Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

A comparative study of the ovicidal and larvicidal activities of aqueous and ethanolic extracts of pawpaw seeds *Carica papaya* (Caricaceae) on *Heligmosomoides bakeri*

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ARTICLE INFO

Article history:
Received 7 March 2011
Received in revised form 17 April 2011
Accepted 15 May 2011
Available online 20 June 2011

Keywords:
Carica papaya
Extracts
Heligmosomoides bakeri
Larvicidal
Ovicidal

ABSTRACT

Objective: To assess the ovicidal and larvicidal activities of aqueous and ethanolic extracts of pawpaw seeds *Carica papaya* (Caricaceae) on the eggs and first stage larvae (L_1) of *Heligmosomoides bakeri*. **Methods:** Eggs of this parasite were obtained from experimentally infested mice (*Mus musculus*) and larvae were from eggs after incubation at 25 ℃ for about 72 hours. The eggs and larvae were exposed to ten different concentrations (0.125, 0.25, 0.375, 0.5, 0.75, 1.0, 1.25, 1.75, 2.25 and 2.75 mg/mL) of both aqueous and ethanolic extracts respectively for 72 hours. Distilled water and 0.05% ethanol used as placebo and negative control, respectively. **Results:** Placebo and negative control group all showed average 92% embryonnation, 98% egg hatching and 2% larval mortality, and did not affect development and larval survival. The extracts inhibited embryonic development, egg hatching and larval survival. In general, the ovicidal and larvicidal activities increased with increasing concentration of different extracts. The aqueous extract was found to be more potent on eggs than on larvae. At 2.75 mg/mL, only 8% of eggs embryonnated and 50% hatched to L_1 vs 57% embryonic development and 79% hatching occurred in the ethanolic extract. However, this later extract was more efficient in preventing larval development producing 96% mortality as against 68% with the aqueous extract. **Conclusions:** These results shows the ovicidal and larvicidal properties of aqueous and ethanolic pawpaw seeds extracts.

1. Introduction

In recent times, gastro-intestinal helminthiases (GIH) seem to be lossing interest due to the fact that available resources are being diverted to other priorities such as HIV/AIDS[1]. However, these infections constitute not only a public health problem but also a development concern in the countries of the South. High prevalence and intensity have been reported from China where million people harbour gastro-intestinal nematodes[2]. In Cameroon, the Permanent Secretary of the National Programme of schistosomiasis and 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 affected especially children of school age in their growth, intellectual development and their school performance and increase their vulnerability to other diseases. GIH also affect domesticated animals and have an impact on their production which results in some economical losses. In Kenya for example, losses in the agricultural sector due to haemonchosis are estimated US$ 26 million per year[3]. The misuse of anthelmintics for decades has led to the development of resistant strains of worms. Some side effects are noted and the use of disinfectants to control free stages of parasites is harmful to the environment[4]. The use of available local resources in low cost and efficient phytotherapy remains the most pertinent alternative to modern anthelmintic. The discovery of new natural and cheaper drugs which are relatively less toxic seem to be a desirable solution to the drawbacks of modern anthelmintic[5,6]. The pawpaw (*Carica papaya*-Caricaceae)

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seeds have been widely used as worm medicine but their anthelmintic properties have rarely been quantified *in vitro*. This study reports on a comparative assay of ovicidal and larvicidal properties of aqueous and ethanolic extracts of this plant seeds.

2. Materials and methods

Pawpaw seeds of "*solo*" variety were collected in Dschang, Menoua division, West Region of Cameroon, from fruit sellers. In deed, the fruits were brought down from Manjo and Njombé in the Mungo Division. The seeds were spred on a large plastic sheet and air dried for 4 hours daily for 14 days. They were then ground and stored in airtight plastic bags for further treatment (extraction) in the laboratory.

2.1. Preparation of extracts

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

For ethanolic extract, the procedure used is as described by Wabo Poné *et al*[7,8]. Briefly, 1 400 g of stored powder were macerated in 2.5 L of ethanol 95% which helps to remove the principal natural compound (polyphenol, alkaloids salts, saponines, carotenoids, carbohydrate) of plants[9]. The mixture was daily stirred and 72 hours later, this solution was filtered through a filter paper of pore size 2.5 μm. Ethanolic extract was obtained using the procedure described by Ciulei[9]. This was followed by the dilution of 100 mg of the concentrated extract (using a rotating evaporator) with 0.5 mL of ethanol 95% which helps to dilute the extract and to facilitate the mixture with water. After 5–10 min, 9.09 mL of distilled water was added to obtain a stock solution of 11 mg/mL from which a series of dilutions were made to obtain solutions of different concentrations: 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 4.5 and 5.5 mg/mL.

For the aqueous extract, a procedure similar to that of ethanol extraction was applied except that the distilled water was used and the maceration took 48 hours (to avoid growth of fungi). Also, the drying of the solvent was done in 24 hours in ventilate oven heated at 50 ℃. After, the same stock solution was prepared, followed by series of dilutions and the range of concentrations as for the organic extract was obtained.

