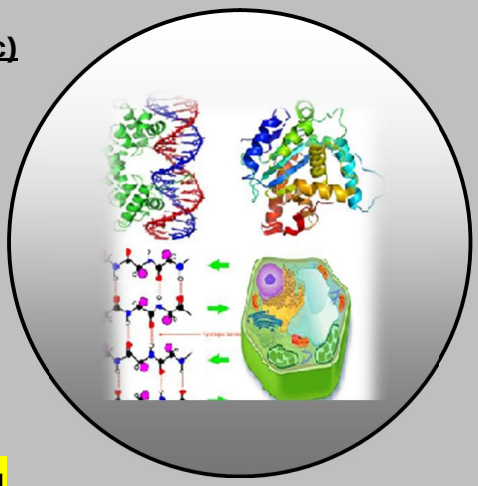
ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Volume 30
No.1 (2013)

J. Biol. Chem. Research
Volume 30 (1) 2013 Pages No. 98-106



Journal of Biological and Chemical Research

(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 30, No. 1: 98-106 (2013)
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)
Published by Society for Advancement of Science®

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RESEARCH PAPER

Received: 17/11/2012 Revised: 25/01/2013 Accepted: 26/01/2013

Ovicidal and Larvicidal Activities of Aqueous and Ethanolic Extracts from *Erythrina sigmoidea* (Fabaceae) against *Heligmosomoides bakeri*

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ABSTRACT

The rapid development of parasite resistance due to indiscriminate use of conventional anthelminthics associated with the high cost of available anthelminhtic drugs has limited the success of gastro-intestinal parasites control and thus led to the evaluation of medicinal plants as an alternative source of anthelminthics. . In the present study, the anthelminthic activity of Erythrina sigmoidea stem bark extracts was evaluated against Heligmosomoides bakeri, using in vitro embryonation, egg hatch and larval mortality tests. To achieve our objective, dried extracts were diluted in distilled water to obtain five different concentrations: 625, 1250, 2500, 3750 and 5000 µg/ml. Fresh eggs obtained from artificially infected mice feces were exposed to these different concentrations for 48 hours. Time of contact for embryonated eggs was 6 hours while L_1 and L_2 larvae were exposed for 24 hours. Distilled water (placebo) and 1.5 % DMSO were used as negative controls.

Distilled water, and 1.5 % DMSO had no effect on embryonation, hatching and larval survival. Aqueous extracts of E. sigmoidea showed a weak activity against all stages of the parasite at all concentrations tested. On the contrary, the ethanolic extract of E. sigmoidea showed low IC_{50} and LC_{50}, indicating their good efficacy. This extract inhibited 87.5 % of fresh eggs embryonation with an IC_{50} of 62.9 µg/ml, 77.4 % of egg hatch and induced the mortality of 96.2 % and 90.8 % of L_1 and L_2 larvae respectively at 5000 µg/ml.

The overall findings of the present study have shown that ethanolic extracts of E. sigmoidea contains possible anthelminthic compounds and further in vivo and toxicity tests should be carried out.

Keywords: Erythrina sigmoidea, Heligmosomoides bakeri, In Vitro, Eggs and Larvae.

