Veterinary Parasitology 169 (2010) 198–203

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Short communication

In vitro anthelmintic activity of *Combretum molle* (R. Br. ex G. Don) (Combretaceae) against *Haemonchus contortus* ova and larvae

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ARTICLE INFO

Article history:
Received 13 August 2009
Received in revised form 16 December 2009
Accepted 21 December 2009

Keywords:
Anthelmintic activity
Combretum molle
Haemonchus contortus
Solvent:solvent fractions

ABSTRACT

Parasitic nematodes, especially *Haemonchus contortus* (Rudolphi), are among the most common and economically important causes of disease in sheep and goats owned by pastoralists and small holder farmers in Africa. The control of these infections relies mainly on the use of anthelmintic drugs. However, herbal preparations are widely used by pastoralists and small holder farmers for the treatment of their livestock against helminth parasites. The anthelmintic effect of acetone leaf extract and fractions of *Combretum molle* was investigated to determine the relative efficacy of the components against gastrointestinal sheep nematodes. The fractions were obtained by solvent:solvent extraction from the acetone extract. These were evaluated for nematocidal activity by means of an egg hatch (EHA) and larval a development and viability assay (LDVA) *in vitro*. The effect of the test extracts on the hatchability of eggs and development of first to third stage larvae and the survival rate of the third stage larvae. *H. contortus*, were used to determine the relative bioactivities. Best-fit LC_{50} values were computed using global model of nonlinear regression curve-fitting. The extracts inhibited egg hatching and development of the larvae of *H. contortus* in a concentration-dependent manner. Best-fit LC_{50} values for the egg hatch test were 0.866, 0.333, 0.833, 0.747, and 0.065 mg/mL for acetone extract, n-butanol, hexane, chloroform, and 35% water in methanol fractions, respectively. The best-fit LC_{50} values for the LDVA were 0.604, 0.362, 1.077, 0.131 and 0.318 mg/mL for the acetone extract, butanol, hexane, chloroform, and 35% water in methanol fractions, respectively. In the EHA the 35% water in methanol fraction was significantly more active than all the other fractions ($p < 0.05$); however the activity was not significantly different with the LDVA. *C. molle* leaf could find application in anthelmintic therapy in veterinary practice.

1. Introduction

Gastrointestinal parasites are of major economic importance in livestock because they cause clinical and sub clinical infections that reduce animal survival and depress growth rates, wool and milk production, and reproductive performance. Animal deaths due to nematode infections are very common in tropical and subtropical regions, where marginal levels of nutrition exacerbate the detrimental effects of infection (Waller, 1997). Control programs based solely on the use of synthetic anthelmintics are no longer considered sustainable because of an increased prevalence of gastointestinal nematode resistance (Barry and Mc Nabb, 1999), the slow development of new anthelmintics, high costs to poor

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doi:10.1016/j.vetpar.2009.12.036

farmers and concerns regarding residue in food and the environment. Alternative methods of control are thus required that are both practical and realistic for introduction into farm production systems.

Traditional health practitioners in many parts of Africa usually employ the leaves and barks of *Combretum* species as remedies for a variety of human ailments, including abdominal discomfort, body pains, respiratory disorders, colds and fevers, ear and eye ailments, schistosomiasis, hookworms, dysmenorrhoea and infertility in women, leprosy, syphilis, microbial infections and general body weakness (Hutchings et al., 1996). It is regarded as a medicine for both humans and animals. The leaves of *Combretum molle* contain steroidal acids and saponins (Pegel and Rogers, 1985). Many triterpenoids and their glycosides have been isolated from the leaves of South African *Combretum* species (Pegel and Rogers, 1985; Rogers and Verotta, 1996).

The aim of this study was to investigate the anthelmintic effect of acetone leaf extract and fractions of *C. molle* and the relative efficacy of the components against gastrointestinal sheep nematodes.