The controls used for the bioassay were 0.05% ethanol (maximum concentration of ethanol in test dishes) and distilled water.

2.2. Recovery of nematode eggs

Heligmosomoides bakeri (*H. bakeri*)(previously known as *Nematospiroides dubius* and *Heligmosomoides polygyrus*) fresh eggs were obtained from the faeces of experimentally infected mice according to Michael *et al*[10]. Briefly, 3 g of faeces were collected, homogenised in a mortar, suspended in saturated salt solution (0.4% NaCl), and cleaned of organic debris by filtration through sieves (1 mm and 150 μm) into a 100 mL beaker. The content of the later was poured into four tubes and centrifuged at 1 000 *g* for 5 min. The supernatant was poured through a 45 μm sieve. The retained material on the sieve containing eggs was washed with tap water to remove the salt solution. It was then turned and the opposite side was washed with tap water. The eggs were collected in a Petri dish (Ø=16 cm)[11].

2.3. 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 a diluted egg solution containing about 31 parasite eggs was distributed in each of the 12 Petri dishes (35 mm Ø × 10 mm) and mixed with the same volume of a specific extract giving the following final tested concentrations: 0.125, 0.25, 0.375, 0.5, 0.75, 1.0, 1.25, 1.75, 2.25 and 2.75 mg/mL. The dishes were covered and the eggs incubated at room temperature for 24 hours, after which the number of embryonated eggs per Petri dish was counted using a microscope (at 4× magnification). The percentage of embryonation (EM%) was determined using the formula below[8].

$$EM\% = \frac{\text{Number of embroynated eggs}}{\text{Number of eggs in nature}} \times 100$$

After 24 hours, to assess the effects of the extracts in the second experiment, all embryonated eggs and first-stage larvae (L_1) were counted using a microscope (at 4×magnification). The hatching rate or eclodibility (E%) was calculated by the formula below[7]:

$$E\% = \frac{\text{Number of } L_1 \text{ larvae}}{\text{Number of embroynated eggs in culture}} \times 100$$

2.4. 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 one Petri dish. This later was then covered to maintain a high relative humidity (65%–67%) to prevent the dish from drying out, and was stored at 24 ℃. After 3 days of incubation, L_1 larvae were observable in Petri dish and were concentrated with a Baermann apparatus[12,13].

2.5. Evaluation of the larvicidal activity

To assess the effects of the extracts on L_1 larvae, 1 mL of a solution containing about 15–20 parasite larvae was distributed in each of the 12 Petri dishes (35 mm Ø×10 mm) and mixed with the same volume of a specific extract giving the same experiment. The dishes were covered and the larvae incubated in at room temperature for 24 hours, after which the number of death or immobilization of the larvae was assessed under a microscope (at 4×magnification). The percentage of mortality (Mc%) was determined using the

Abott's formula[14].

$$Mc\% = \frac{Mce - Mt}{100 - Mt} \times 100$$

where: Mc is the corrected mortality (%); Mce, the mortality obtained during the test and Mt the mortality registered in the negative controls dishes. If the mortality rate in the later dishes is lesser than 5%, Mc = Mce[15].

2.6. Statistical analysis

The different rates (means embryonation, hatching and larval mortality) due to the two types of extracts were compared using the chi-square test at the $P<0.05$ significance level. The lethal concentration 50 (LC_{50}) was determined using the regression lines of the probit according to the decimal logarithm of the concentration. All tests were repeated six times for each treatment and control.

3. Results

The effect of extracts of seeds of papaya on mean embryonation rate of eggs of *H. bakeri* is shown in Figure 1. The mean embryonation rate was 99% in placebo (distilled water) and 0.05% ethanol. For aqueous extract at 0.125–1.0 mg/mL, mean embryonation rate was higher than 90%. In contrast, from the concentration 1.25 mg/mL the number of eggs that embryonated reduced significantly ($P<0.05$) with increasing concentration of aqueous extract. The low embryonation rate was observed in Petri dishes treated with 2.75 mg/mL concentration of aqueous extract.

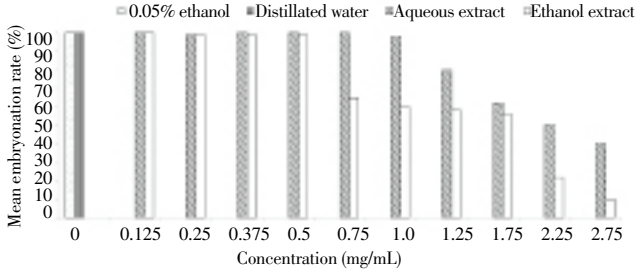

Figure 1. Effect of aqueous and ethanolic extract of papaya seeds on the mean embryonation rate of *H. bakeri* eggs.

The same profile was observed with the ethanolic extract. In fact, it is from the concentration 1.25 mg/mL that the embryonation rate began to reduce progressively and significantly. The low proportion of embryonated eggs was also observed in Petri dishes containing the 2.75 mg/mL. Also, for the concentration greater or equal to 0.75 mg/mL, the ethanol extract showed stronger inhibitive effect on the embryogenic development of *H. bakeri* than the aqueous extract.