INTRODUCTION

In tropical and developing countries, the prevalence of intestinal nematode infection is apparently very high, due to climatic, hygienic, poverty and demographic conditions which favour the development and transmission of parasites (**Wabo Poné *et al*., 2011a).** Basically, three classes of helminths exist: Cestoda, Trematoda and Nematoda (**Adamu *et al*., 2010).** The class Nematoda contains the most pathogenic helminths to livestock and humans and impact negatively on their health. In livestock, helminthiasis causes appetite depression, damage in gastric function and alterations in total protein content, energy and mineral metabolism production leading to enormous economic losses particularly in areas where extensive grazing is practiced (**Camila *et al*., 2012).** In humans, gastro-intestinal nematodes are causes of maternal and child morbidity. Infections in children can result in intellectual and growth retardation whereas maternal infections induce prematurity, intrauterine growth retardation and low birth weight among newborns due to iron deficiency anemia (**Michelle et al., 2009**). Helminthiasis control is performed by the use of synthetic anthelminthics, however there are some disadvantages, such as the development of resistant strain of parasites (**Kamaraj and Abdul, 2011**). The first case of resistance to anthelminthics was accurately described by **Drudge *et al*. (1964)**. Thereafter, several studies reporting decreased anthelminthic effectiveness have been published (**Camila *et al*., 2012).** Moreover, anthelminthic drugs needed for the treatment of gastro-intestinal parasites of livestock and humans are usually expensive, and often out of reach for the poor populations of developing countries (**Nchu *et al*., 2011**). One of the novel approaches investigated as alternative for treatment of parasitic infection is the use of indigenous plant preparations commonly utilized in traditional medicine. The benefit of using these as possible dewormers is that they are inexpensive and available in developing countries. Furthermore, plant-derived anthelminthics are advantageous as they are less toxic, biodegradable and environmentally friendly (**Hernandez-villegas *et al*., 2011**). Some of the medicinal plants claimed by traditional healers have been screened and evaluated for their anthelminthic activities with variable findings (**Tadesse *et al*., 2009; Eguale *et al*., 2007**). However, there are several other claimed medicinal plants for which no scientific evaluation has been conducted such as *Erythrina sigmoidea*. *E. sigmoidea* is a perennial shrub belonging to the family Fabaceae and distributed in tropical and subtropical regions. It has been the subject of *in vitro* studies that have noted its anti-cholinergic (**Nkeh *et al*., 2006**), antibacterial (**Kouam *et al*., 2007**) and antimicrobial (**Nkengfack *et al*., 1997**) activities. The aim of this study was to investigate the *in vitro* anthelminthic activity of the aqueous and ethanolic extracts from stem bark of *E. sigmoidea*, using fresh eggs, embryonated eggs, L_1 and L_2 larvae of *Heligmosomoides bakeri*, a gastro-intestinal nematode parasite of laboratory mice commonly used as a model to test new antiparasitic substances since it is resistant to a number of available anthelminthics.

MATERIAL AND METHODS

The stem bark of *E. sigmodea* used in this work was harvested in Dschang, Menoua Division in the West Region of Cameroon. They were dried in the shade for 1 to 4 hrs/day for one week, ground and stored in airtight plastic bags in the laboratory for further use.

Preparation of extracts

Three types of extracts [aqueous (infused, macerated) and ethanolic] were prepared to compare their activities. The procedure described by **Wabo Poné *et al*. (2006, 2010)** was used to obtain 05 different solutions of concentrations 1250, 2500, 5000, 7500 and 10000 µg/ml. The final concentrations tested were 625, 1250, 2500, 3750 and 5000 µg/ml.

Recovery of Nematode eggs

Fresh eggs of *Heligmosomoides bakeri* (previously known as *Nematospiroides dubius* and *Heligmosomoides polygyrus*) were obtained from the feces of experimentally infected mice according to **Ngangout *et al*. (2012).**

Recovery of Nematode larvae

Eggs obtained from the above were allowed in a beaker with water for 2 days and 4 to 5 days to obtain L_1 and L_2 larvae respectively. The solution containing the larvae was then distributed in test tubes and allowed for 10 minutes. The supernatant was decanted and Ringer solution was added in the test tubes to optimize the survival of larvae.

Evaluation of ovicidal and larvicidal activities

Fresh and embryonated eggs were used to evaluate the ovicidal efficacy, while L_1 and L_2 larvae were used for larvicidal activity according to **Wabo Poné *et al*. (2011b, 2011c)**

Statistical Analysis

At equal concentration, the mean embryonation rates, hatching rates and larval mortality rates due to the extracts were compared using the chi-square at the $P \leq 0.05$ significance level. The 50 % inhibitory concentrations (IC_{50}) and lethal concentrations (LC_{50}) were determined using probit regression lines of the according to the decimal logarithm of the concentration. All tests were repeated four times for each treatment and control [distilled water (DW) and 1.5 % DMSO].

RESULTS

The yields obtained after extraction with ethanol, hot and cold water solvents from 100 g of *E. sigmoidea* stem bark powder were 6.1 g, 14 g and 10.25 g respectively.

The variation of the mean embryonation rate of *H. bakeri* according to the different concentrations of *E. sigmoidea* extracts is shown in **Figure 1**. It shows that DW and 1.5 % DMSO allowed normal embryonation of the parasite eggs, with the mean embryonation rates of 100 % and 96.2 % respectively. On the other hand, aqueous extracts of *E. sigmoidea* presented a weak activity with mean embryonation rates which remained higher than 50 % in all concentrations tested. On the contrary, the ethanolic extract exhibited a high activity with inhibition rates of embryonation increasing from 70 ± 1.9 % at 625 µg/ml to 87.5 ± 0.1 % at 5000 µg/ml, with a significant difference ($P < 0.05$). The IC_{50} obtained on fresh eggs after transformation of the embryonation rate to probit (not illustrated) were 11249.3, 7349.2 and 62.9 µg/ml for infused, macerated and ethanolic extracts respectively.

