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Full Length Research Paper

The *in vitro* effects of *Chenopodium ambrosioides* (Chenopodiaceae) extracts on the parasitic nematode *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae)

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The aim of the study was to evaluate the ovicidal and larvicidal efficacy of hexane and ethanolic extracts of *Chenopodium ambrosioides* (Chenopodiaceae) leaves on the eggs and larvae of *Heligmosomoides bakeri in vitro.* The eggs of the parasite were obtained from feaces of artificially infected mice, and after 3 and 4 to 5 days incubation at 25°C, L_1 and L_2 larvae were obtained respectively. The different stages were exposed to 5 concentrations (0.625, 1.25, 2.5, 3.75 and 5 mg.ml^{-1}) and expositions periods were 48 and 24 h for eggs and larvae respectively. Distilled water, 10% gelatine and mebendazole were used in bioassay as negative and positive controls respectively. The hexane and ethanolic extracts of *C. ambrosioides* had a negative effect on the development of embryos, hatching rate and larval survival of *H. bakeri.* 61.59 and 39.06% embryonation rates were obtained at 3.75 and 5 mg.ml^{-1} for hexane extract while 0% hatching rate was obtained at concentration of 5 mg.ml^{-1} with the same extract. The 50% inhibitory concentration (IC_{50}) on embryonation was 2.84 and 13.53 mg.ml^{-1} for hexane and ethanolic extracts respectively while the same values were 0.966 and 1.4 mg.ml^{-1} on embryonated eggs respectively. At the highest concentration larvicidal activity was 100% on both L_1 and L_2 larvae. As concern larval mortality, the LC_{50} obtained with hexane and ethanolic extracts and mebendazole was 2.963, 5.208 and 2.243 for L_1 larvae and 0.8, 1.661 and 1.602 for L_2 larvae respectively. These data show that both extracts of *C. ambrosioides* possess nematicidal activity justifying its use as worm medicine all over the world.

Key words: Anthelmintic, *Chenopodium ambrosioides,* extracts, *Heligmosomoides bakeri.*

INTRODUCTION

Parasitic infections constitute a major public health problem in developing countries where climatic, hygienic, poverty and demographic conditions favour the development and transmission of parasites. In Haiti for example, more than 39% children of school age are infected with gastro-intestinal helminths (Champetier de Ribes et al., 2002). The same findings were observed in Ivory-Cost (Menan et al., 1997). In Cameroon, the Permanent Secretary of the National Programme of schistosomiasis and gastro-intestinal helminthiasis (GIH) control reported in January 2006 that, 2 million people are infected with schistosomes and more than 10 million with various intestinal worms. These infections affect mostly children of school age and compromise their growth, intellectual development and their school performance as well as increase their vulnerability to other diseases. GIH also affect domesticated animals and have an impact on production which results in some economical losses. In Kenya for example, losses to the agricultural sector due

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to haemonchosis are estimated at US$ 26 million per year (Githiori, 2004). The use of synthetic anthelmintic drugs has been the main method of control by farmers and the population. Misuse of these drugs for decades has led to the development of resistant worm strains. In addition, some side effects have been noted and the use of disinfectants to control free living stages of parasites is harmful to the environment (Elard, 2000). The use of natural local resources which are less expensive and with better efficiency remains the most pertinent alternative to modern anthelmintic drugs. Unfortunately the activities of these natural substances have rarely been shown in laboratories. Thus, the discovery of new and cheaper drugs which are relatively less toxic seem to be a desirable solution to the drawbacks of modern anthelmintic (Cirak et al., 2004; Wolstenholme et al., 2004). This study reports on a comparative assay of ovicidal and larvicidal properties of hexane and ethanolic extracts of *Chenopodium ambrosioides* (Syn. *C. anthelminticum*). This aromatic herb (Quarles,1992) belonging to the family chenopodiaceae is being widely used as worm medicine with some common name such as “ipazote” in Mexico, “ambrosi” in USA, “paîco” in Spain, “goosefoot” in UK or “elo’o nson” (Bulu) in Cameroon.

