

Efficacy of *Peltophorum africanum* Sond. (Fabaceae) Extracts on *Haemonchus contortus* and *Trichostrongylus colubriformis* in Sheep

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Abstract: The search for sustainable and easily affordable herbal medicines is on worldwide, prompted by emergency of anthelmintic resistance. Following substantially positive *in vitro* research, the efficacy and safety of extracts of *Peltophorum africanum* was determined against the parasitic gastrointestinal nematodes in sheep. Twenty four weaned male Dorper lambs were induced with infections (L₃ infective stages) of *Haemonchus contortus* (2,800 each) and *Trichostrongylus colubriformis* (3,500 each). Four weeks later, the sheep were dosed with acetone extracts of *P. africanum* at doses of 50, 500 and 750 mg kg⁻¹. The control group received no treatment. There was no significant (p = 0.073) faecal egg counts reductions at concentrations of up to 750 mg kg⁻¹, or reduction in total worm burden. However, the extracts showed no deleterious effects on the sheep. Feed consumption remained the same for both treated and untreated controls. Further research on appropriate dosage form or on isolation of effective anthelmintic compounds from *P. africanum* is called for.

Key words: Efficacy, safety, sheep, extracts, *Trichostrongylus colubriformis*, *Haemonchus contortus*, *Peltophorum africanum*

INTRODUCTION

One of the important restraints in livestock production is parasitic diseases. In the tropics and subtropics, parasitic gastrointestinal nematodes are prevalent and remain a major constraint to ruminant productivity. Parasitic nematodes rank as number one in causing production losses arising from stock mortality, severe weight loss and poor production, especially in small ruminants of resource-poor farmers in tropical Africa and South Eastern Asia (Perry and Randolph, 1999; Chiejina, 2001; Perry *et al.*, 2002). In various surveys, *Haemonchus contortus* and *Trichostrongylus colubriformis*, have been listed among the top ten most common nematodes hampering production of goats and sheep in tropical countries (Anon, 1992; Arosemena *et al.*, 1999; Horak, 2003; Horak *et al.*, 2004). Haemonchosis (caused by *H. contortus*) is characterised by anaemia, haemorrhagic gastroenteritis, hypoproteinaemia, sudden death or chronic emaciation, whereas infection with *T. colubriformis* causes protracted diarrhoea with dark stool, weakness, loss of production and death (Soulsby, 1982; Urquhart *et al.*, 1996). Adult female *H. contortus* have high egg-producing capacity of 5000-15000 eggs per day (Hansen and Perry, 1994),

whereas the infective larvae (L₃) of *T. colubriformis* have high capacity to survive even in adverse weather conditions (Urquhart *et al.*, 1996). The high fecundity, combined with the high rainfall and temperatures of the tropics, favour permanent larval development in the environment leading to the development of the free-living stages to infective larval forms (L₃) throughout the year.

Control of gastrointestinal nematode infections of small ruminants is almost exclusively by use of proprietary anthelmintics. However, these drugs are expensive and sometimes unavailable or unaffordable by subsistence rural farmers or remote pastoralist communities in developing countries, who as a result end up using adulterated or poor quality products (Monteiro *et al.*, 1998). Alternatively, the widespread intensive use of anthelmintic drugs by commercial farmers has created multiple drug resistance that has led to a failure to control helminths in ruminants (Prichard, 1990; Van Wyk *et al.*, 1997; Wolstenholme *et al.*, 2004; Jabbar *et al.*, 2006). In South Africa, the anthelmintic resistance situation in commercial farms has been described as very serious (Van Wyk *et al.*, 1999). These constraints have made the reliance on pharmaceutically derived anthelmintics at present difficult and necessitate alternative strategies of helminth control (Jabbar *et al.*, 2006; Waller, 1997;

Danø and Bøgh, 1999; Sanyal, 2001). Amongst these strategies, is use of plants as an alternative anthelmintic opportunity. Use of effective indigenous plant preparations as livestock dewormers would appear to be sustainable and affordable method readily adaptable to rural farming communities (Danø and Bøgh, 1999; Hammond *et al.*, 1997). However, due to concerns for scientific evidence of efficacy and safety, there is need for scientific validation of herbal medicines before their acceptance and use worldwide.