The variation of the mean hatching rate of embryonated eggs of *H. bakeri* according to concentrations of the extracts is illustrated in **Figure 2**. As on fresh eggs, both DW and 1.5 % DMSO had no effect on embryonated eggs, with mean hatching rate of 90 %. In all the concentrations, hatching rate remained higher than 60 %, with aqueous extracts.

The mean rates of hatching decreased with increase in concentration of ethanolic extract. We obtained 51.5 ± 0.4 % at 625 µg/ml which decreased to 22.6 ± 0.3 % at 5000 µg/ml with a significant difference ($P < 0.05$). The IC_{50} obtained from embryonated eggs were 57173, 29788.3 and 860.5 µg/ml for infused, macerated and ethanolic extracts respectively.

Figure 3 shows the effects of different extracts of *E. sigmoidea* on L_1 larvae of *H. bakeri* after 24 h of contact. Distilled water and 1.5 % DMSO allowed the normal development of L_1 larvae. Ethanolic extract showed a concentration - dependent activity, with mortality rates increasing from 59.3 ± 0.8 % at 2500 µg/ml to 96.2 ± 0.9 % at 5000 µg/ml. The LC_{50} obtained with L_1 larvae were 1.4 x 10^{19} and 1231 µg/ml for macerated and ethanolic extracts respectively.

The effect of different extracts of *E. sigmoidea* on L_2 larvae of *H. bakeri* after 24 h of contact is presented in **Figure 4**. As on L_1 larvae, DW and 1.5 % DMSO had no effect on L_2 larvae, with the mortality rate of 0 %. Activity of aqueous extracts remained relatively low whereas, ethanolic extract registered mean mortality rates increasing from 71 ± 2.2 % at 2500 µg/ml to 90.8 ± 0.9 % at 5000 µg/ml. The LC_{50} obtained with L_2 larvae were 3705.9 and 1062.7µg/ml for infused and ethanolic extracts respectively. High values of IC_{50} and LC_{50} were obtained with aqueous extract indicate the inefficacy of these extracts against all stages of the parasites.

DISCUSSION

Due to high cost of *in vivo* tests, majority of researchers prefer *in vitro* tests for preliminary screening of anthelminthic activity of plant extracts using free living stages of parasitic nematodes (eggs and larval stages) (**Eguale *et al*., 2011**). These *in vitro* tests measure the effects of anthelminthics directly on physiological processes such as hatching, development and motility of parasites (**Assisa *et al*., 2003**). Yield of extraction obtained with water was higher than that obtained with ethanol. **Wabo Poné *et al*. (2010)** obtained similar results with aqueous and ethanolic extracts of *Canthium mannii*. This could be due to the fact that ethanol extracted more active compounds than impurities. The high rates of embryonation (100 % and 96.2 %) egg hatch (90 %) and low mortality rates (0 %) obtained with negative controls (DW and 1.5 % DMSO) indicated their non-effects on development of eggs and larvae of *H. bakeri*. Aqueous extracts of *E. sigmoidea* were either ineffective or had a weak activity against all stages of the parasite at all tested concentrations. This result is in agreement with findings of other studies which suggest that water extracts of some plant species are not effective anthelminthics (**Worku *et al*., 2009**). **Aremu *et al*. (2010)** also reported that, water extracts generally exhibit poor pharmacological activities. Although traditional healers often used water for extracting medicinal plants, studies have shown that the use of water for plant extraction naturally limits the type and amount of compounds extracted. According to **Taylor *et al*. (2001),** the absence of significant activity could be due to factors such as dilution of active compounds and antagonistic effects by other compounds and not necessarily the inactivity of the plant extracts.

The results of this study also revealed that the ethanolic extract of *E. sigmoidea* inhibits embryonation, egg hatch and larval survival at various concentrations when compared with the control.

