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In vitro ovicidal and larvicidal activity of methanolic leaf extract of *Manihot esculenta* (cassava) on susceptible and resistant strains of *Trichostrongylus colubriformis*

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ABSTRACT

This study aimed to represent the first report of the ovicidal and larvicidal activity of the methanolic leaf extract of *Manihot esculenta* (cassava) against eggs and larvae of susceptible and resistant strains of *Trichostrongylus colubriformis*. As well as, to determine the total tannin compounds, antioxidant activity and toxicity of the extract. The egg hatch test was used to evaluate ovicidal activity against unembryonated eggs, whereas larval feeding inhibition assay and MTT-formazan assay were used to evaluate larvicidal activity against first (L_1) and infective (L_3) larvae, respectively. The results showed no significant differences were detected between the sensitivities of susceptible and resistant strains of *T. colubriformis* to the extract. Eggs, L_1 and L_3 were significantly affected ($P < 0.001$) compared with negative control, and L_1 were more sensitive than the eggs and L_3. The total tannin compounds were investigated using tannin quantification assay and determined by 254.44 TAE/mg. The antioxidant activity was evaluated using the DPPH radical scavenging assay and the median inhibition concentration (IC_{50}) was determined by 2.638 mg/ml. Acute oral toxicity at dose of 5000 mg/kg, and sub-chronic oral toxicity at 500 and 1000 mg/kg of the extract were observed in male and female Sprague-Dawley (SD) rats. The acute oral toxicity revealed that the median lethal dose (LD_{50}) of methanolic extract of cassava leaves on SD rats was greater than 5000 mg/kg, whereas the sub-chronic oral toxicity did not show observed adverse effects at 500 and 1000 mg/kg per day for 28 days. In conclusion, the methanolic extract of cassava leaves has direct ovicidal and larvicidal activity against *T. colubriformis* strains with a safety margin for animals, and it may be potentially utilized as a source of natural antioxidants.

1. Introduction

Trichostrongylid nematodes of small ruminants are a global problem, particularly in developing countries (Gura, 2008). These parasitic worms may lead to a reduction in weight gain, decreased supplies of wool, milk, and meat, increased mortality, as well as increased costs associated with anthelmintic treatments and other control measures, thereby causing significant annual economic losses (Sissay et al., 2007). The annual economic loss resulting from the increased cost of controlling parasitic nematodes using anthelmintic drugs is estimated at billions of dollars worldwide. McLeod (1995) found that nematode parasites in sheep inflict the greatest cost to the livestock industry in Australia compared with ectoparasites (lice, ticks, and flies). De Haan and Bekure (1991) estimated the annual economic loss caused by production losses and livestock mortality at US$ 2 billion within sub-Saharan Africa, whereas Waller (2004) estimated it at US$ 26 million for Kenya. In Indonesia, the production losses caused by these

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parasitic nematodes in goats and sheep are estimated at US$ 7.1 and 5.6 million, respectively (Sani et al., 2004). The corresponding economic losses in India and Australia have been estimated at US$ 103 and 111 million, respectively (McLeod, 2004). The development of the resistance of trichostrongylid nematodes to anthelmintics, which has been detected globally, mostly among goats and sheep, has further exacerbated this problem (Jabbar et al., 2006; McKellar and Jackson, 2004).

Trichostrongylus colubriformis is a parasitic nematode of goats, sheep, camels, and cattle. It has also been detected in pigs, rabbits, dogs, and humans (Soulsby, 1982). Adult parasites infect the anterior part of the small intestine and sometimes the abomasum. Infected animals may exhibit numerous symptoms and lesions, such as loss of appetite, weight loss, emaciation, dry skin, dark-colored diarrhoea, fluid accumulation in tissue, myocardial atrophy, and skeletal muscular atrophy, weakness of the legs, hypoalbuminemia, hypophosphataemia, slight haemorrhaging, and enteritis. Trichostrongylosis is common among young goats and sheep, and infections with only 3000–4000 worms can cause death in two- to three-month-old animals (Hoste, 1989; Kusiluka and Kambarage, 1996; Love and Hutchinson, 2003; Maclean et al., 1987).