Peltophorum africanum (weeping wattle), a plant widespread in southern Africa and other tropical regions, is traditionally used to treat, *inter alia*, 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). Condensed flavonoids, a novel cyanomaclurin analogue (Bam *et al.*, 1988), profisetinidin-type 4-arylflavan-3-ols and related δ -lactones (Bam *et al.*, 1990) were found in the heartwood. Mebe and Makuhunga (1992) isolated bergenin, norbergenin and 11-0-(E)-*p*-coumaroylbergenin from the bark, while Khattab and Nasser (Khattab and Nasser, 1998) isolated coumarins from the leaves. *P. africanum* extracts have inhibitory effects against human immunodeficiency virus (HIV-1) reverse transcriptase and integrase (Bessong *et al.*, 2005), antibacterial (Obi *et al.*, 2003; Samie *et al.*, 2005) and antioxidant activities (Bizimenyera *et al.*, 2005, 2007) and ovicidal and larvicidal activities (Bizimenyera *et al.*, 2006 a, b) against *Haemochus contortus* and *Trichostrongylus colubriformis*. The latter was against both egg hatching and larval development.

As *in vitro* efficacy does not necessarily imply corresponding *in vivo* effect, the aim of the present study was to determine the efficacy and safety of acetone extracts of *P. africanum* on the reduction of faecal egg and total worm counts of the gastrointestinal parasites *Haemochus contortus* and *Trichostrongylus colubriformis* in sheep.

MATERIALS AND METHODS

Collection, storage and preparation of plant material: The plant material (root bark) was collected from mature *Peltophorum africanum* Sond. (Fabaceae) trees growing naturally (and labelled S.A Tree No. 215) at the Onderstepoort campus of University of 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 room temperature

before grinding to powder using a Mascalab mill (Eriez®, Bramley). The powdered material, weighing 1.2 kg, was stored in dark tightly closed glass bottles before investigation.

Plant extraction: The plant material was extracted overnight in acetone (in ratio of 1:10, weight for volume) in glass bottles on a shaker. To circumvent the problem of incomplete solubility in acetone, the extracts of *P. africanum* were not dried (Eloff, 2004). Known volumes of the extract containing 370 mg mL⁻¹ were put in bottles, which were sealed and stored in a refrigerator as stock solution before use. Other than use of capsules in the anthelmintic treatment tests (due to the residual acetone), the stock extract was diluted with distilled water and administered by stomach tube to sheep at 3 dose concentrations, 50, 500 and 750 mg kg⁻¹ body weight for three consecutive days.

Experimental animals: Twenty four young (6 months) weaned male Dorper lambs were bought from reputed breeders. After weighing on a calibrated scale, the lambs were dosed with albendazole (Valbazen, Pfizer) at the dose of 22 mg kg⁻¹ before the start of the study. The lambs were housed indoors in four groups of 6 each at the University of Pretoria Biomedical Research Centre (UPBRC), Onderstepoort, on concrete floors, under quarantine conditions. The animals were provided with a commercial ration, lucerne and hay and given free access to water. They were under supervision of a veterinarian on a daily basis throughout the study. The health of animals was observed and recorded daily. The cut-off point for salvage treatment for anaemia was a Packed Cell Volume (PCV) value of 15%.

Experimental design: The experimental design was a slight modification (use of plant extract instead of drug) of the method recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (Wood *et al.*, 1995).

Preparation and administration of infective larvae: Mono-specific larval suspensions of *H. contortus* and *T. colubriformis* were obtained from cryopreserved infective larval (L₃) stocks from Onderstepoort Veterinary Institute. After thorough mixing with a magnetic stirrer, 20 aliquots of the larval suspension were counted to establish the number of viable L₃ per mL. The required inocula of L₃ for both *H. contortus* and *T. colubriformis* were mixed in one syringe and orally administered. The L₃ were administered over 5 consecutive days yielding an

estimated total dose of 3,500 larvae of *T. colubriformis* and 2,800 of *H. contortus* per animal (Wood *et al.*, 1995). Treatment was effected 28 days after infection, after the infection had been confirmed through faecal egg counts using sugar floatation (Hansen and Perry, 1994).

Treatment procedures: Each animal was identified by an ear tag and distinctive coat marks. A day before treatment, nine days later and before necropsy the sheep were weighed along with a full haematology analysis of each. The sheep were randomly allocated according to body weight to 4 treatment groups, following a table of random numbers (Beyer, 1968) and according to the treatment groups: I-Control (no treatment), II-750 mg kg⁻¹, III-50 mg kg⁻¹ and IV-500 mg kg⁻¹ (Table 1). Extracts (prepared as in 2.2 above) were administered by stomach tube according to body weight on three consecutive days. All animals were subsequently, observed daily for any adverse effects of treatment. Feed intake was also noted.