The effect of extracts of seeds of papaya on the mean hatching rate of L_1 larvae of *H. bakeri* is illustrated in Figure 2. Independently of the type of extract, the mean hatching rate of L_1 larvae was higher than 90% in Petri dishes with the concentration ranged from 0.125 to 1.0 mg/mL. From 1.25 mg/mL, the hatching rate reduced with the increase in concentration of extracts. The low proportion of hatching rate (44%) was registered in Petri dishes containing 2.25 mg/mL of aqueous extract. For the ethanolic extract, the overall hatching rates were closer or greater than 80%. However, this variation was not significant ($P>0.05$).

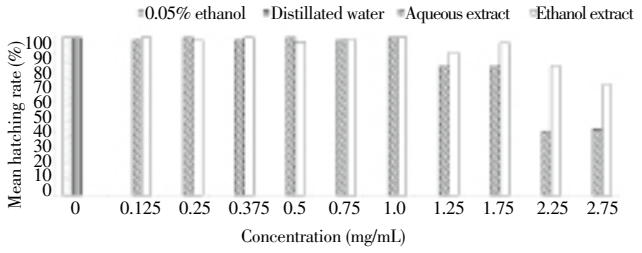

Figure 2. Effect of aqueous and ethanolic extract of papaya seeds on the mean hatching rate of *H. bakeri* eggs.

The effects of different extracts of papaya seeds on L_1 larvae of *H. bakeri* are shown in Figure 3. The larval mortality rate was low (<6%) in negative control and in extracts with concentrations lesser or equal to 1.0 mg/mL. Above 1.0 mg/mL, the mortality of the rhabditids larvae increased with increasing concentration of the extract. The difference in mortality rate was significant ($P<0.05$) between concentrations 1.25 and 1.75 mg/mL, while it was not significant between 1.75 to 2.25 mg/mL. For the ethanol extract the same finding was observed at same concentrations. The lively larvae were still moving after 24 hours. After the transformation of the larval mortality to probit, a linear relationship was observed with the logarithm of the concentration greater or equal to 1.25 mg/mL. The LC_{50} obtained were 2.25 mg/mL and 1.8 mg/mL for aqueous and ethanol extracts, respectively.

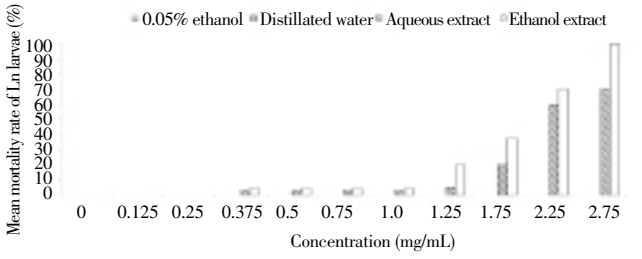

Figure 3. Effect of aqueous and ethanolic extract of papaya seeds on the mean mortality rate of L_1 larvae of *H. bakeri* after 24 hours of exposure.

4. Discussion

In our study 1 422 eggs treated with aqueous extract, 1 109 (88%) embryonated, in which 1 052 (94.87%) hatched. This finding was identical with the ethanolic extract. In fact, 1 274 (78.74%) of 1 618 eggs treated with this extract embryonated,

and in these 1 274 eggs, 1 199 (94.12%) hatched. These data indicates that a high proportion (78%–88%) of egg released in the nature succeed to embryonation and finally hatch despite the physical and chemical variation of the environment. Our results suggested that, the two extracts seem to be more active on embryonation mechanism than on the hatching. These products may pass through the different layers of the egg and inhibited the blastomer mitosis. This mode of action is similar to that reported on compounds of the benzimidazole family[16-19]. The blockage of the blastomers segmentation of the egg that failed to embryonate conforted this finding. In fact, unembryonated eggs found in solutions containing the two extracts had their blastomer completely destroyed. The fact that the aqueous solution seem to be more efficient against embryonation of eggs than the ethanolic one, while the later is much larvicidal than the former extract could be due to the composition of each extract. In fact, Ciulei[8] stated that, aqueous extract contained hydro-soluble compounds compared to ethanolic extract which possesses hydro-soluble substances lipid substances, alkaloids and polyphenols. Also, since the layers of the egg shell are hydrophobic, the aqueous extract has the facility to penetrate the layers than the ethanol extract which has an oil aspect in this work. Conversely, the greater larvicidal activity of ethonolic extract could be due to alkaloid. The later creats basidicity condition. It is uncomfortable for the survival of the larvae which prefers acid conditions. In general, we observed that, the increase of the concentration of each extract led to the increase of the anthelmintic activities. These observations showed that, an increase in concentration represent a supplementary input of different active compounds. In conclusion, the *in vitro* anthelmintic activities of aqueous and ethanolic extracts of seeds of papaya were shown in this work. Further experiment incorporating *in vivo* and toxicological studies should be carried out.

Conflict of interest statement

We declare that we have no conflict of interest.

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