2. Materials and methods

2.1. Preparation plant extracts

The leaf of the plant *C. molle* was collected in Zaria, Nigeria. Voucher specimens (No. 900191) were identified and deposited by the Herbarium Section of the Biological Sciences Department, Ahmadu Bello University, Zaria. The plant material was air dried and ground to a powder (170 g) using a Macsalab Model 200 LAB grinder. The extract was prepared by maceration with shaking (Labotec Model 20.2 shaker) for 24 h in 70% acetone (A) with a 10:1 solvent to dry weight ratio (Eloff, 1998) and the extract was filtered through Whatman No. 1 filter paper using a Buchner funnel, and the acetone removed by air drying.

A solvent:solvent group separation procedure used by the USA National Cancer Institute as described by Suffness and Douros (1979) was adopted to fractionate the acetone extract with a slight variation. The acetone extract (15.80 g) was dried in a rotary evaporator under reduced pressure and this extract was dissolved in a 1:1 mixture of chloroform and water. The water fraction was extracted with an equal volume of butanol in a separating funnel to yield the water and butanol fractions. The chloroform fraction was dried in a rotary evaporator under reduced pressure and extracted with a 1:1 mixture of hexane and 10% water in methanol. The hexane fraction was recovered with a separating funnel. The 10% water in methanol extract was diluted to 35% water in methanol and extracted by adding equal volume of chloroform to yield the chloroform fraction and the 35% water in methanol fractions.

2.2. Haemonchus contortus egg recovery

H. contortus eggs were recovered from faeces according to Hubert and Kerboeuf (1992). Sample of faeces (10–15 g) were collected from sheep experimentally infected with mono-specific larval suspensions of fresh *H. contortus*. The faecal samples were suspended in water and cleared of organic debris by filtration through 1 mm and 150 μm sieves. Eggs were collected on a 25 μm sieve and further cleared of organic debris by centrifugation in magnesium sulphate (density 1.10) for five minutes at 1000 × *g*. The supernatant was filtered through 100 μm and 63 μm sieves and the eggs were washed in water and collected on a 25 μm sieve. The concentration of eggs was estimated in 200 μL samples and adjusted to 500 eggs/mL. 5 μg/mL amphotericin B solution (Sigma, Germany) was added to the egg suspension to avoid fungal development.

2.3. Egg hatch assay

The *in vitro* egg hatch assay was based on the method described by Coles et al. (1992). Egg suspension (0.2 mL) was distributed in a 48-flat-bottomed microplate so that each well contain100 fresh eggs and mixed with the same volume of plant extract dissolved in acetone at concentrations of 10 mg/mL in 8 serial dilutions. Albendazole (99.8% pure standard reference) (Sigma, USA) was used as a positive control. The albendazole was dissolved in dimethyl sulfoxide (DMSO) and diluted at concentrations between 1 μg/mL and 0.0075 μg/mL. The control plates contained the diluents water and acetone or 0.3% DMSO and the egg solution. The eggs were incubated in this mixture for 48 h at 27 °C and 70% relative humidity. After this time a drop of Lugol's iodine solution (Reidel de Hae, Germany) was added to stop the eggs from hatching. All the eggs and first-stage larvae (L_1) in each plate were counted. There were three replicates for each concentration and control.

2.4. Larval development and viability assay

The procedures used were a modification of the technique described by Hubert and Kerboeuf (1992). 150 μL aliquots of egg suspension which contained approximately 100 eggs and 20 μL of filtrate obtained by faecal washing during egg recovering were distributed to wells of a 48-well flat-bottomed microtiter plate. This suspension was supplemented with 30 μL of the nutritive medium described by Hubert and Kerboeuf (1984) and comprised of Earle's balanced salt solution (Sigma, Germany) plus yeast extract (Sigma, Germany) in saline solution (1 g of yeast extract/90 mL of saline solution) at a ratio of 1:9 (v/v). The plates were incubated at 27 °C and 70% relative humidity. After 48 h, 200 μL of the extract, albendazole (Sigma, USA) or diluent (control) were added. The third stage larvae were recovered six days later. At this time the parasites were counted by separating the larvae into two classes, third-stage larvae (L_3) and other developmental stage larvae (L_1 and L_2). There were three replicates for each concentration and control.

2.5. Statistical analysis

The LC_{50} was determined by calculating the concentration of extract that gave a response halfway between the minimum and maximum responses in a concentration–