MATERIALS AND METHODS

The *C. ambrosioides* leaves used in this study were harvested in Dschang, Menoua division, West Region of Cameroon. They were dried in an oven heated at 50°C for 7 days. The leaves were ground and stored in airtight plastic bags for further use in the laboratory.

Preparation of extracts

Two types of extracts (hexane and ethanolic) were prepared to compare their activities.

Hexane extract

The procedure used is as described by Wabo Poné et al. *(*2006, 2010). Briefly, 445 g of stored powder were macerated in 4 L of hexane which helps to remove apolar compounds of plants (Ciulei, 1982). The mixture was stirred daily and 72 h later, this solution was filtered through a filter paper of pore size 2.5 µm. The hexane extract was obtained using the procedure described by Ciulei (1982.). This was followed by the dilution of 100 mg of the extract (concentrated using a rotary evaporator) with 1 ml of 10% gelatine which helps to dilute the extract and to facilitate the mixing with water. After 5 to 10 min, distilled water was added to obtain a total volume of 10 ml which produced a stock solution of 10 $mg.ml^{-1}$ from which a series of dilutions were made to obtain solutions of: 1.250, 2.5, 5.0, 7.5 and 10 $mg.ml^{-1}$ concentrations. The final tested concentrations were 0.625, 1. 25, 2.5, 3.75 and 5 $mg.ml^{-1}$.

Ethanolic extract

The same procedure was used for the ethanolic extract and 95% ethanol was used as solvent.

Reference drug

Mebendazole (MBZ) was used as positive control only in larval test since the larva is structurally close to adult which is the main target of this synthetic drug. The same concentrations as organic extracts were used, while 10% gelatine and distilled water (DW) were used as negative controls.

Recovery of nematode eggs

The recovery of *H. bakeri* (previously known as *Nematospiroides dubius* and *Heligmosomoides Polygyrus)* (Cable et al., 2006; Behnke and Harris, 2010) fresh eggs were obtained from the faeces of experimentally infected mice following Wabo Poné et al. *(*2010).

Evaluation of the ovicidal activity

The ovicidal efficacy test of the different extracts was performed using two different procedures. To assess the effects of the extracts on fresh eggs, 1 ml of suspension containing 30 parasite eggs was distributed in each of 12 Petri dishes (35 × 10 mm) and mixed with the same volume of a specific concentration of a given extract. The dishes were covered and the eggs incubated at room temperature for 24 h, after which 2 to 3 drops of lugol’s iodine were added in each Petri dish which help to fix the different life cycle stages and the number of embryonated eggs per Petri dish was counted under a microscope (at 4× magnification). The percentage of embryonation (EM %) was determined as follows (Wabo Poné et al., 2010):

$$E\,M(\%) = \frac{Number\ of\ embryonated\ eggs}{Number\ of\ eggs\ in\ culture} X\ 100$$

To test the effects of the extracts in the second procedure, the same number of fresh un-embryonated eggs distributed in Petri dishes as above, was allowed at room temperature for about 24 h until the eggs had developed to the fully embryonated pre-hatch stage. When the first stage larvae become transparent and were actively moving within the egg envelope (> 90% in the control Petri dish), 1 ml of a range of each concentration of extract was added to each Petri dish. The Petri dishes were then covered and incubated for a further 6 h at room temperature (24°C) to allow hatching to be completed in the control dish. Thereafter, 2 to 3 drops of lugol’s iodine were added to each Petri dish to help to fix the different life cycle stages and all embryonated eggs. The first-stage larvae (L_1) were counted under a microscope (at 4× magnification). The hatching rate or eclodibility (E%) was computed as follows (Wabo Poné et al., 2006):

$$E\ (\%) = \frac{Number\ of\ L_1\ larvae}{Number\ of\ embryonated\ eggs\ in\ culture} X\ 100$$

Recovery of nematode larvae

Eggs were cultured using the technique described by Smyth (1996). Briefly, 3 ml of the egg suspension was poured on filter paper covering the bottoms of two Petri dishes. This later were then covered to maintain a high relative humidity (65 to 67%) to prevent the dishes from drying out, and was stored at 24°C. After 3 and 4 to 5 days of incubation, L_1 and L_2 larvae respectively were observable in Petri dishes and were concentrated with a Baermann apparatus