Full haematology and liver enzyme analysis: Along with the full haematology, blood samples were analysed for liver enzymes, Aspartate Aminotransaminase (AST) and Gamma-Glutamyl Transpeptidase (GGT), the enzymes that may reflect liver function (pathology or hepatobiliary dysfunction) in herbivores.

Evaluation

Faecal egg counts: From 3 weeks after infection and on day 1 before treatments, day 1 and 6 after treatment, faecal

egg counts were determined on faecal samples collected per rectum with a lubricated gloved hand. The number of eggs per gram (epg) was determined in the standard manner using the McMaster slide. The faecal samples were collected at the same time each day to reduce the number of variables in egg counts.

Larval cultures and identification: From faecal eggs collected as 2.5.1, an egg suspension (with about 100 eggs per 200 µL water) was incubated in 48-well microtitre plates at room temperature for 48 hours. A drop of Lugol's iodine was added to each well and the larvae (L₁) were counted and identified according to established identification keys (Wood *et al.*, 1995; Van *et al.*, 2004). This helped to determine the establishment of infection and evaluation of possible effect of *P. africanum* extracts on the different worm species under investigation.

Adult worm counts: At necropsy, the abomasums and small intestines were tied off and freed of excess fat and mesenteric attachments. Each abomasum and small intestine was opened and its contents thoroughly washed into a graduated bucket and the volume of the wash made up to 2-3 L. After thorough mixing, 5% aliquots of the contents were fixed in formalin. The nematodes in the 5% aliquots were stained with Lugol's iodine for ease of counting. The contents were poured into flat dishes for counting.

Calculations and statistical analysis: The efficacy of anthelmintic action was determined by comparing the parasite/eggs populations in groups of treated and untreated animals and dose relationship determined. The following formula (Wood *et al.*, 1995) expresses the percent efficacy (E%) of a dose given against a given parasite species (S) in a given treatment group (T) when compared with an untreated control group (C):

$$E (\%) = \frac{\text{Mean of S in C} - \text{mean of S in T}}{\text{Mean of S in C}} \times 100$$

T-Test and ANOVA were used to determine whether there were differences between treated and untreated groups over time schedules.

RESULTS

Other than transient cough in some animals, no abnormal behavioural changes were observed after treatment of the animals with the extracts. None of the animals required salvage treatment in the 4 weeks post-inoculation with L₃. There was general decline of the PCV in all the infected lambs. There was no significant

Table 1: Treatment groups and individual doses

Group*	No.	Weight (kg)	Dosage (mg kg ⁻¹)	Total dose (mg)	Volume (mL)
Control (I)	1	32.5	Nil	0	-
	3	30.6	Nil	0	-
	4	23.3	Nil	0	-
	5	28.4	Nil	0	-
	17	25.8	Nil	0	-
	26	33.2	Nil	0	-
II	7	33.3	750	24975	67.5
	8	26.5	750	19875	53.7
	9	24.6	750	18450	50.0
	14	30	750	22500	60.8
	21	26.2	750	19650	53.1
	23	33	750	24750	66.9
III	2	25.7	50	1285	3.57
	6	31.5	50	1575	4.3
	11	29.8	50	1490	4.0
	12	26.2	50	1310	3.5
	19	27.5	50	1375	3.7
	22	34.6	50	1730	4.9
IV	10	25.7	500	12850	34.7
	13	26.1	500	13050	35.3
	15	31.3	500	15650	42.3
	16	21.7	500	10850	29.3
	24	28.1	500	14050	40.0
	25	31.6	500	15800	42.7

* Randomly grouped according to Table of Random Units (Beyer, 1968)

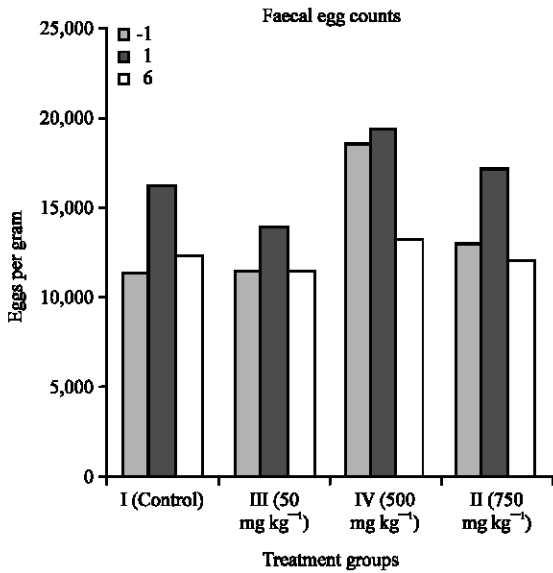


Fig. 1: Faecal egg counts (-1 = one day before, 1, 6 = one and 6 days after treatment)

