Chapter

Antiparasitic Efficacy of the Root Bark Powder of *Oldfieldia Dactylophylla* (Welw. Ex Oliv.) J. Léonard on the Digestive Strongyles of Grazing Goats in Lubumbashi (DR Congo)

Victor Okombe Embeya, Gaël Nzuzi Mavungu, Welcome Muyumba Nonga, Célestin Pongombo Shongo, Amandine Nachtergael and Pierre Duez

Abstract

In order to evaluate the efficacy of the root bark powder of *Oldfieldia dactylophylla* (Welw. ex Oliv.) J. Léonard (a Picrodendraceae), 32 locally breed grazing goats naturally infested with various gastrointestinal helminths were randomly assigned to four groups of eight animals: one untreated control, one positive control group treated with a reference anthelmintic (albendazole, 5 mg/kg), and two groups treated *per os* with *O. dactylophylla* root bark powder (100 and 200 mg/kg body weight, respectively). Four doses of these respective treatments were administered monthly. To evaluate parasitological, blood and zootechnical parameters, samples were taken on day 0, just before administration of the first treatment and on 14, 31, 45, 62, 76, 92 and day 126. *O. dactylophylla* was effective on day 14 after treatment with 69% strongle egg fecal excretion (both doses) versus 90% albendazole. However, efficacy was stabilized at 85, 86 and 89% for *O. dactylophylla* (100 and 200 mg/kg) and albendazole, respectively. These data support the ethnoveterinary use of this plant in the control of digestive parasitism in goat breeding. However, phytochemical studies support that the plant should make contributions to human studies in the future.

Keywords: gastrointestinal parasite, *Oldfieldia dactylophylla*, *Haemonchus contortus*, goat, Haut-Katanga

1. Introduction

In Lubumbashi (Haut-Katanga Province, southeastern DR Congo) and in its green belt, as in most tropical areas, goat husbandry has low productivity because of

inadequate management and poor animal health [1]. Parasite infections by gastrointestinal helminths remain among the main causes of this weak production [2–4]. *Haemonchus contortus* is one of the nematode species that dominate the parasite spectrum of goats in sub-Saharan Africa [5–7].

It is therefore of great importance, because of its prevalence and pathogenicity [8].

Control of these infections is generally based on the strategic use of anthelmintics [9, 10]. However, these anthelmintics are not always available or, when they are, their costs are so high that they are not readily available to farmers and breeders [11]. And so, in developing countries, that are heavily affected by these parasitic infections, the traditional methods of control used by herders remain largely dependent on medicinal plants [12–14]. It is therefore essential to find means of control that would be inexpensive and accessible to breeders.

In a survey we conducted in the regions of Kamina and Kaniama (Haut-Katanga province, DR Congo), *Vitex thomasii* De Wild (Verbenaceae) was the most cited traditional plant-based remedy applied to the control of gastrointestinal disorders in livestock [13, 15]. We have however recently determined that this species name is incorrect, the plant being locally confused with *Oldfieldia dactylophylla* (Welw. ex Oliv.) J.Léonard (Picrodendraceae); all the "*Vitex thomasii* " samples we could examine so far are in fact *Oldfieldia dactylophylla* [15].

O. dactylophylla is found in Africa, south of the Sahara (DR Congo, Tanzania, Angola, Zambia, Malawi), at altitudes from 1000 to 1800 m [16]. Our survey among breeders showed that root bark powder is used in animals on wounds or given, *per os*, against gastrointestinal parasitosis [13]. In humans, it is administered, always *per os*, against gastrointestinal parasitosis, back pain, hip or various joint pain. Its decoction is also administered orally in case of threats of miscarriage, asthenia, rheumatism, gonorrhea and diarrhea [17].

The aim of the present work was to evaluate the purported antiparasitic efficacy of the root bark powder of *O. dactylophylla* in grazing goats raised under natural conditions in DR Congo.

