In vitro anthelmintic activity of Cassava (Manihot esculenta) extract on Trichostongyloid larvae

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Abstract

Anthelmintic resistance has become a serious world-wide problem and has affected ruminant productivity in many countries. Thus the search for alternative methods of worm control has been an important priority in many small ruminant industries. *Manihot esculenta* had been shown to reduce trichostrongyloid eggs in infected sheep fed with such fresh leaves for prolonged period of time. Thus, this study was carried out to evaluate the anthelmintic activity of Cassava (*Manihot esculenta*) leaves extract against larvae of Trichostrongyloid nematodes using larval paralysis assay. Phytochemical analysis was carried out to determine the presence of tannin, saponin, phenols and alkaloids. The results of this study showed that *Manihot esculenta* extract can be used to control the infective stage of trichostrongyloid parasites of small ruminants.

Keywords: trichostrongyloid, larvae, *Manihot esculenta* (Cassava), phytochemical analysis.

Introduction

In Malaysia, trichosrongyloid worms showed a resistance to wide range of anthelmintics in sheep and goats (Dorny *et al*., 1994 and 1995; Rahman, 1994; Pandey and Sivaraj, 1994; Chandrawathani *et al*., 1999; Sivaraj *et al*., 1994). In recent years a lot of research had focused on using of traditional medical herbs to control these worms and most of them showed significant effects on the egg-hatching and larval development of *Haemonchus contortus* and other trichostrongyloids (Ademola *et al.*, 2004, Al-Shaibani *et al.*, 2009, Marie-Magdeleine *et al*., 2010).
Manihot esculenta (Cassava) and locally known as Ubi kayu is a perennial shrub of the Euphorbiaceae and native to tropical and subtropical regions. It is used in human and animal food because it is rich in carbohydrates and it is considered as one of the main source of starch together with rice and corn (Grace, 1977). *Manihot esculenta* hyad been shown to reduce gastrointestinal nematodes eggs in infected sheep fed with fresh leaves for prolonged time (López, 2007, Sokerya, 2009). The present research will study the effect of cassava leaf extracts against the infective larvae of trichostrongyloids worms in infected goats.

Materials and Methods

(i) Plant collection and extracts preparation

Leaves of *M. esculenta* were collected in Bayan Lepas, Pulau Pinang, northwest Peninsular Malaysia. Voucher specimens were identified by botanists and deposited at the herbarium of School of Biological Sciences, Universiti Sains Malaysia, Penang and given a reference number 11182.
The leaves were washed and dried at room temperature and the milled to powder using an electrical blender and stored in dark tightly closed glass bottles. The crude leaf extract were prepared with four organic solvents: hexan, chloroform, ethyl acetate and methanol (80%) using Soxhlet's apparatus. Solvents were evaporated using a rotary evaporator and the residue extracts were dried at room temperature and then stored at 4ºC in small tightly closed universal glass bottles. *In vitro* assays were conducted at concentrations of 3.1, 6.2, 12.5 and 25 mg/ml with three replicates for each concentration after dissolving the dried extract in Tween 20 to improve the solubility of extract in water (Maciel *et al*., 2006).

(ii) Larvae collection and identification

Faecal samples were collected from the rectum of naturally infected goats in a private farm in Bayan Lepas, Penang, northwest Peninsular Malaysia. Larvae were cultured according to MAFF (1986), and 100 larvae were identified according to MAFF (1986). Five replicates were carried out and the percentage of each species calculated.

(iii) *In vitro* assay

In vitro assays were carried out using Larval Paralysis Assay (LPA) according to the recommendations of the World Association for Advancement of Veterinary Parasitology (WAAVP) (Taylor *et al*., 2002; and Coles *et al*., 2006). Briefly, 100µl containing approximately 100 larvae were added to 100µl of extract in 96-well plate and kept at room temperature for 24 h. The living (motile) and dead larvae were counted. Two controls were used; one positive (Tween 20) and one negative (ivermectin 0.01 mg/ml).

(iv) Phytochemical study

Phytochemical testes were carried out to detect the presence of alkaloids, flavonoids, steroids, phenols and tannins in the methanol extract of *M. esculenta* leaves (Jack & Okorosaye-Orubite 2008; Egwaikhide & Gimba, 2007).