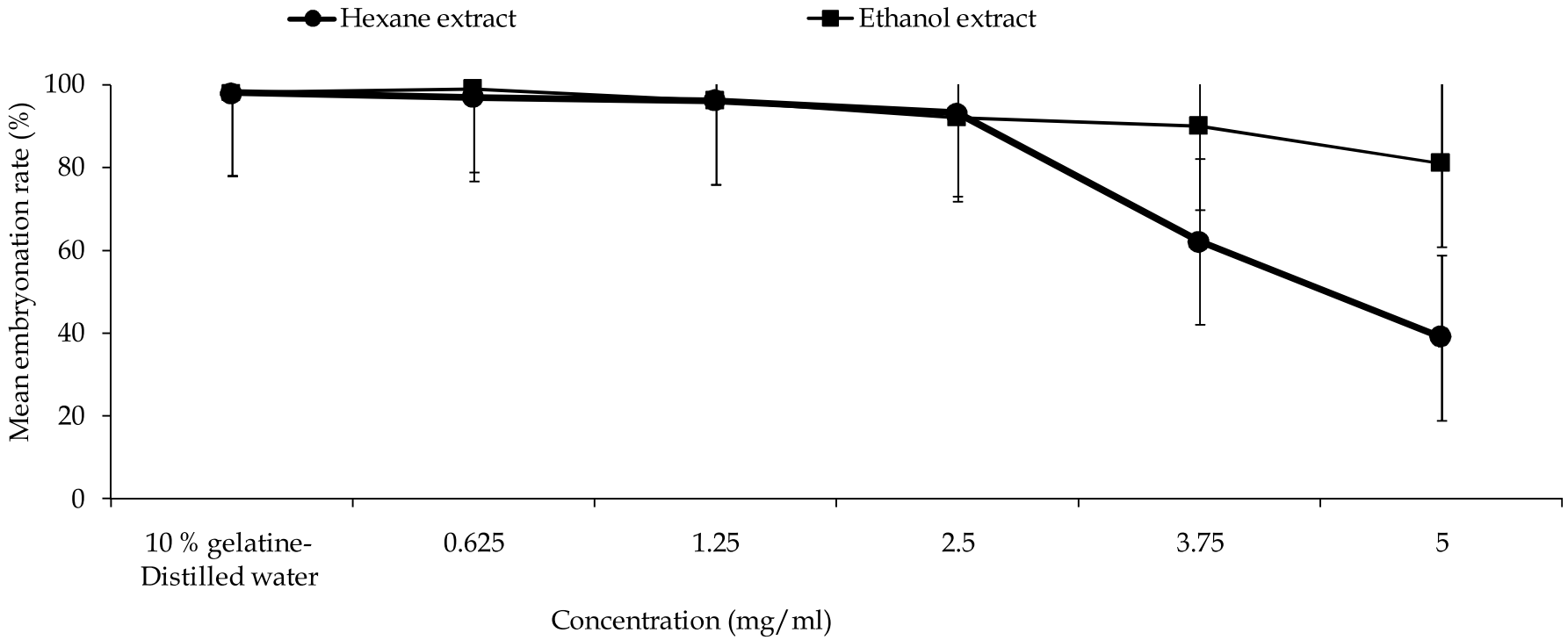

Figure 1. Variation of the mean embryonation rate of *Heligmosomoides bakeri* eggs according to the concentration (mg.ml^{-1}) of hexane and ethanolic extracts of leaves of *Chenopodium ambrosioides*.

(Cheesbrough, 1987; Smyth, 1996).