2. Material and methods

2.1 Plant material

The root barks of *O. dactylophylla* (vernacular name, kikoto muchi); previously wrongly identified as were harvested in Kelambwe (8°35′335 S; 24°414′422 E), Kankundwe (8°45′636 S; 24°50′491 E) and Kiabukwa (8°44′270 S; 24°54′253 E). A voucher sample was deposited in the Kipopo herbarium (Botanic Laboratory of the University of Lubumbashi; specimen N°9-OKOMBE). The plant was identified by Nsenga Nkulu (Université de Lubumbashi, UNILU) and confirmed by P. Meerts (Université Libre de Bruxelles, ULB). The material was dried in the shade and powdered. The powder was cohesive enough to be compressed "*as is*" into 500 mg tablets that were administered *per os* to the animals.

2.2 Chromatographic profiling of *Oldfieldia dactylophylla* root bark

O. dactylophylla root bark powder (1.0 g) was extracted with 10 mL of n-heptane; after vortexing, ultrasonication and centrifugation, the supernatant was evaporated

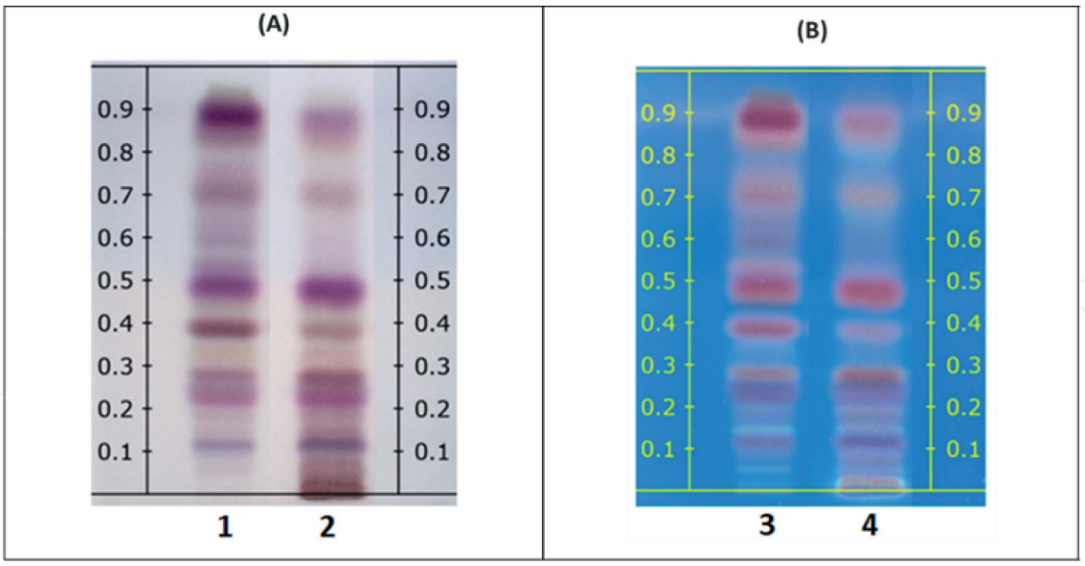

Figure 1.
Chromatographic profiles of Oldfieldia dactylophylla *root bark. HPTLC plate (20 × 10 cm), silica gel 60 F254. Mobile phase, petroleum ether (40–60°C)—acetone—ethyl acetate (85: 15: 5, v/v/v); derivatization with vanillin reagent and visualization (A) under white light; (B) under UV light (365 nm). Tracks 1 and 3,* O. dactylophylla *root bark n-heptane extract; Tracks 2 and 4,* O. dactylophylla *root bark dichloromethane extract.*

to dryness and dissolved in 1 mL of methanol. The plant residue was then similarly extracted with 10 mL of dichloromethane and the extract was dissolved in 1 mL of methanol. The high-performance thin-layer chromatography (HPTLC) of extracts was performed according to the procedure of the European Pharmacopoeia 10 [18] using Camag Automatic TLC Sampler (ATS 4), Automatic Developing Chamber 2 (ADC 2), Derivatizer and TLC Visualizer 2. The Camag systems were driven by the software visionCATS version 2.5. The HPTLC was performed on silica gel 60 F254 HPTLC plates (Merck, Germany); 5 µL of samples were applied in 8-mm wide bands, the plates were activated on MgCl2 (~33% RH) and the tank saturated for 20 min; the solvent system was petroleum ether (40–60°C)—acetone—ethyl acetate (85: 15: 5, v/v/v). The plate development was performed at 70 mm from the lower edge of the plate. 5 mL of a vanillin reagent (2 mL of sulfuric acid added to 100 mL of a 10 g/L solution of vanillin in 96% ethanol) was applied on the plate that was then heated at 110°C for 3 min. The plate was examined under visible light and UV_{366nm} (**Figure 1**).

