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Short communication

Ovicidal and larvicidal activity of extracts of *Opuntia ficus-indica* against gastrointestinal nematodes of naturally infected sheep

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ABSTRACT

This study describes the *in vitro* anthelmintic activity of extracts from *Opuntia ficus indica* against gastrointestinal nematodes of sheep. The anthelmintic activity was evaluated by inhibition of egg hatching, larval development and larval migration assays. The residual aqueous fractions from cladodes and fruits showed higher ovicidal activity with EC_{50} values of 7.2 mg/mL and 1.5 mg/mL, respectively. The aqueous, hexane, and ethyl acetate fractions from fruits and the aqueous fraction from cladodes inhibited 100% of larval development at the lowest concentration tested (1.56 mg/mL). The crude cladode and fruit ethanolic extracts inhibited larval migration and showed EC_{50} values of 0.74 mg/mL and 0.27 mg/mL, respectively. Phytochemical screening detected high concentrations of alkaloids, tannins, flavonoids, and saponins in the fruits and cladodes. The results demonstrated that *O. ficus* exhibits anthelmintic activity *in vitro*, suggesting that, beyond its nutritional potential, this plant can also be an ally for parasite control in sheep.

1. Introduction

The indiscriminate use of synthetic anthelmintics has selected resistant parasites, further aggravating the problem of parasitosis in small ruminants (Botura et al., 2013).

Medicinal plants have emerged as an alternative to the use of synthetic anthelmintics worldwide, but there is a lack of scientific evidence regarding their effectiveness and their excess ingestion may pose a risk to animals (Rajeswari, 2014). Therefore, it is mandatory to evaluate whether medicinal plants are safe to use for the control of parasitosis in small ruminants.

Opuntia ficus-indica, belonging to the family Cactaceae, is a common plant in the Cerrado and Caatinga biomes (Griffith, 2004). This plant displays anti-inflammatory, analgesic, antioxidant and antiviral activities (Stintzing and Carle, 2005). Furthermore, a review on folk veterinary medicine demonstrated the use of *O. ficus* as a plant for ethnoveterinary medicine in Italy (Viegi et al., 2003), and in Brazil this species is widely used in feeding sheep (Veras et al., 2002). Considering that sheep feed on *O. ficus* in several places, and that finding alternative ways to control parasitosis is necessary, this study evaluated the ovicidal and larvicidal activity of *O. ficus* by means of *in vitro* assays.

2. Materials and methods

2.1. Plant materials

Plant materials were collected in the municipality of Ilha Solteira in the state of São Paulo, Brazil in September 2014. Cladodes were dried in a circulating air oven (50 °C). The pulp was removed from ripe fruits and frozen. The crude cladode ethanolic extract (CCE) was prepared from 28 g of powdered cladodes in 200 mL of ethanol (70%) by the exhaustive maceration method for seven days. The crude fruit ethanolic extract (CFE) was obtained by centrifuging the pulp (2054g) with 10 mL of ethanol (70%) for 15 min. The supernatant was filtered and evaporated. The partition of the extracts was performed by diluting 1.5 g of the crude extract with 20 mL of ethanol (70%) and successively extracting with hexane, dichloromethane, and ethyl acetate. The extraction process was repeated three times for each solvent. All extracts were frozen.

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Table 1
Percentages of inhibition of egg hatching (mean ± SD) of sheep gastrointestinal nematodes (95% *Haemonchus contortus*) by the *O. ficus* extracts and EC_{50} of the *O. ficus* extracts.