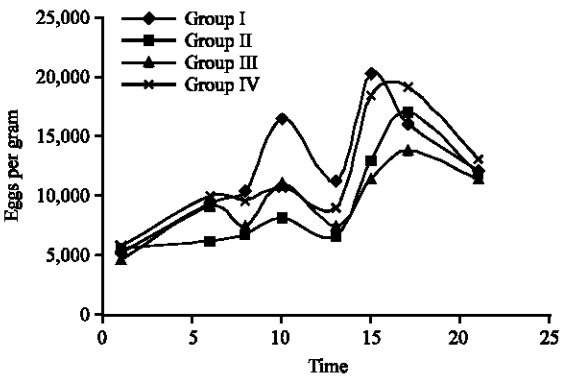


Fig. 2: Mean EPG per day of trial (I-untreated control)

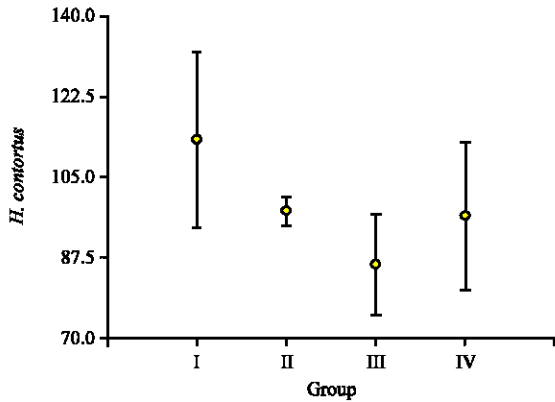


Fig. 3: *H. contortus* adult worm counts (I-untreated control)

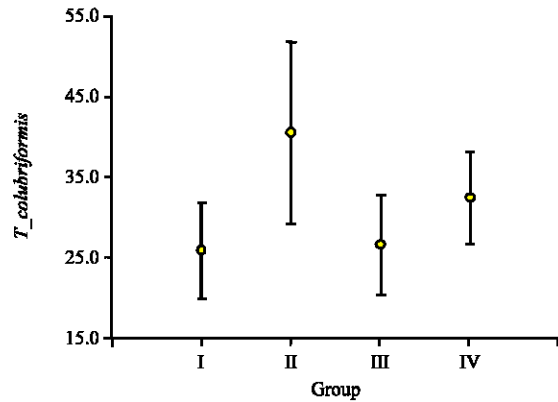


Fig. 4: *Trichostrongylus colubriformis* adult worm counts (I-untreated control)

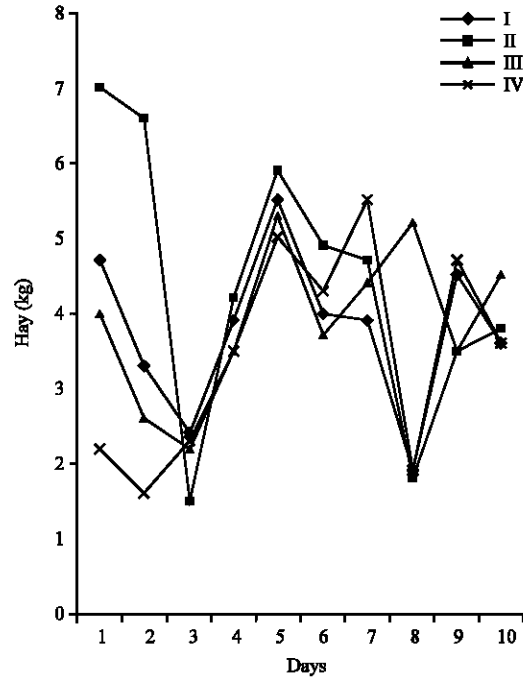


Fig. 5: Daily hay consumption (kg) per group, after treatment (I-untreated control)

difference in faecal egg counts between the treated and control groups (Fig. 1). By the time the experiment was terminated only a few animals had PCV values below 20%, but none below 15%.