Evaluation of the larvicidal activity

To test the effects of the extracts on L_1 and L_2 larvae, 1 ml of a solution containing about 30 to 40 parasite larvae was distributed in each of 12 Petri dishes (35 ×10 mm) and mixed with the same volume of a specific concentration of each extract. The dishes were covered and kept at room temperature for 24 h, after which the number of dead or immobile larvae was counted under a microscope (at 4 × magnification). The percent mortality (Mc %) was determined using Abott's formula for corrected mortality (Wabo Poné et al., 2005).

$$Mc(\%) = \frac{Mce - Mt}{100 - Mt} X\ 100$$

where Mce is the mortality obtained during the test and Mt the mortality registered in the negative control dishes. It is considered that when the mortality rate in the latter dishes is less than 5%, Mc = Mce (Busvine, 1981).

Statistical analysis

At equal concentrations, the mean embryonnation rates, hatching rates and larval mortality rates were compared using ANOVA. Data after transformation was done using arc-sinus and with the t paired test at the $p < 0.05$ significance level. The 50 per cent inhibitory concentration (IC_{50}) and larvicidal concentration (LC_{50}) were determined using the regression lines of the probit according to the decimal logarithm of the concentration. All tests were repeated four times for each treatment and control.

RESULTS

The variation of mean embryonation rate of *H. bakeri* eggs according to the concentration of extracts of *C. ambrosioides* leaves is shown in Figure 1. The mean embryonnation rate was