2.3 Experimental setup and treatment

The applied sampling protocols met the Unified Ethical Principles and an Animal Research 'Helsinki' declaration [19]. The study was conducted at the Naviundu Farm of the University of Lubumbashi (RD Congo). It included 32 locally breed goats, at least 2 months old, naturally infected with various gastrointestinal helminths and randomly assigned to four groups of eight homogeneous animals. All animals graze together on the same pastures and the groups were not physically separated throughout the study period. No anthelmintics were received in the 4 months prior to the experiment.

Four consecutive treatments (excluding the control group) were administered once a month to the different groups for four consecutive months according to the scheme described below:

- group 1 was the untreated control group (control);
- group 2 was treated with albendazole orally (Vermitan®, Laprovet) at a dose of 5 mg/kg body weight (positive control); albendazole is a broad-spectrum benzimidazole anthelminthic, effective against most nematodes, but at these doses would have little efficacy against cestodes [20];
- group 3 was treated with a tablet containing bark powder from the root of *O. dactylophylla per os*, 100 mg/kg body weight;
- group 4 was treated with a tablet containing bark powder from the root of *O. dactylophylla per os*, 200 mg/kg body weight.

The treatments were administered on the same day by two experimenters used to handling animals, one of whom assisted with the restraint by raising the neck of the animal and the other by inserting the drug into the pocket of the animal's cheek with a small amount of water to facilitate the administration of the treatment. Our ethnopharmacological study indicated that, in traditional veterinary treatments, the doses of kikoto muchi (vernacular name of *O. dactylophylla* root bark) are not standardized and depend on the experience of individuals [13]; we determined the doses to be tested by considering the dosages indicated by interviewed farmers. All procedures performed were in accordance with the ethical standards committee of the University of Lubumbashi (license N° UNILU/CEM/167/2011). Fecal, blood samples and weight measurements were taken on day 0 (before administration of the first treatment) and on days 14, 31, 45, 62, 76, 92 and 126 post-treatment (**Figure 2**).

2.4 Stool specimens and coprological analyzes

Individual fecal samples were taken directly from the rectum of animals early in the morning and immediately placed in separate plastic bags, clearly identified with the animal identification number. When the stool examination did not directly follow the collection, samples were temporarily placed at a temperature of 4°C for not longer than 24 h.

For qualitative coprological analyzes, the Willis flotation method [21] was applied. The eggs per gram of fecal matter (EPG) were quantified by the Mc Master technique with a solution of NaCl of density 1.2 (sensitivity: 1 egg observed = 50 EPG, i.e. 50 EPG).

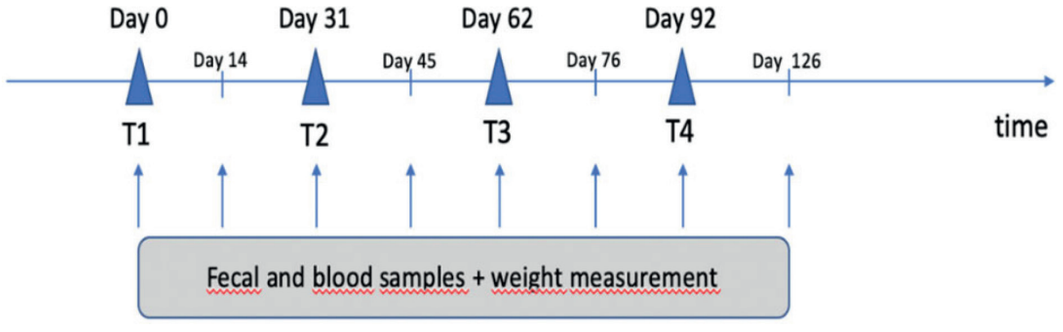

Figure 2.
Time scale of the experiment.

