Veterinary Parasitology 179 (2011) 100–106

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Ovicidal and larvicidal activity of the crude extracts from *Phytolacca icosandra* against *Haemonchus contortus*

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ARTICLE INFO

Article history:
Received 19 November 2010
Received in revised form 16 February 2011
Accepted 23 February 2011

Keywords:
Egg hatch assay
Larval migration inhibition assay
Haemonchus contortus
Phytolacca icosandra

ABSTRACT

The development of anthelmintic resistance has impacted on the success of conventional anthelmintics (AH) for the control of gastrointestinal nematodes in grazing/browsing sheep and goats. Medicinal plants from the traditional herbolary in Mexico may provide new candidates that can be explored as alternative sources of AHs for ruminants. This study evaluated the leaf extracts derived from *Phytolacca icosandra* against infective L_3 larvae and eggs from *Haemonchus contortus* collected from sheep. Three extracts of different polarities were obtained from the leaf plants using ethanol, *n*-hexane and dichloromethane as the solvents. The effectiveness of the *in vitro* AH activity of the plant extracts was evaluated using larval migration inhibition (LMI) and egg hatch (EHA) assays. For the LMI assays, the ethanolic extract of *P. icosandra* showed 55.4% inhibition of larval migration at 2 mg/mL ($p < 0.05$). The dichloromethane extract of *P. icosandra* showed 67.1% inhibition of migration at 3 mg/mL ($p < 0.05$) and a dose-dependent response with an LD_{50} of 0.90 mg/mL. The *n*-hexane extract failed to show inhibition of larval migration at any concentration explored. In the EHA for the ethanol extract, the lowest concentration tested (0.15 mg/mL) resulted in inhibition of egg hatching greater than 72.6%. Therefore, the LD_{50} could not be calculated for this extract. The LD_{50} of the dichloromethane extract of *P. icosandra* was 0. 28 mg/mL. An egg hatch inhibition greater than 90% was observed with both the ethanolic and dichloromethane extracts when using a concentration of 0.90 mg/mL or higher. The *n*-hexane extract failed to show egg hatch inhibition at any concentration tested. The AH activity reported for *P. icosandra* could be attributable to the flavonoids, steroids, terpenoids, coumarins and/or saponins that were present in the ethanolic and dichloromethane extracts. A combination of more than one component may also explain the observed AH activity against the *H. contortus* life stages that were evaluated. In conclusion, the ethanolic and dichloromethane extracts of *P. icosandra* showed clear *in vitro* AH activity against the *H. contortus* eggs and the L_3 larvae. However, the hexanic extract of the plant leaves failed to show any *in vitro* AH activity.

1. Introduction

Gastrointestinal nematodes (GINs) are a major threat to the health and welfare of small ruminants raised on pasture worldwide (Athanasiadou and Kyriazakis, 2004; Sackett et al., 2006). They can affect the reproduction, and production through mortality, weight loss, and reduce milk and

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doi:10.1016/j.vetpar.2011.02.019

wool production (Sackett et al., 2006). To date, the control of parasitism due to GINs has relied on the repeated and often frequent use of commercial anthelmintic (AH) drugs. However, the worldwide emergence of drug resistant GIN populations has motivated the search for alternative approaches for the control of GINs in infected ruminants (Jackson and Miller, 2006).

One of the novel approaches investigated is the use of indigenous plant preparations commonly used in the herbolary against human parasites. The benefit of using these as possible livestock dewormers is that they are non-toxic and inexpensive, features that are important for farmers in developing countries (Taylor et al., 2001). These possible ethnoveterinary alternatives would be viable for small-scale livestock farmers who cannot afford the allopathic drugs and/or for larger, conventional farmers who cannot rely on the use of conventional veterinary products in their flocks (Gradé et al., 2008). Furthermore, plant-derived AH products are advantageous as they are less toxic, biodegradable and environmentally friendly (Hammond et al., 1997).