Statistical Analysis

The lethal concentration (LC_{50}) of methanol extract was calculated from the linear regression ($y = ax + b$). Data from larval paralysis assay were compared using ANOVA and Tukey's test ($p<0.05$ using SPSS).

Results and Discussion

The examination of fecal samples revealed that *Haemonchus* was the predominant genus (72 ± 2.20%), followed by *Oesophagostomum* (20.2 ± 1.00%), *Trichostrongylus* (6.2 ± 0.86%) and *Ostertagia* (1.6 ± 0.68%).

Table 1 shows the mean efficacy of *M. esculenta* leaf extracts on trichostrongyloid larvae using larval paralysis assay. Methanol extract had higher efficacy against the larvae than others ($p<0.05$); concentraction of 25 mg/ml was more effective than other concentrations ($p<0.05$).

Table 1: Mean efficacy ± S.E. of *Manihot esculenta* leaves extract on Trichostrongyloid larval paralysis assay.

Concentrations mg/ml	*M. esculenta* leaves extracts			
	hexan	chloroform	ethyl acetate	methanol 80%
25	10.33 ± 1.20	4.33 ± 2.33	5.00 ± 2.31	59.33 ± 8.96
12.5	2.33 ± 0.67	1.67 ± 0.88	1.33 ± 0.67	57.33 ± 6.89
6.2	0.67 ± 0.67	1.33 ± 0.33	0.33 ± 0.33	24.00 ± 3.60
3.1	0.33 ± 0.33	0.33 ± 0.33	0.00 ± 0.00	12.67 ± 3.18
1.6	0.00 ± 0.00	0.33 ± 0.33	0.33 ± 0.33	1.67 ± 0.33
Ivermectin 0.01	99.67 ± 0.33			
Tween 20 (3%)	1.33 ± 0.40			

The relationship between the concentration of methanol extract and the number of dead larvae was seen as linear despite a concentration of 25mg/ml. As result of this linear regression, it has been used to determine the equation that governs the relation. The equation is given as $y = 4.981x - 5.268$ with $R^2 = 0.993$. The effective concentration of methanol extract (LC_{50}) that killed half of infective larvae was 11.1 mg/ml.

Phytochemical tests revealed the presence of alkaloids which were detected by producing of orange red precipitate after adding of Dragendroff's reagent. The presence of tannins were detected by the dark green colour formed after adding of 2 drops of 5 % ferric acid solution whereas saponins were detected by using frothing test.

Results and Discussion

Cassava decreased in the number of nematodes eggs and Coccidia oocysts in the faeces (Tien Dung *et al*., 2005). However, there are no previous reports on the efficacy of the plant on larvae of trichostrongyliod nematodes in goats.

In the present research, 11mg/ml of methanol extract killed half of the larvae and that was less effective than the control because the control was a synthetic anthelmintic which included pure active substances, while the extract contained several compounds and the active compound may be present in small amounts.

Phytochemical tests revealed the presence of phenols, tannins, alkaloids, and saponins. These compounds revealed anthelmintic activity in some plants (Maciel *et al*., 2006, Costa *et al*., 2008, Olivrira *et al*., 2008). The activity of *M. esculenta* against trichostrongyloid larvae may differ from that of other plants like *Melia azedarach* (Maciel *et al*., 2006), *Fumaria parviflora* (Al-Shaibani *et al.*, 2009), *Azadirachta indica* (Costa *et al*., 2008) and *Tabernaemontana citrifolia* (Marie-Magdeleine *et al*., 2010) and thus may differ in their respective active compounds.

Generally, the presence of condensed tannins in cassava extracts gives a good anthelmintic activity because it has direct and indirect effects against gastrointestinal nematodes (Athanasiadou *et al*., 2001, Iqbal *et al*., 2007). The mode of action of tannins as anthelmintic is attributed to their capacity to bind to some proteins of the metabolism or larva's organs and muscles causing a change in their functions and resulting in the paralysis or death (Martínez *et al*., 2010). The mechanism of saponin action can cause changes in the permeability of the cell membrane and pore formation, resulting in the disintegration of teguments of the parasites. Nevertheless, the mode of action of these organic substances still needs to be further investigated.

Conclusion

In this study, *M. esculenta* revealed anthelmintic activity due to the active substances in methanol leaf extract. However, further work should be carried out to isolate the active compounds responsible for anthelmintic activity and to understand their mode of action as well.

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