

Anthelmintic Effect of *Dichrostachys cinerea* on Infectious L3 Larvae of Cyathostomes Resistant to Albendazol

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ABSTRACT

In vitro anthelmintic effect of raw aqueous extracts from leaves and bark of *Dichrostachys cinerea* on infectious L₃ larvae of Cyathostomes resistant to Albendazol was evaluated. An inhibition assay for larval hatching was made. Third-stage larvae (L₃) were exposed to raw aqueous extracts from leaves (4 mg/ml), and bark (6.8 mg/ml) of *Dichrostachys cinerea*, for three hours, then hatching was induced. Observations were made every 10 min. Paired T-Student was used to determine the differences in per cent of hatched larvae between the control and the treatment groups. GraphPadPrism 5.0.0 was used in the study. Significant inhibition levels were observed in all the extracts ($P < 0.05$) during the process; whereas hatching in the control group was 100 %, for the leaf and bark extracts of *D. cinerea* were 24.05, and 12.56 %, respectively. The results suggest the use of *D. cinerea* as an alternative anthelmintic treatment to Albendazol-resistant Cyathostome populations.

Key words: parasitic nematodes, Cyathostomes, hatching, third-stage larvae

INTRODUCTION

Worldwide, horses are exposed to a variety of parasites, the most commonly found are in the cyathostome group (Kuz'mina, 2012; Relf *et al.*, 2013). When the parasitic loads of cyathostomes are high, animal health can be seriously compromised (Mair, 1994; Matthews, 2008 and Matthews, 2014). Significant loads may encyst in the walls of the large intestine and remain there for years (Murphy and Love, 1997). These stages, particularly, the early L₃, are sensitive to most existing anthelmintic treatments available today (Monahan *et al.*, 1996).

Since 1960, nematode control has been made using frequent administration of synthetic anthelmintics (Parry *et al.*, 1993; Kaplan and Nielsen, 2010), with proven effectiveness in substantially reducing strongyle infection. However, they have likewise, contributed to antihelminth resistance, particularly by cyathostomes (Kaplan, 2004).

There is an international need to find alternatives for nematode control, that can contribute to reducing antihelminth use. One of them is plants with antihelminth properties (Molento *et al.*, 2011). Antihelminth activity may be caused by the presence of different secondary metabolites (Athanasiadou and Kyriazakis, 2004), such as cyanogenic glycosides, sterols, tannins (Alonso-Díaz *et al.*, 2008), alkaloids, nonprotein amino acids (Githiori *et al.*, 2006), or terpenoids and saponins (Marie-Magdeleine *et al.*, 2009).

In vitro tests may be used as a preliminary step to assess the possible anthelmintic effects of plant extracts (Costa *et al.*, 2002). Several me-

thods are commonly used to examine anthelmintic activity, both from drugs and plants. In that sense, *in vitro* assays have proved their relevance and higher cost effectiveness than *in vivo* assays. Currently, the larval exsheathment inhibition assay (Bahuaud *et al.*, 2006) is used to achieve that goal (Alonso-Díaz, Torres-Acosta, Sandoval-Castro and Hoste, 2011).

Dichrostachys cinerea (sickle bush) is a shrub or small tree of about 4 to 5 m high, very widespread in Cuba, particularly in the province of Camagüey. Its fruit and bark are used as antiseptic because the plant is rich in tannins (Roig, 1974), and farmers use it commonly to treat elephantiasis, syphilis, gonorrhoea, leprosy, and as vermifuge in Sierra Leone (Dalziel, 1948). The aim of this paper is to evaluate *in vitro* the antihelminth effect of aqueous extracts of *Dichrostachys cinerea* on infecting larvae (L₃) of Albendazol-resistant cyathostomes, through the larval exsheathment inhibition assay.

MATERIALS AND METHODS

Extract preparation

All plant parts used in the experiment were collected at the University of Camagüey. To prepare the raw aqueous extract from *D. cinerea* leaves, 4 g of fresh leaves were crushed in mortar, and then mixed with 20 ml distilled water. After twelve hours of rest, it was filtered through successive sieves of 50 and 25 μm and, finally, through Watman 0.45 μm . The filtrate was used in the experiment, and the concentration was calcu-

lated using 100 µl oven-dried and previously weighed. For preparation of the raw aqueous extract, 8 g from the *Dichrostachys cinerea* trunk's bark was mixed with 30 ml of water and the same treatment was applied.

Infecting larvae

The larvae (L₃) were obtained by cropoculture, from equine feces previously diagnosed with Albendazol resistant nematodes by the fecal egg count reduction tests.

Larval exsheathment inhibition assay

The assay was made according to Aguilera and Delgado (2015).

Statistical analysis

A paired T-student was used to determine the percent of exsheathed larvae, between the control and the treatment groups. GraphPadPrism 5.0.0, was used for statistical analysis.

