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In vitro activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*

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Abstract

Trichostrongylus colubriformis is an important cause of parasitic gastroenteritis in ruminants, where it causes protracted diarrhoea, rapid loss of weight, loss of production and death. The *in vitro* efficacy of extracts of *Peltophorum africanum* was determined against this parasitic nematode. Eggs and larvae of *T. colubriformis* were incubated at 23 °C in the extracts of the leaf, bark and root of *P. africanum* at concentrations of 0.008–25 mg ml^{-1} for 2 and 5 days, respectively. Thiabendazole and water were used as positive and negative controls, respectively. Inhibition of egg hatching and larval development increased significantly ($P < 0.05$) with increasing concentrations of the extracts. Concentrations of 0.2–1.0 mg ml^{-1} of the extracts of leaf, stem bark, and root bark of *P. africanum* completely inhibited the hatching of eggs and development of larvae. No eggs and larvae of *T. colubriformis* could be observed in wells incubated with all the three extracts at concentrations of 5 and 25 mg ml^{-1}. The *in vitro* model results support the traditional use of *P. africanum* against nematode parasites. Further research is required to isolate and structurally identify the active anthelmintic compounds, and to improve methods of plant extraction of the effective anthelmintic components that will be readily adaptable for use by rural communities against helminthosis.

Keywords: Ovicidal; Larvicidal; Extracts; *Trichostrongylus colubriformis*; *Peltophorum africanum*

1. Introduction

Gastrointestinal nematodes remain a major constraint to economic productivity of livestock throughout the world, being the chief parasitoses responsible for disease-related production losses arising from stock mortality, severe weight loss and poor production, especially in small ruminants (Perry and Randolph, 1999; Chiejina, 2001). *Trichostrongylus colubriformis*, an intestinal nematode, is one of the most important causes of parasitic enteritis causing protracted diarrhoea, weakness, loss of production and death. Infestation of sheep with *T. colubriformis* causes a severely infected animal to pass dark diarrhoea that has earned the parasite the name of ''black scours worm'' (Soulsby, 1982). The parasite is frequently identified in large numbers in infected sheep and cattle in South Africa (Horak, 2003; Horak et al., 2004). The infective larvae (L_3) of *T. colubriformis* have a high capacity to survive even in adverse weather conditions (Urquhart

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et al., 1996). In tropical areas, the high temperatures and rainfall favour the development of the free-living stages to infective stage throughout the year.

In the last 30 years, control of gastrointestinal nematode infections of ruminants has been achieved almost exclusively by use of pharmaceutically derived anthelmintics. Indeed, synthetic and semi-synthetically produced anthelmintics have for long been considered the only effective method of controlling helminthosis. However, in the extreme situations of subsistence farming where anthelmintics are either unavailable or unaffordable, massive mortalities of young stock are tragically commonplace in tropical Africa and Asia (Anon., 1992; Griggs, 1996). At the other extreme, misuse and or widespread intensive use of sometimes poor quality synthetic or semi-synthetic anthelmintics has led to development of a high level multiple anthelmintic resistance that may lead to failure of control of worm parasites in ruminants (Prichard, 1990; Maciel et al., 1996; Monteiro et al., 1998; Wolstenholme et al., 2004). These constraints indicate that entire reliance on pharmaceutically derived anthelmintics may present difficulties in the management of gastrointestinal parasitic infections in livestock, necessitating alternative methods of helminth control (Waller, 1997; Sanyal, 2001).

In recent times, there has been an increasing interest in ethnomedical and ethnoveterinary practices across the world, especially as it relates to the use of medicinal plants in treating various ailments. Use of indigenous plant preparations as livestock dewormers is gaining ground as one of the alternative and sustainable methods readily adaptable to rural farming communities (Hammond et al., 1997; Danø and Bøgh, 1999). Important opportunities exist through research on the traditional use of herbal medicine, since 80% of people in developing countries rely on phytomedicine for primary healthcare in both humans and animals (Plotkin, 1992; McCorkle et al., 1996). As ethnomedicine does not follow western paradigms of scientific proof of efficacy and safety, most medical and veterinary professionals distrust the use of herbs, and know little about them (Sofowora, 1982; Thompson, 1997).

Peltophorum africanum (weeping wattle) is a plant that is traditionally used to treat among other conditions, diarrhoea, dysentery, helminthosis and promotion of well being and resistance to diseases in man and animals (Watt and Breyer-Brandwijk, 1962; Van der Merwe, 2000; Van Wyk and Gericke, 2000). Several authors have investigated the phytochemistry of this species without testing the activity of extracts or isolated components (Bam et al., 1988, 1990; Mebe and Makuhunga, 1992; Khattab and Nassar, 1998). The extracts of *P. africanum*, however, have inhibitory effects against human immunodeficiency virus (HIV-1) reverse transcriptase and integrase (Bessong et al., 2005), and also antibacterial and antioxidant activities (Bizimenyera et al., 2005). Nevertheless, much work remains in the study and characterization of bioactive compounds extracted from *P. africanum*.

The aim of the present *in vitro* study was to establish the effects of acetone extracts of leaf, stem bark and root bark of *P. africanum* on the egg hatchability, larval viability and larval development (to infective stage, L_3) of the intestinal parasite of sheep and goats, *T. colubriformis*. This study is part of the ongoing work on isolation and characterisation of bioactive compounds from *P. africanum*.

2. Materials and methods

2.1. Collection, storage and preparation of plant material

Leaves (L), stem bark (B), and root bark (R) (referred to further as leaf, bark and root) were collected in spring from mature *P. africanum* Sond. (Fabaceae) trees growing naturally (and labelled S.A. tree number 215) at Onderstepoort, Pretoria, South Africa. A voucher specimen (PM 001) was stored in the medicinal plant herbarium, Department of Paraclinical Sciences, University of Pretoria. The collected plant material was dried in the shade, at ambient temperature. Dried plant material was ground to powder using a Mascalab mill (Model 200 LAB), Eriez®, Bramley. The powdered material was separately stored in dark tightly closed glass bottles before investigation.

2.2. Plant extracts preparation

Acetone has been shown to be a good extractant of compounds from plants (Eloff, 1998). A previous study on extraction of bioactive compounds from *P. africanum* (Bizimenyera et al., 2005) showed that acetone extracted the largest amount (quantity) of extracts from the plant material when compared to ethanol, dichloromethane, and hexane. Three grams (3 g) of each plant part (L, B, and R) was extracted in triplicate with 30 ml of technical grade acetone in glass bottles on a shaking apparatus for 1 h. Typically, extracts have to be dried and subsequently re-dissolved in suitable solvents to make up known concentrations for bioassays. As preliminary work had shown that there is incomplete solubility in acetone of the acetone extracts of *P. africanum*, the extracts were not dried to circumvent