2.5 Blood sampling and analyzing

The blood was collected from the jugular vein using a syringe and transferred in test tubes with or without anticoagulant. The blood collected on tubes with anticoagulant (sodium citrate) was used for the establishment of the hematocrit and the count of total leukocytes. Blood collected on dry tubes was used to obtain serum after centrifugation at 3000 rpm for 10 min for biochemical assays. The hematocrit was determined by a microhematocrit method [22]. The biochemical parameters (total proteins, albumin, creatinine and transaminases) were measured using standard kits from Biomérieux.

2.6 Measure of efficacy of treatments

The efficacy of the treatment was calculated according to the method of Presidente [23], that considers the average EPG before and after the treatments:

$$E\% = \left\{1 - \left(\frac{T1}{T2} \times \frac{C2}{C1}\right)\right\} \times 100 \quad (1)$$

With E% = rate of effectiveness;
T1 = EPG on the day after treatment;
T2 = initial EPG of the treated lot;
C1 = EPG on the day after treatment of control batch.
C2 = initial EPG of the control batch.

2.7 Statistical analysis

For each of the four groups, we performed Multiple Factor Variance Analysis (ANOVA) to compare the various parameters. The EPG values did not follow a Gaussian distribution and were therefore transformed according to the log (x + 10) function. These different statistics were calculated using the software R. For each of the tests, the standard criterion of $p < 0.05$ was used to check whether the measured differences were statistically significant. The effects were noted as very highly significant ($p < 0.001$), highly significant ($p < 0.01$), significant ($p < 0.05$) and not significant ($p > 0.05$).

3. Results

3.1 Chromatographic profiling of *Oldfieldia dactylophylla* root bark

Figure 1 presents HPTLC profiles of the n-heptane and dichloromethane sequential extracts of *O. dactylophylla* root bark. These profiles are quite characteristic, yielding bands probably attributable to terpenoids; a phytochemical study is on-going to identify the compounds of interest.

3.2 Animals

Table 1 shows that all four groups were equilibrated at baseline, regarding sexes, weights and EPG.

Groups	Sex	Body weight (kg)	Fecal worm egg count (EPG)
Control	Female (n = 4)	18.9 ± 3.8[a]	500.0 ± 20.5[a]
	Male (n = 4)	17.9 ± 2.3[a]	497.0 ± 19.9[a]
Albendazole (5 mg/kg)	Female (n = 4)	18.6 ± 4.7[a]	491.3 ± 16.5[a]
	Male (n = 4)	18.2 ± 3.6[a]	498.6 ± 17.1[a]
O. dactylophylla (100 mg/kg)	Female (n = 4)	19.3 ± 4.5[a]	496.0 ± 15.9[a]
	Male (n = 4)	19.0 ± 4.6[a]	500.2 ± 13.4[a]
O. dactylophylla (200 mg/kg)	Female (n = 4)	20.2 ± 5.2[a]	487.1 ± 17.5[a]
	Male (n = 4)	19.9 ± 4.3[a]	498.9 ± 17.2[a]

Values expressed as mean ± sd of body weight and EPG for all groups before the experiment. Means with the same letters are not significantly different through ANOVA and the Tukey test ($p < 0.05$).

Table 1.
Baseline characteristics of study animals.

3.3 Effects of *Oldfieldia dactylophylla* root bark powder on the gastrointestinal strongyles and evaluation of efficacy

From the 2nd week after the first treatment (day 14), the EPG study indicated a decrease of more than half for the fecal excretion of strongyle eggs in goats receiving *O. dactylophylla* root bark powder ($p < 0.001$ vs. negative control). There was no dose-effect relationship as the two doses of *O. dactylophylla* root bark powder showed non-significant differences in eggs counts. Although a 90% EPG decrease was obtained in the control group treated with albendazole, the within-group variabilities were such that the differences between all treated groups were non-significant. The effectiveness of treatments was also evaluated using the method of Presidente (**Table 2**) [23]. On day 126, the decrease in EPG stabilized at 85, 86 and 89% compared to the initial infestation in *O. dactylophylla* 100 mg/kg, *O. dactylophylla* 200 mg/kg and albendazole-treated groups, respectively.