RESULTS AND DISCUSSION

Fig. 1 shows the total exsheathment percent of L₃ in the control group, and the groups treated afterwards by contact with hypochlorite solution at 0.0066 %, for 60 min. Exsheathment in the control group was 100 %; however, the 3 h contact with the aqueous extracts caused a significant inhibition ($P < 0.05$) of exsheathment. After 60 min, only 24.05 % and 12.56 % of larvae had exsheathed for *D. cinerea* (leaves) and *D. cinerea* (bark), respectively.

Fig. 2 shows exsheathment kinetics for 60 min. In the control group and the groups under *D. cinerea* (leaves) treatment, exsheathment began at 20 min, and for *D. cinerea* (bark), at 30 min.

This is the first report of anthelmintic activity of aqueous extracts of *D. cinerea* on infecting third-stage cyathostome larvae. Although the tannin contents used in the extracts were not quantified in this research, all the plant parts have, according to Roig (1974), a high level of tannins; therefore, the authors assume that the anthelmintic effects observed are produced by such compounds.

Exsheathment of L₃ represents a transition from the free-life phase to the parasitic one, and it is essential in the life cycle of nematodes. Tannins have been reported to interrupt exsheathment, thus prevent the settlement of infecting larvae in the host, as well as the ensuing infection (Brunet *et al.*, 2007). The mechanism of action of tannins on L₃ remains undetermined, though some data

support the hypothesis of a direct effect (Brunet *et al.*, 2008).

The mode of action of tannins on larval exsheathment might involve tannin ability to bind aminoacids of glycoproteins present in the cuticle, or to shut down enzymes involved in that process, and therefore, affect exsheathment (Hoste *et al.*, 2006; Iqbal *et al.*, 2007).

This result also confirms the effect of tannins on cyathostome species for the first time, which is crucial when dealing with Albendazol resistant nematodes.

CONCLUSIONS

Both extracts had anthelmintic activity, which corroborates *D. cinerea* as a useful treatment against parasites. *In vivo* studies are also necessary to confirm its anthelmintic properties, and assess its use for sustainable management of gastrointestinal nematodes.

RECOMMENDATIONS

Phytochemical studies must be developed to quantify and correlate tannin contents to anthelmintic activity.

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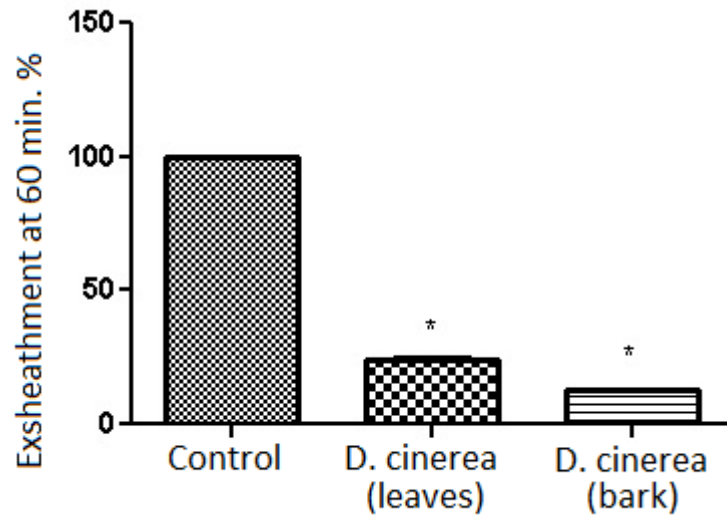


Fig. 1 Effect of raw aqueous extracts of *D. cinerea* on exsheathment percent at 60 min
* (P < 0.05)

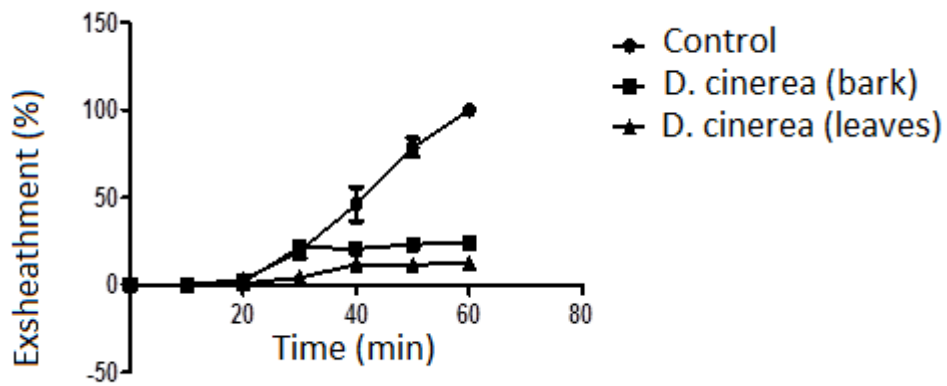


Fig. 2 Effect of raw aqueous extracts of *D. cinerea* on enhanced exsheathment kinetics of third stage infecting cyathostome larvae (L3)