Trichostrongylus colubriformis has developed resistance to the three main broad spectrum anthelmintic drugs, benzimidazole, macrocyclic lactones, and imidazothiazoles (Gopal et al., 1999; Le Jambre et al., 2005; Maingi, 1991; Martin and McKenzie, 1990), and multiple resistance has also been detected in different parts of the world (Almeida et al., 2010; Dash, 1986; West et al., 2004).

In recent years, ethnoveterinary medicine has been receiving considerable attention as an alternative means to control gastrointestinal nematodes of ruminants (Athanasiadou et al., 2007). Researchers have focused on the anthelmintic activity of traditional medicinal plants, particularly those that have high toxic activities against parasitic nematodes but low toxicity in animals (Camurça-Vasconcelos et al., 2007; Iqbal et al., 2005; Katiki et al., 2011; Oliveira et al., 2009).

Attention had been recently focussed on the anthelmintic activity of *Manihot esculenta* (cassava) against gastrointestinal nematodes of ruminants (Bunyeth and Preston, 2006; Phengvichith and Preston, 2011; Sokerya et al., 2009). Cassava which has been reported to have antibacterial, antifungal, antimalarial, leishmanicidal, trypanocidal, and amoebicidal activity (Melo et al., 2009; Moundipa et al., 2005; Punthanara et al., 2009; Zakaria et al., 2006), has also exhibited *in vivo* anthelmintic activity against *Haemonchus contortus* (López et al., 2007) and direct larvicidal activity against its L_1 (Marie-Magdeleine et al., 2010). No published reports studying the ovicidal or larvicidal activity of cassava extracts on *T. colubriformis* could be traced in literature. Therefore, the objectives of the present study were to evaluate the *in vitro* ovicidal and larvicidal activity of the methanolic extract of cassava leaves on the eggs and larvae of susceptible and resistant strains of *T. colubriformis*, and also to determine the antioxidant activity and toxicity of this extract.

2. Materials and methods

2.1. Collection and preparation of plant materials

Cassava leaves were collected from Balik Pulau District, Penang Island, northwest Peninsular Malaysia in February 2009. Voucher specimens of cassava were identified by botanists and deposited at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia (USM), under reference number 11182. The leaves were washed with tap water, dried at room temperature under a ceiling fan, and then in an incubator at 40 °C for 3 days. The dried leaves were milled into powder using an electric blender and stored in individual dark glass bottles until further used.

2.2. Extraction and preparation of concentrations

A total of 100 g of plant powder was extracted in a Soxhlet apparatus using 80% methanol. The extract was collected and filtered using Whatman #1 filter paper, thereafter, the extract was concentrated using a rotary evaporator. The crude extract was collected and kept in a sealed dark glass vial at 4 °C until use.

Five serial concentrations (3.1, 6.2, 12.5, 25, and 50 mg/ml) of crude methanolic extract were used in this study. A stock solution of methanolic extract at 100 mg/ml was prepared by dissolving 10 g of crude plant extract in 3 ml of Tween 20 and then adding 97 ml of PBS (pH 7.2). This stock solution was used to prepare lower serial concentrations by diluting the stock further with distilled water.

2.3. Parasite isolates and experimental infection

The experiment with goats was approved by the Animal Ethics Committee of USM under approval number 68–331.

Two isolates of *T. colubriformis* were used: McMaster, isolated prior to 1960, is susceptible to all anthelmintics, and Arding, isolated in 2005, is resistant to benzimidazoles and levamisole. The L_3 of these parasite isolates were provided by the Commonwealth Scientific and Industrial Research Organization (CSIRO), McMaster Laboratories, Chiswick Research Station, Armidale, NSW, Australia. Two nematode-free goats, more than 6 months old, were kept individually indoors in an experimental farm in Penang Island. These goats were used as experimental animals to serve as sources of *T. colubriformis* eggs for further *in vitro* tests. Each animal was infected orally with 3000 L_3 of one strain according to the guidelines of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) [Efficacy of Anthelmintics: Specific Recommendations for Ovines 1999] (Vercruysse et al., 2001). Faeces were collected two months post infection, and the presence of eggs was verified.

2.4. Ovicidal activity test

Eggs were recovered from faeces as described by Schürmann et al. (2007) and the purified eggs were suspended in distilled water at 1000 eggs/ml. The egg hatch test (EHT) was used to evaluate the ovicidal activity of the