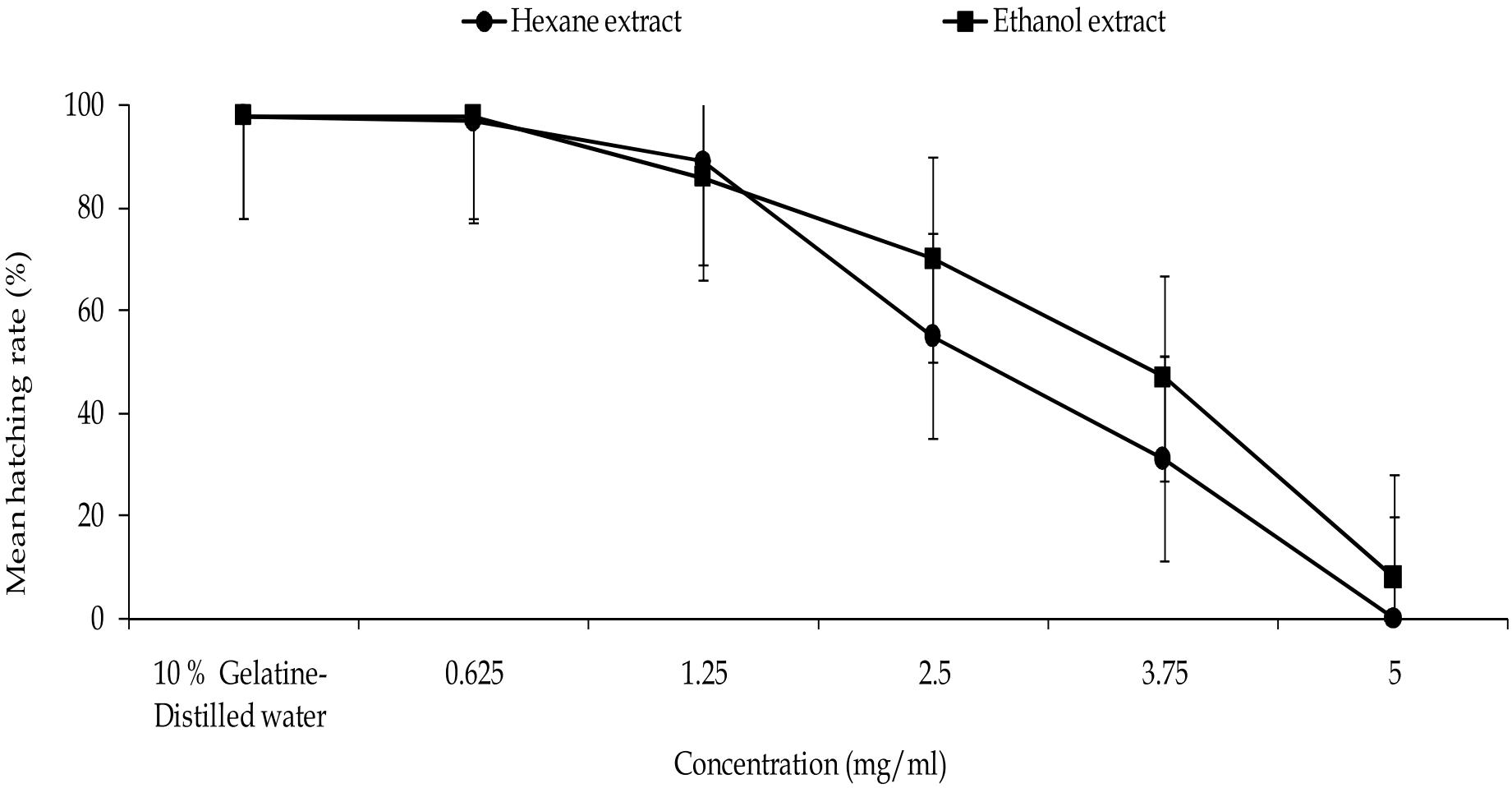

Figure 2. Variation of the mean hatching rate of *Heligmosomoides bakeri* eggs according to the concentration ($mg.ml^{-1}$) of hexane and ethanolic extracts of leaves of *Chenopodium ambrosioides*.

higher (95.17%) in negative control dishes. It was observed that, this rate reduced with increased concentration of the extracts. At concentration higher than or equal to 2.5 $mg.ml^{-1}$, the activity of hexane extract was similar to that of ethanolic extract. At concentration 3.75 and 5 $mg.ml^{-1}$, hexane extract showed the lowest embryonation rates 61.59 and 39.06% respectively. The IC_{50} obtained on the fresh eggs was 2.84 and 13.53 $mg.ml^{-1}$ for hexane and ethanolic extracts respectively.

The variation of mean hatching rate of L_1 larvae of *H. bakeri* according to the concentration of extract is illustrated in Figure 2. As of the first experiment, negative controls had no effect on the hatching rate with 97.97 and 98% eclodibility respectively for DW and 10% gelatine. The activity of the two extracts were similar to that of negative