Concentration(mg/mL)	Fruit extract					Cladodes extract				
	Ethanol	Hexane	Dichloro-methane	Ethyl acetate	Residualaqueous	Ethanol	Hexane	Dichloro-methane	Ethylacetate	Residualaqueous
100	100.0 ± 0.0^{Aa}	–	–	–	–	93.0 ± 2.2^{Bb}	–	–	–	–
50	98.2 ± 0.5^{Aa}	–	–	–	–	90.5 ± 1.5^{Bb}	–	–	–	–
25	58.5 ± 2.6^{Bc}	47.0 ± 2.2^{Bd}	32.2 ± 2.3^{Be}	98.7 ± 1.5^{Aa}	100.0 ± 0.0^{Aa}	77.5 ± 2.1^{Cb}	14.2 ± 1.7^{Bf}	32.7 ± 1.7^{Be}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}
12.5	24.7 ± 1.2^{Ce}	26.5 ± 1.0^{Ce}	22.5 ± 2.1^{Ce}	58.5 ± 1.9^{Bc}	90.0 ± 0.8^{Bb}	58.7 ± 2.3^{Dc}	6.7 ± 0.9^{Cf}	24.5 ± 2.6^{Ce}	51.5 ± 1.3^{Bd}	100.0 ± 0.0^{Aa}
6.25	14.0 ± 0.8^{Ce}	28.2 ± 2.3^{Cd}	15.0 ± 0.8^{De}	32.7 ± 1.9^{Cc}	84.2 ± 0.9^{Ca}	37.0 ± 2.2^{Eb}	7.5 ± 0.9^{Cf}	11.0 ± 0.9^{De}	15.0 ± 0.8^{Ce}	30.7 ± 0.9^{Bc}
3.12	13.0 ± 0.8^{Cc}	7.5 ± 0.3^{Dd}	6.7 ± 0.9^{Ed}	19.2 ± 0.9^{Db}	69.0 ± 0.8^{Da}	13.7 ± 0.9^{Fc}	7.5 ± 0.5^{Cd}	6.2 ± 1.3^{Ed}	7.0 ± 0.8^{Dd}	$16.5 \pm 0.6^{Cb,c}$
Albendazole 0.025	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}	100.0 ± 0.0^{A}
5% DMSO	6.0 ± 1.4^{D}	6.0 ± 1.4^{D}	6.0 ± 1.4^{E}	6.0 ± 1.4^{E}	6.0 ± 1.4^{E}	6.0 ± 1.4^{G}	6.0 ± 1.4^{C}	6.0 ± 1.4^{E}	6.0 ± 1.4^{D}	6.0 ± 1.4^{D}
EC_{50}	9.90	27.3	8.90	55.7	1.50	9.90	71.5	11.9	50.7	7.12

Small letters compare mean between lines and capital letters between columns ($p < 0.05$). – Not tested.

Table 2
Percentages of inhibition of larval development (mean ± SD) of sheep gastrointestinal nematodes (95% *Haemonchus contortus*) by the *O. ficus* extracts and EC_{50} of the *O. ficus* extracts.

Concentration (mg/mL)	Fruits extracts					Cladodes extracts				
	Ethanol	Hexane	Dichloro-methane	Ethylacetate	Residualaqueous	Ethanol	Hexane	Dichloro-methane	Ethylacetate	Residualaqueous
100	100.0 ± 0.0^{Aa}	–	–	–	–	100.0 ± 0.0^{Aa}	–	–	–	–
50	100.0 ± 0.0^{Aa}	–	–	–	–	100.0 ± 0.0^{Aa}	–	–	–	–
25	96.2 ± 0.9^{Aa}	100.0 ± 0.0^{Aa}	85.0 ± 0.8^{Bb}	85.7 ± 1.7^{Bb}	100.0 ± 0.0^{Aa}	99.7 ± 0.5^{Aa}	6.1 ± 0.5^{Bc}	5.6 ± 1.5^{Bc}	100.0 ± 0.0^{Aa}	100.0 ± 0.00^{Aa}
12.5	86.7 ± 2.2^{Bb}	100.0 ± 0.0^{Aa}	74.5 ± 1.7^{Cc}	83.2 ± 0.7^{Bb}	100.0 ± 0.0^{Aa}	98.7 ± 1.0^{Aa}	5.1 ± 0.8^{Bd}	5.9 ± 0.5^{Bd}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}
6.25	68.5 ± 2.1^{Cc}	100.0 ± 0.0^{Aa}	68.3 ± 1.3^{Dc}	77.1 ± 1.1^{Cc}	100.0 ± 0.0^{Aa}	73.7 ± 1.7^{Bb}	5.8 ± 0.3^{Bd}	4.9 ± 1.3^{Bd}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}
3.12	49.2 ± 0.9^{Dc}	100.0 ± 0.0^{Aa}	63.2 ± 0.9^{Db}	67.0 ± 2.1^{Db}	100.0 ± 0.0^{Aa}	51.2 ± 0.96^{Cc}	6.2 ± 1.0^{Bd}	5.2 ± 0.8^{Bd}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}
1.56	12.5 ± 0.7^{De}	86.0 ± 1.4^{Bb}	53.2 ± 1.7^{Ec}	44.2 ± 1.7^{Ed}	100.0 ± 0.0^{Aa}	50.3 ± 1.9^{Cc}	5.9 ± 0.7^{Bf}	5.0 ± 0.7^{Bf}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}
Ivermectin 0.010	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}
5% DMSO	5.1 ± 1.4^{E}	5.1 ± 1.4^{C}	5.1 ± 1.4^{F}	5.1 ± 1.4^{F}	5.1 ± 1.4^{B}	5.1 ± 1.4^{D}	5.1 ± 1.4^{B}	5.1 ± 1.4^{B}	5.1 ± 1.4^{B}	5.1 ± 1.4^{B}
EC_{50}	3.70	<1.56	1.70	1.20	<1.56	3.40	>25	>25	<1.56	<1.56

Small letters compare mean between lines and capital letters between columns ($p < 0.05$). – Not tested.