In general, the extract with higher concentrations showed more anthelminthic activity when compared to extract with lower concentration. This observation showed that, an increase in concentration represent a supplementary input of different active compounds **(Wabo Poné *et al*., 2011c)**. Ethanolic extract of *E. sigmoidea* inhibited the embryonation of 87.5 % of fresh eggs, hatching of 77.4 % of embryonated eggs and induced mortality of 96.2 % of L_1 larvae and 90.8 % of L_2 larvae at 5000 µg/ml with IC_{50} and LC_{50} of 62.9 µg/ml, 860.5 µg/ml, 1231 µg/ml and 1062.7 µg/ml respectively. Based on IC_{50} and $LC_{50,}$ larvae were more resistant than eggs. These results do not conim with earlier reports of **Katiki *et al*. (2011**) in which they observed that eggs are more resistant than L_1 larvae due to their hard and resistant shell, confirming the order of resistance of free stage parasites describe by Soulsby, 1982. On the contrary, **Adama *et al*. (2009)** reported that the possible explanation for the better activity of extracts of *Anogeissus leiocarpus* and *Daniella oliveri* on eggs compared to first stage larvae could be due to their easier diffusion through eggshells than the cuticles. The activity of *E. sigmoidea* may be related to active substances previously reported from the plant such as flavanone, isoflavone, pterocarpans (**Nkengfack *et al*., 1997**), flavonoïds and triterpenoids saponins (**Kouam *et al*., 2007). Doligaska *et al*. (2011)** found that triterpenoid saponin inhibited egg hatching, distorted larvae morphology and disturbed moulting of all free living larvae of *H. bakeri* even though the mechanism of action of these saponins is not yet understood. However, it is possible that secondary metabolites contained in the plant extracts exhibited the same mechanism of uptake of anthelminthic drugs described by **Alvarez *et al*. (2001).** By this mechanism, they could bind to the free proteins available for the first stage larvae nutrition and thus depriving the larvae of food, then provoking death in embryonated eggs by paralysis as does Levamisole (**Athanasiadou *et al*., 2001**).

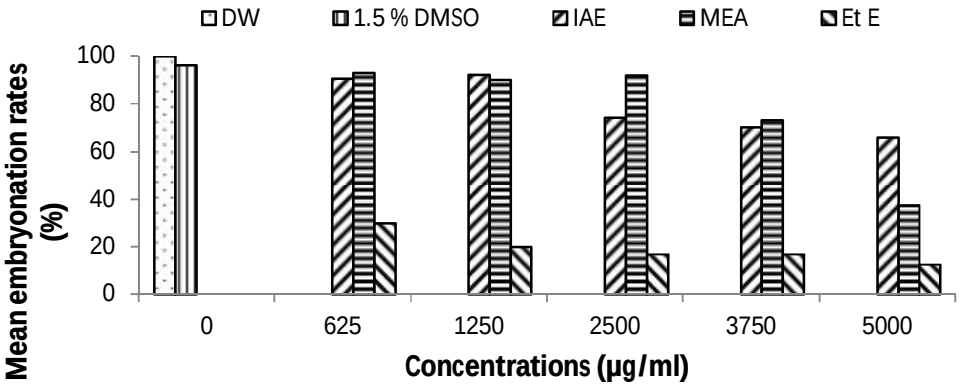

Figure. 1. Variation of the mean embryonation rate of *Heligmosomoides bakeri* eggs according to the concentrations of the extracts of *Erythrina sigmoidea*.

Legend: DW: Distilled water; DMSO: Dimethylsulfoxide; IAE: Infused aqueous extract; MAE: Macerated aqueous extract; Et. E: Ethanolic extract.

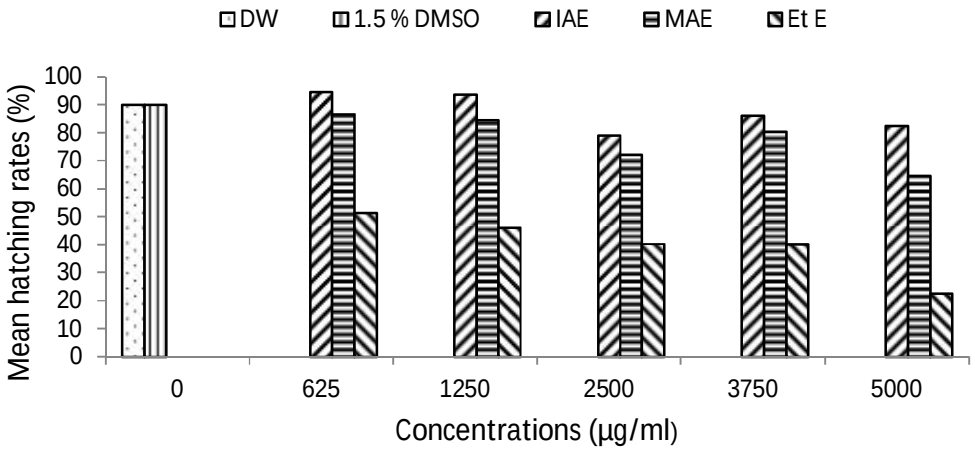

Figure. 2. Variation of the mean hatching rate of *Heligmosomoides bakeri* eggs according to the concentrations of the extracts of *Erythrina sigmoidea*.
Legend: DW: Distilled water; DMSO: Dimethylsulfoxide; IAE: Infused aqueous extract; MAE: Macerated aqueous extract; Et E: Ethanolic extract.

