Veterinary Parasitology 189 (2012) 284–290

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

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

# *In vivo* anthelmintic activity of *Phytolacca icosandra* against *Haemonchus contortus* in goats

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ARTICLE INFO

*Article history:*
Received 28 October 2011
Received in revised form 4 April 2012
Accepted 12 April 2012

*Keywords:*
***Phytolacca*** *icosandra*
Ethanolic extract
*Haemonchus contortus*
*In vivo* anthelmintic activity
Plant secondary metabolites
Goats

ABSTRACT

The *in vivo* anthelmintic (AH) activity of the ethanolic extract from leaves of *Phytolacca icosandra* was evaluated in goats artificially infected with *Haemonchus contortus*. Parasite naïve goats were artificially infected with 3000 *H. contortus* infective larvae per animal. Once the infection was patent (day 28 post-infection) all the animals were sampled to determine the faecal egg counts (FEC) for five consecutive days. Two groups of animals were formed balanced for their FEC and body-weight (BW) ($n = 6$/group): the non-treated control group and the treated group in which goats were individually administered with the ethanolic extract of *P. icosandra*. The extract was administered orally using gelatin capsules (250 mg/kg BW) which were dosed on two consecutive days using a pill-dispenser. Faecal samples were collected from each animal from the day of dosage (Day 0) on a daily basis to determine the number of eggs per gram of feces (EPG) for 15 days post-treatment (PT). The FEC of the two groups were compared using the repeated measures analyses of variance using the log transformed data Ln (FEC + 1). The presence of saponins, coumarins, flavonoids, steroids and terpenoids were detected by standard methodologies in the extract. The *P. icosandra* ethanolic extract was further analyzed by gas chromatography (GC) coupled to a mass spectrometry (GC-MS). A significant reduction in FEC was observed in the treated group compared to the control from day 7 until day 15 PT ($P < 0.05$). The highest percentage reduction (72%) was found on day 11 PT. No adverse reactions were observed in all treated animals for the entire trial. The GC-MS analysis of the organic extracts revealed the presence of three fatty acids as compounds with highest abundance. The three compounds that were identified by their mass fragmentation patterns were: 2-Pentadecanone, 6, 10, 14-trimethyl (RT 10.3 min), Pentadecanoic acid, 14-methyl-, methyl ester (RT 10.8 min) and Hexadecanoic acid, ethyl ester (RT 11.2 min). It is concluded that the *P. icosandra* ethanolic extract obtained from leaves showed *in vivo* anthelmintic activity against *H. contortus* when administered orally to goats at a dose of 250 mg/kg BW on two consecutive days. The dose used did not cause any negative effects on the health of goats.



## 1. Introduction

The nematode parasite, *Haemonchus contortus*, is an important nematode pathogen of small ruminants in the production systems based on grazing or browsing (Waller et al., 2006). The mortality rates due to gastrointestinal nematode (GIN) infections, in which *H. contortus* can

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0304-4017/$ – see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.vetpar.2012.04.017