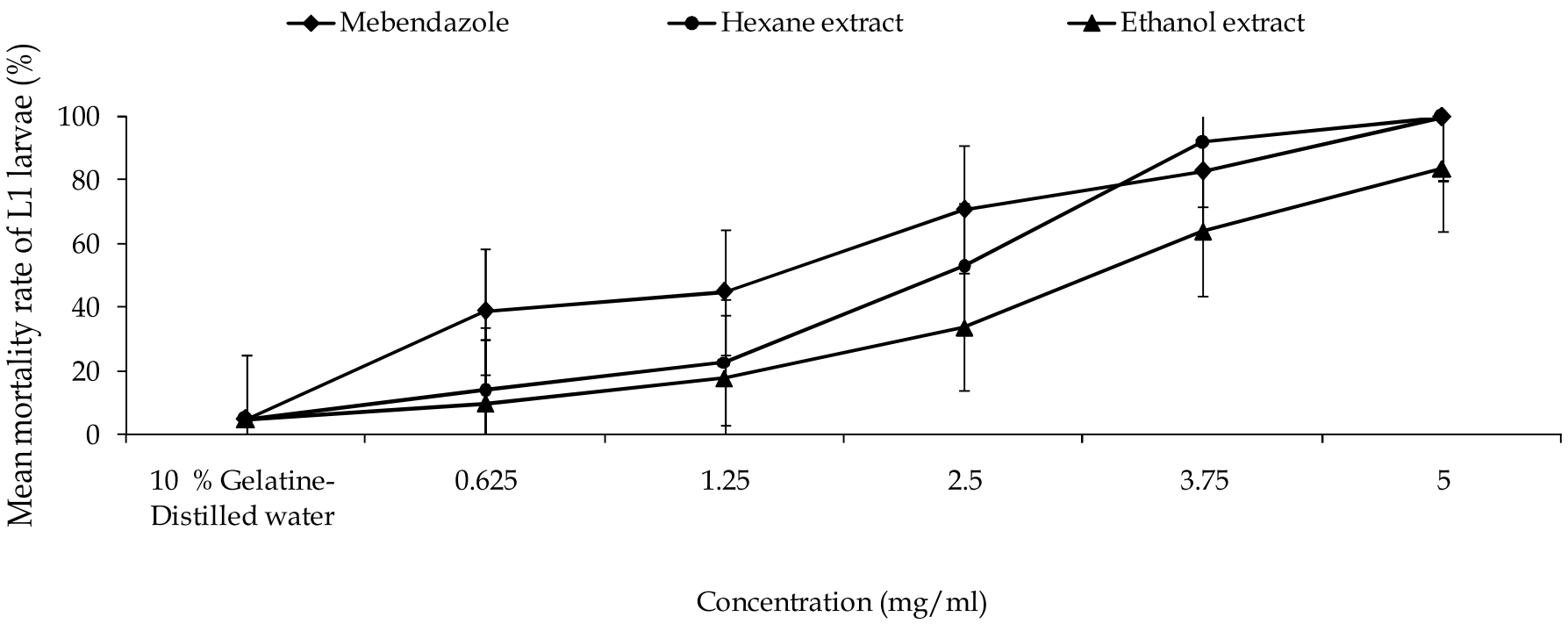

Figure 3. Mean mortality rate of *Heligmosomoides bakeri* L_1 larvae incubated in increasing concentrations ($mg.ml^{-1}$) of hexane and ethanolic extracts of *Chenopodium ambrosioides* compared to Mebendazole.

control at concentrations less than or equal to 1.25 $mg.ml^{-1}$. At concentrations higher than or equal to 2.5 $mg.ml^{-1}$ the two extracts showed highest ovicidal activities. Maximum activity (0% eclodibility) was obtained with hexane extract at the concentration 5 $mg.ml^{-1}$.

This finding was significantly different ($p < 0.05$) to that of ethanolic extract. The IC_{50} obtained on embryonated eggs was 0.966 and 1.4 $mg.ml^{-1}$ for hexane and ethanolic extracts respectively.

The effects of different extracts and MBZ on L_1 larvae of *H. bakeri* after 24 h of contact are shown in Figure 3. The larval mortality rate was high (91.74%) in Petri dishes containing 3.75 mg of hexane extract per millilitre. This larvicidal activity was slightly greater than that of mebendazole (83.33%). At concentration 5 $mg.ml^{-1}$ the larvicidal activity of hexane extract was maximum (100% observed with logarithm of the concentration. The LC_{50} obtained were 2.963, 4.365 and 2.243$mg.ml^{-1}$ for hexane, ethanolic extracts and mebendazole respectively.

After 24 h of incubation of L_2 larvae in the three treatments, the larval mortality increased with that of the concentration Figure 4. The mean mortality rate due to the hexane extract and MBZ was 100% at concentration 5 $mg.ml^{-1}$. The LC_{50} obtained were 0.800, 1.661 and 1.602 $mg.ml^{-1}$ for hexane, ethanolic extracts and mebendazole respectively. These findings show that, the hexane extract was more active on L_2 larvae than L_1 one showing that L_2 larvae of *H. bakeri* were more sensible to the substances.

DISCUSSION AND CONCLUSION

The extracts of *C. ambrosioides* affect embryonic development and hatching of *H. bakeri.* This