In Mexico, a number of plant species have been used as traditional ethnobotanical medicines to cure human diseases in the indigenous communities; these plants have mainly been used for treating intestinal worm infection in children (Aguilar et al., 2008). The search for plants with AH activity against the GINs of livestock has emerged from ethnoveterinary surveys and from the observation of AH activity in the tannin-rich fodder commonly used for feeding ruminants (Alonso-Díaz et al., 2010). The AH activity may be due to the presence of different plant secondary metabolites in the feed such as sterols, tannins, condensed tannins (Alonso-Díaz et al., 2008), coumarins (Hoskin et al., 1999), alkaloids, non-protein amino acids (Githiori et al., 2006) or terpenoids and saponins (Marie-Magdeleine et al., 2009). This activity is attributed to secondary metabolites that may be extracted from the raw plant material by different solvents according to the polarity of the molecules being extracted. For example, as used in the current experiment, ethanol extracts polar compounds, dichloromethane is used to extract medium polar compounds and *n*-hexane, non-polar compounds (Domínguez, 1979; Balansard et al., 1991).

The tropical pokeweed, *Phytolacca icosandra*, belongs to the Phytolaccaceae family. It is a flowering plant that is native to tropical America from Mexico to Peru and is found in the Caribbean islands. The berries from *P. icosandra* have traditionally been used as soap for washing cotton clothes and, also have molluscicidal, spermicidal and haemolytic properties (Treyvaud et al., 2000). The leaves and roots are used for various human and animal ailments such as scabies, ringworm, dandruff, itching, headache, rheumatism, skin irritations, stomach pain and intestinal roundworms of children (*Ascaris* spp.) (Fonnegra and Jimenez, 2007).

P. icosandra leaves contain high concentrations of triterpenoids and saponins (Treyvaud et al., 2000; Lavaud et al., 2001). With these features, *P. icosandra* appeared to be a good candidate to evaluate as an AH against nematode parasites of small ruminants. This study evaluated the *in vitro* AH activity of three different *P. icosandra* leaf extracts (polar, non-polar and medium polarity) against eggs and infective (L_3) larvae of *Haemonchus contortus* from sheep.

2. Materials and methods

2.1. Plant materials

P. icosandra leaves were collected in Yaxcabá, Yucatan, Mexico (20°33′N and 88°49′W) from February to April, 2009. The voucher specimen was authenticated and deposited in the herbarium of the Centro de Investigación Científica de Yucatán (CICY) under the following code number: *P. icosandra* (MMendez 1497).

2.2. Plant extraction

Polar, medium polar and non-polar compounds were extracted from the leaf extracts with different solvents (ethanol, dichloromethane and *n*-hexane, respectively). The extracts were obtained from a sample of 134.5 g of *P. icosandra* leaves that were dried at 40 °C for 72 h and ground in a grinder with a 5 mm diameter mesh. For the ethanolic extract, the ground material was immersed in 100% ethanol for 72 h (using 1.0 mL of ethanol per 0.83 g of ground material). The ethanol extract was filtered and evaporated at 45 °C in a vacuum rotary evaporator (Buchi®) to give the crude ethanolic extract. The process of maceration was repeated three times. The crude plant extract was transferred to glass vials and kept at 4 °C until use.

A portion of the crude ethanolic extract (4.3 g) was diluted with 150 mL of methanol and 225 mL of water to obtain a 2:3 ratio. The solution was stirred for 10 min with an electromagnetic stirrer and filtered using a Buchner funnel. *n*-Hexane (375 mL) was added to obtain the *n*-hexane extract, which was filtered and evaporated at 45 °C using a vacuum rotary evaporator to give the non-polar extract. The extraction process was repeated twice.

The dichloromethane extract was obtained from the aqueous portion of the *n*-hexane procedure (375 mL). The same amount of dichloromethane (375 mL) was added to the solution and placed in a separation funnel, where it was agitated manually. The filtrate was evaporated to give the dichloromethane extract. The extraction process was repeated twice.

The percentage yield of plant materials after extraction with each solvent was calculated.

2.3. Phytochemical study

The phytochemical tests to detect the presence of flavonoids, steroids, terpenoids and alkaloids were performed with the ethanolic, hexanic and dichloromethane extracts following the methods previously described by Domínguez (1979). These tests are based on visual observation of colour change or the formation of precipitates after the addition of specific reagents. To detect the presence of different metabolites in the three extracts of *P. icosandra* leaves, the Shinoda (flavonoids), Salkowsky (steroids and terpenoids) and foam-haemolysis (saponins) tests were