Judging from the analysis of enzymes that reflect liver damage (results not shown), Gamma-Glutamyl Transpeptidase (GGT) and Aspartate Aminotransaminase (AST), there was no significant pathological or toxic effect on the animals by the extract treatment.

The faecal egg counts fluctuated, with no significant (with $p = 0.073$) difference between the treated and control groups after the various treatments (Fig. 2). More of the *H. contortus* than *T. colubriformis* developed (Fig. 3 and 4), despite the fact that the respective infecting doses with L_3 were 2,800 and 3,500.

The extracts did not effect a significant adult worm reduction in treated compared to control animals (Fig. 3 and 4). The administration of extracts did not affect the feed consumption in both treated and control groups significantly (Fig. 5).

Results of the experiment show that *P. africanum* extracts are safe but not effective against the *H. contortus* and *T. colubriformis* infections in sheep at doses and dosage forms administered in the current work.

DISCUSSION

The objective of the study was to establish efficacy and safety of *P. africanum* extracts on faecal egg and adult worm count reduction in sheep artificially induced with *H. contortus* and *T. colubriformis*. Anthelmintic efficacy was evaluated on faecal egg and adult worm count reduction. This is the first report of such anthelmintic trial in livestock involving *P. africanum*. Other investigators have either used plant powder or plant extracts in analogous work (Githiori *et al.*, 2002; Hördegen *et al.*, 2003; Hounzangbe-Adote *et al.*, 2005; Iqbal *et al.*, 2006a) all modifications of the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (Wood *et al.*, 1995).

In the *in vivo* model, up to 750 mg kg^{-1} of acetone extracts did not significantly reduce faecal egg counts of *T. colubriformis* and *H. contortus*. Githiori *et al.* (2004) found that seven plant species had no significant effect on faecal egg counts on lambs infected with *H. contortus* and fed aqueous extracts of the plants. Some factors could explain why extracts that were effective *in vitro* failed to show positive results *in vivo*. The dosage rate (750 mg kg^{-1}) used in the sheep experiments may not have provided sufficient knock-down effect. Some workers reported good effect when extracts were administered at doses of 1-3 g kg^{-1} body weight (Akhtar *et al.*, 2000; Suleiman *et al.*, 2005; Iqbal *et al.*, 2006b). The stock extract solution was very concentrated (370 mg mL^{-1}) and some of the extract adhered to the bottle, possibly limiting the effective concentration in the administered extract. There could also be stability factors of anthelmintic components, if plant material had overstayed. However, Eloff (1999) reported that some chemical components did not change and biological activity did not decrease significantly in over 100 year-old herbarium plant specimens. Was three days administration of the

extracts too short a period? Ademola *et al.* (2004, 2005) administered ethanolic extracts for 2 days.

Whereas, ovicidal and larvicidal action of *P. africanum* extracts on eggs and larval (L_1) forms of *H. contortus* and *T. colubriformis* has been reported (Bizimenyera *et al.*, 2006a, b), the extracts did not have a similar action on the adult worm parasites in the current work. The relevance of *in vitro* studies to *in vivo* efficacy, in regard to anthelmintic activity, is greatly influenced by the differences in the physiology and the bioavailability of plant preparations within animal hosts (Githiori *et al.*, 2005). The reservoir effect, whereby the extract gets dissolved in the large volume of fluid in the rumen and an increase in the mean residence time in the rumen due to the extract binding to rumen material, with little passing on to the abomasums may have reduced the bioavailability. What about the potential degradation of the extract by rumen microflora? Possibly different results could have been obtained if the extracts had been administered by direct injection into the abomasums or small intestine; Van Wyk and Gerber (1980) injected *H. contortus* and *T. colubriformis* infective larvae L_3 to establish infections in abomasums and small intestine, respectively. Athanasiadouou *et al.* (2001) reported direct anthelmintic astringent effects of condensed tannins on gastrointestinal nematodes. Alternatively, the extracts could be administered orally after the oesophageal groove is closed by suitable methods, such as pre-administration of copper sulphate solution (Gibson, 1975). Hence, improved methods of extraction to get an anthelmintic rich extract need to be developed.

P. africanum extracts however were found to be safe in the treated animals. Gamma-Glutamyl Transpeptidase (GGT) and Aspartate Aminotransaminase (AST), levels used to assess liver pathology, were not statistically different between treated and control lambs. Haematological analysis of these enzymes gives a good indication of liver pathology in herbivores (Reyers, 2005).

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