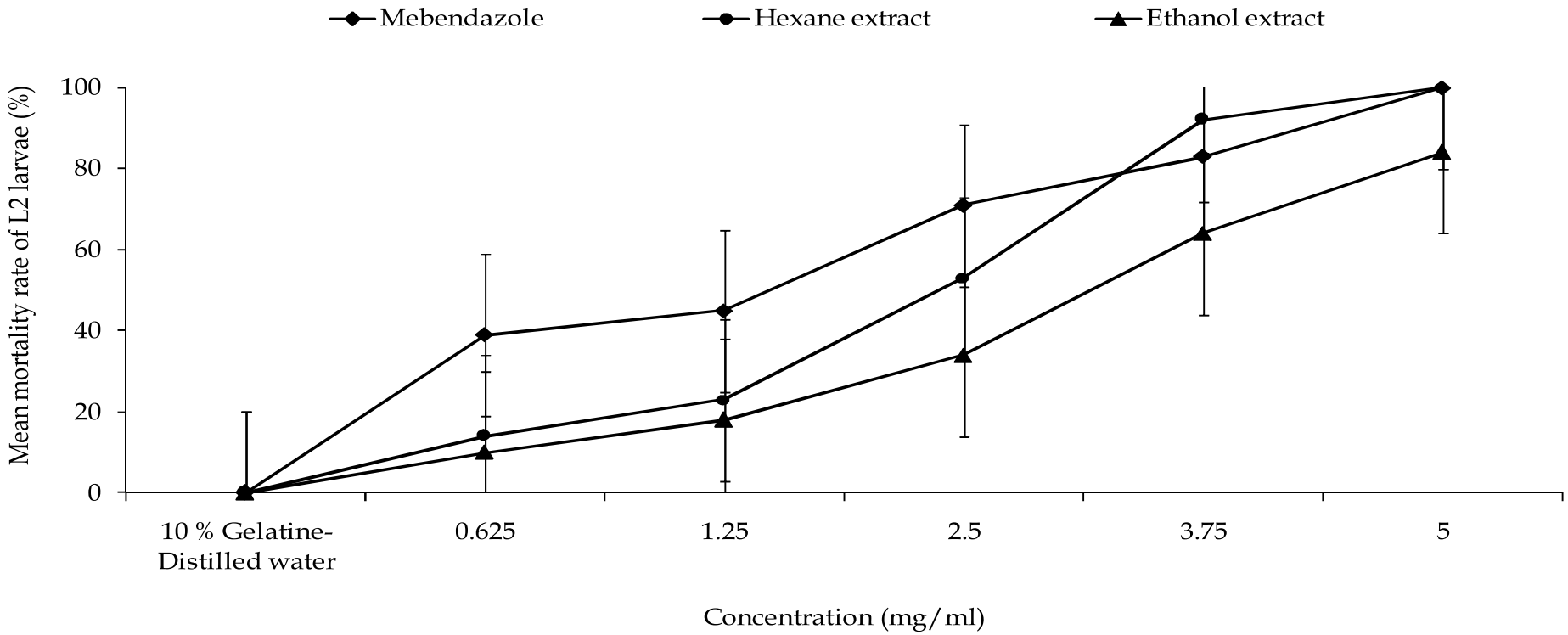

Figure 4. Mean mortality rate of *Heligmosomoides bakeri* L_2 larvae incubated in increasing concentrations ($mg.ml^{-1}$) of hexane and ethanolic extracts of *Chenopodium ambrosioides* compared to Mebendazole.

property was more visible in hatching rate, and the hexane extract was more active than the ethanolic extract. This finding could be due to the polarity of the 2 solvents. Hexane removes apolar compounds such as oil. It is known that, the essential oil from the leave of *C. ambrosioides* have nematicidal and antileishmanial properties (Monzote et al., 2009; Reis et al., 2010). The ovicidal activities of the extracts could be due to the fact that, the active compounds passed through the egg shell Such as ascaridole (Kliks, 1985) and stopped segmentation of blastomers or paralysed larvae inside the embryonated egg. The higher activity of both extracts on L_2 larvae may be due to the fact that, these free living stages were coming from their first moult in which they have lost energy and their cuticle is not yet had. For this reason the active compound may easily penetrate the cuticle and prevent the absorption of glucose, or block post synaptic receptors thus paralysing the larvae (Enriquez, 1993). Active compounds may also induced the release of gamma aminobutyric acid (GABA) which blocked transmission of nerve impulses or decoupling the phosphorylation oxydative reaction which can lead to the exhortion of the energy of the larvae (Chartier et al., 2000).

In general, we observed that, increase in concentration of each extract led to the increase in ovicidal and larvicidal activities. These increases in concentrations represent a supplementary input of different active compounds. Thus, the *in vitro* activities of hexane and ethanolic extracts of *C. ambrosioides* have been proven in this work. Further experiment incorporating *in vivo* and toxicological studies is recommended.

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