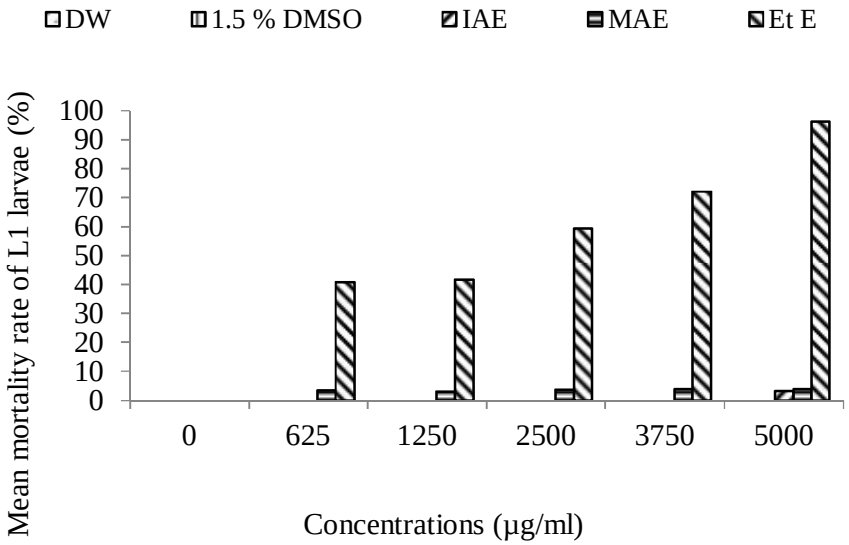

Figure. 3. Variation of the mean mortality rate of L_1 larvae of *Heligmosomoides bakeri* according to the concentrations of the extracts of *Erythrina sigmoidea*.
Legend: DW: Distilled water; DMSO: Dimethylsulfoxide; IAE: Infused aqueous extract; MAE: Macerated aqueous extract; Et E: Ethanolic extract

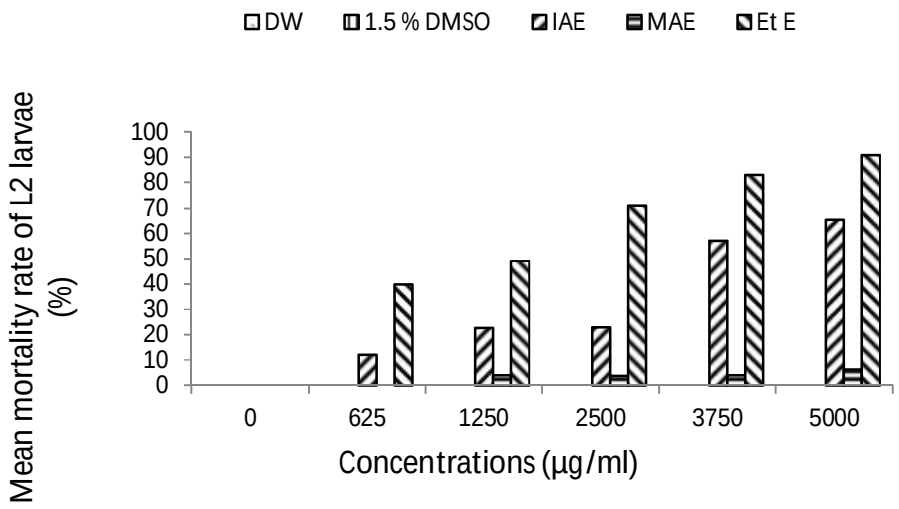

Figure. 4. Variation of the mean mortality rate of L_2 larvae of *Heligmosomoides bakeri* according to the concentrations of the extracts of *Erythrina sigmoidea*.
Legend: DW: Distilled water; DMSO: Dimethylsulfoxide; IAE: Infused aqueous extract; MAE: Macerated aqueous extract; Et E: Ethanolic extract.

ACKNOWLEDGEMENTS

The authors wish to express sincere gratitude to the Laboratory of Applied Biology and Ecology (LABEA) in the Faculty of Science at the University of Dschang where this study was carried out. We also thank the traditional healers of Dschang – Cameroon, particularly Mr NDAM souleman for information on the plant specimen used in this work.

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