make an important contribution, may exceed 40%, while weight losses of 50% may occur under tropical conditions (Githigia et al., 2001; Torres-Acosta et al., 2004). Conventional anthelmintic (AH) drugs have been used to minimize the effect of those parasites in ruminants. However, the sustainability of this control method has been compromised due to the emergence of *H. contortus* strains resistant to different AH families (Kaplan, 2004; Canul-Ku et al., 2012). The anthelmintic resistance problem and the growing demand for animal products free from chemical residues, has led to the development of alternative methods to the control of helminths in small ruminant production systems (Jackson and Miller, 2006; Torres-Acosta and Hoste, 2008). The use of traditional medicinal plants, or ethnoveterinary alternatives (based on medicinal plants), is considered a viable options for the control of GIN amongst small-scale goat farmers who cannot pay for allopathic drugs (Tarik et al., 2009). Studies from various parts of the world have shown that certain plant species effectively reduce the parasite burden and are promising alternatives to conventional AH (Githiori et al., 2006). The tropical pokeweed, *Phytolacca icosandra*, belongs to the Phytolaccaceae family. It is a flowering plant that is native to tropics of North, Central and South America (from Mexico to Peru) and it can be found in the Caribbean islands. The berries from *P. icosandra* have traditionally been used as soap for washing cotton clothes and also have molluscicidal and spermicidal activity (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). A previous study reported the *in vitro* AH efficacy against *H. contortus* eggs (egg-hatch assay) and larvae (larval migration inhibition test) when using the ethanolic or the dichloromethane extracts from the leaves of *P. icosandra* (Hernández-Villegas et al., 2011). The *in vivo* efficacy of this plant extract has not been evaluated. The aim of the present study was to evaluate the *in vivo* AH efficacy of the ethanolic extract obtained from *P. icosandra* leaves against *H. contortus* in goats.

## 2. Materials and methods

### 2.1. Plant materials

The *P. icosandra* leaves were collected in Yaxcabá, Yucatán, Mexico (20°33′ N and 88°49′ W) in January 2011. 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* MMéndez 1497.

### 2.2. Production of the plant extract

The extract was obtained from a sample of 1000 g of dry *P. icosandra* leaves. The leaves were dried at 40 °C for 72 h and ground in a grinder with a 5 mm diameter mesh. 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 (liquid form). This process of maceration was repeated three times. The crude plant extract was transferred to glass vials and was kept at 4 °C until its use.

### 2.3. Phytochemical study

Phytochemical tests to detect the presence of flavonoids, steroids and terpenoids were performed on the ethanolic extract following the methodology previously described by Domínguez (1979). The tests were based on the visual observation of color change or the formation of precipitates after the addition of specific reagents. To detect the presence of different metabolites in the ethanolic extracts of *P. icosandra* leaves, the Shinoda (flavonoids), Salkowsky (steroids and terpenoids) and foam-haemolysis (saponins) tests were used. In addition, the determination of the color under UV light was used to detect the presence of coumarins (Lock, 1994). Gas chromatography coupled to a mass spectrometer detector (GC–MS) was used to separate and identify the volatile, semi-volatile and thermally stable molecules (or metabolites, or compounds) of a mixture; this technique allows the evaluation of the number of compounds that could be vaporized in the ethanolic extract of *P. icosandra*. The GC–MS equipment was an, Agilent Technologies 6890N Gas Chromatography Apparatus coupled with a mass selective detector 5973, using the following chromatographic conditions: split injection of 1 mL of a 1% concentration sample: column HP5 MS of phenyl methyl silicone 30 m × 0.25 mm, flow rate 1.0 mL/min (helium as carrier gas). A sample was analyzed with the column held initially at 80 °C for 1 min, increasing 15 °C/min, up to 300 °C end of the run time.

The results related to the number of compounds present on a sample can be presented as a graph with an *x*-axis that shows the retention time (RT) of a compound, and the *y*-axis that shows the intensity (abundance) of the signal (compound). Information related to the mass fragmentation pattern of each compound (signal) can be obtained as a graph where each peak represents an ion derived from the molecular ion (molecular weight) after its fragmentation in proportion of their abundance. The identification of components in the extract was performed by a computer-based library search. The fragmentation patterns of the mass spectra were compared with those from the NIST05 Libraries.

### 2.4. Infective larvae

Third-stage larvae of *H. contortus* were obtained from a donor sheep infected with a pure strain (CENID-INIFAP, sheep strain, México). When infection became patent (presence of nematode eggs in faeces) the faecal material was collected twice daily from each animal. The faeces obtained from the donor sheep were cultured at 28 °C for 7 days. The resulting third stage larvae ($L_3$) were harvested using a modified Corticelli-Lai (Rodríguez-Vivas and Cob-Galera, 2005). The infective larvae were counted and stored