3.4 Effects of *Oldfieldia dactylophylla* root bark powder on serum total proteins and albumin

The total proteins and albumin of the control group showed non-significant differences throughout the study and remained significantly lower ($p < 0.001$)

Groups	Day of treatment							
	T1		T2		T3		T4	
	0	14	31	45	62	76	92	126
	Effectiveness (%)							
Control	0	0	0	0	0	0	0	0
Albendazole (5 mg/kg)	0	90	89	89	90	87	90	89
O. dactylophylla (100 mg/kg)	0	69	78	80	82	83	83	85
O. dactylophylla (200 mg/kg)	0	69	78	77	79	83	84	86

Table 2.
Effectiveness rate of Oldfieldia dactylophylla *root bark powder treatment.*

compared to those of the groups treated either with albendazole or *O. dactylophylla* root bark powder. The total proteins and albumin in the treated groups significantly increased ($p < 0.001$) from day 14 of treatment and remained steady over the study, with no differences between the two dosages of *O. dactylophylla* and albendazole.

3.5 Effects of *Oldfieldia dactylophylla* root bark powder on total leucocytes count

Total leucocytes of the treated animals and control group showed a marked and significant ($p < 0.05$) decrease in all treated groups throughout the study. Comparison of data at different dates revealed non-significant differences between the two dosages of *O. dactylophylla* root bark powder and albendazole.

3.6 Effects of *Oldfieldia dactylophylla* root bark powder on the hematocrit

The mean values of hematocrit of the control group compared to those of the batches treated with *O. dactylophylla* root bark powder or albendazole showed significant differences ($p < 0.001$) throughout the study.

3.7 Effects of *Oldfieldia dactylophylla* root bark powder on transaminases

The base levels of alanine aminotransferase (ALT) slightly differ ($p < 0.001$) in the groups treated with *O. dactylophylla* (100 mg and 200 mg/kg) compared to the other groups whereas the aspartate aminotransferase (AST) levels slightly differ from group to group ($p < 0.001$). The levels of these enzymes in all groups remained stable throughout the study.

3.8 Effects of *Oldfieldia dactylophylla* root bark powder treatments on serum creatinine

There was no difference in serum creatinine levels between the different groups throughout the experimentation.

3.9 Effects of *Oldfieldia dactylophylla* root bark powder on animal weight

Mean weight values compared at different dates between the four groups of animals involved in our study showed no significant difference throughout the experiment.

4. Discussion

This study indicates that the levels of parasitic eggs fecal excretion were similar in the four groups of goats before onset ($p > 0.05$). After 2 weeks of treatment, there was a significative decrease in the fecal excretion of strongyle eggs in goats receiving *O. dactylophylla* root bark powder at either 100 or 200 mg/kg or albendazole. From the 14th day to the 126th day, we observed in the animals treated monthly with *O. dactylophylla* a significant decrease of infestation in comparison to the untreated group ($p < 0.001$). Compared to the group treated monthly with albendazole, the fecal excretion of eggs in animals treated with *O. dactylophylla* monthly at dosages of 100 and 200 mg/kg showed non-significant differences 14th day after the first treatment and the levels of infestation remained similar until the end of the study. There was no statistical difference between the two doses of *O. dactylophylla* tested ($p > 0.05$),

indicating that the lower dosage is sufficient; dose-finding trials are worth conducting to determine the lowest efficient dosage. These results are comparable to those found in several studies using bioactive plants against digestive strongyles: *Eucalyptus staigeriana* leaves [24], *Eucalyptus citriodora* leaves [25] and *Moringa oleifera* leaves [26].

Indeed, several families of molecules present in the root bark of *O. dactylophylla* (tannins, flavonoids, iridoids and triterpenoids) have been evoked in previous works as possessing anthelminthic properties. The action of these molecules, alone or in combination, may explain this decrease of infestation. Similar activities have been demonstrated for sesquiterpenes and lactones in sheep [27], triterpenes and alkaloids in sheep [28–30], anthraquinones in goat [25] saponins [14] and condensed tannins in sheep and goats [14, 31, 32]. In these studies, the use of bioactive plants containing these substances (condensed tannins, triterpenes, alkaloids, anthraquinones or saponins) has been shown to be an alternative to the use of synthetic anthelmintics. As to the efficacy of *O. dactylophylla* monthly administration in our work, we observed a considerable reduction in EPG in the three treated groups (up to 86%). These results are comparable to those obtained by Kommuru et al. [33] with condensed tannins. These molecules have also been identified as effective on some parasites in humans [34]. Thus, to some extent, the use of *O. dactylophylla* may well be extrapolated in the treatment of parasitic nematodes in humans.

Compared to the control group, the serum levels of total proteins and albumin, measured 1 month after the administration of the four monthly treatments, are markedly higher in the treated groups compared to measurements taken 14 days after the first treatment ($p < 0.001$), and similar for the 2 *O. dactylophylla* dosages and albendazole. This suggests that the different treatments have improved the availability of amino acids from dietary proteins. These results are in line with those of Hassan et al. [35] who observed a remarkable increase in serum proteins during treatment with ivermectin; it is likely that the reduction of intestinal parasites burden reduces the diversion of proteins, improving their bioavailability. On the other hand, condensed tannins have been proposed to explain such an increase by an indirect mechanism, the precipitation of food proteins; this would prevent protein degradation in the rumen, improving their intestinal bioavailability [36]. However, certain types of condensed tannins are known for a high toxicity on the digestive mucosa with a consequent reduction of nutrient absorption [37]. But, in goats, physiological adaptations allow them to consume large quantities of plants rich in secondary metabolites, notably tannins [38]; these adaptations include an increase of salivary glands size with the secretion of salivary proteins and three amino acids (glutamine, glycine and proline) with high affinity for tannins [39, 40]. Goats also possess a particular rumen flora that secretes enzymes, such as tannases, active in the rumen [41]. These enzymes specifically break the ester linkages of hydrolyzable tannins, reducing their molecular weight and thus their ability to bind proteins, probably increasing the bioavailability of proteins farther in the digestive tract; such enzymes were notably isolated from the ruminal enterobacteria of goat [42].

From day 14th onwards after the first treatment, the hematocrit of groups treated with albendazole and *O. dactylophylla* show a statistically significant increase compared to the control group. This increase probably corresponds to an anthelmintic activity of tested treatments toward hematophagous parasites [43], including *Haemonchus contortus* that was diagnosed in our study animals. The values of the transaminases (ALT and AST) remained stable in all groups along the study, indicating no hepatotoxicity of tested treatments. There was no difference in serum creatinine levels between the different groups throughout the experimentation, indicating a probable absence of renal toxicity.

Despite the effectiveness of albendazole and *O. dactylophylla* treatments, indicated by a marked decrease in *Haemonchus contortus* bioburden (as seen from EPG data), the evolution of corporeal weights was similar in the treated and control groups throughout the experiment. In a previous study on goats' anthelminthic treatment in Tanzania, during a long dry season, goats were orally dosed with 7.5 mg/kg albendazole and the weight gains were quite limited [20]. At that dosage, albendazole is effective against nematodes but may not be effective against trematodes. The authors explained that their animals may have been infected either with a low level of nematodes, so clearing these would have a small effect, or with a mix of different worms, some of the infections not being cleared by albendazole. In our study, goats are grazing and so are also likely co-infected with different worms, which may explain the non-effectiveness of albendazole on weights. For *O. dactylophylla* treatments, an anti-nutritive effect of tannins on energy production by the rumen flora could yield an additional explanation.

5. Conclusion

The significant decrease, in field conditions, in fecal excretion of parasite eggs after administration of *O. dactylophylla* root bark powder as observed in this study is in line with data obtained in many other earlier works on secondary plant metabolites possessing anthelmintics activities. Moreover, this decrease in fecal excretion underlines the interest that can be gained using *O. dactylophylla* in the control of the digestive parasitism in goat breeding. The goat is an animal particularly well adapted by its physiological and digestive particularities. In view of these initial results, the plant seems to present an ability to be chosen among the alternatives to the control of parasites in agriculture, but it is necessary to complete the analytical approach and to better specify the optimal conditions of its use in farming. The present study has currently highlighted the antiparasitic properties of *O. dactylophylla* on animals; however, phytochemical studies support that the plant should make contributions to human studies in the future.

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Conflict of interest

The authors declare no conflict of interest regarding the publication of this chapter.

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