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Assessment of *in Situ* Anthelminthic Activity of Ethanolic Extract of *Bidens pilosa* against Adult's Gastro-Intestinal Strongyle of Small Ruminants

Mbogning Tayo Gertrude[1*], Yondo Jeannette[2], Tsila Henri Gabriel[1], Mpoame Mbida[1]

[1]Department of Animal Biology, Faculty of Science, University of Dschang, Dschang, Cameroon
[2]Department of Microbiology, Haematology and Immunology, Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Dschang, Cameroon
Email: *mbotager@yahoo.com

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Abstract

Infections caused by gastrointestinal nematodes are major threats to livestock industry in the developing countries. They cause direct effects in form of loss in production and indirect economic losses due to high cost of anthelmintic drugs. Various alternative strategies are in practice to control parasitism which includes the usage of medicinal plants. This study evaluates the *in situ* anthelminthic activity of *Bidens pilosa* ethanolic extract on gastro-intestinal strongyles of small ruminants. Twenty (20) naturally infested goats of both sexes were distributed into 5 groups (n = 4) corresponding to groups receiving Tween 80 at 2.8%, Albendazole at 5 mg/kg and the three doses of extract (125, 250 and 500 mg/kg). The doses of extract and 2.8% Tween 80 were administered twice daily for 3 consecutive days while Albendazole was administered in a single dose. Faecal eggs count reduction, body weight, packed cell volume (PCV) and total worm count reduction were determined. Results showed that the ethanolic extract of *B. pilosa* was not effective against adult worms while Albendozole inflicted a 100% reduction of eggs count and total worm count of digestive strongyle. Body weight and PCV remain almost constant. This study indicates that ethanolic extract of *B. pilosa* at tested doses has no anthelminthic activity. However, further optimization and standardization of the ethanolic extract of *B. pilosa* could enhance its anthelminthic activity.

Keywords

Gastro-Intestinal Strongyle, *Bidens pilosa*, Anthelminthic Activity, *in Situ*

1. Introduction

In small ruminants breeding, diseases caused by gastrointestinal strongyles constitute a major obstacle to the development of profitable livestock industry in the world. These parasites not only affect the health of animals but also the productivity, decrease resistance to other diseases and cause severe mortality leading to heavy economic losses [1]. Various strategies are in practice to control parasitism which include pasture management, biological control, dietary management, vaccination and use of anthelmintic drugs [2]. The most common practice being followed these days is the use of chemical anthelmintic [3]. However, several problems in relation to the use of synthetic anthelminthic have emerged including anthelmintic resistance, e.g. multi resistant *H. contortus* had been already isolated. Parasite resistance increases costs, reduces production efficiency along with the risk of contamination of the animal products [4] and increases the risk of environmental contamination [5]. In addition, commercially available anthelmintics are relatively expensive and smallholder farmers are unable to spend meager income on purchase of drugs to continue regular treatment [6]. Face to this, a quest for a suitable alternative for better control of gastrointestinal infestations is necessary. Numerous plants are used by pastoralists and smallholder farmers as deworming agents for livestock when they could not have access to conventional anthelminthic. The lack of scientific validation of efficacy of all these plants remains a lagging factor in the development of traditional medicine. Screening and proper evaluation of medicinal plants could reveal bioactive compounds that may be sustainable and environmentally acceptable [7]. In Cameroon, *Bidens pilosa* is used by smallholder farmers as deworming agent for livestock. Few phytochemical studies have revealed in *B. pilosa* the presence of bioactive compounds with prooved anthelminthic properties. Okoli *et al.* [8] revealed the presence of saponins, alkaloid and steroids in that plant and Khemraj *et al.* [9] demonstrated the presence of flavonoids, tannins, sesquiterpenes, esculin, diterpenes, chalcones and phytosterols. Tannins, which are glycoprotein-rich compounds, can bind to free proteins, thus reducing nutrient availability which in turn results in the death of the parasite by starvation [10]. Alkaloids have the ability to interfere with DNA synthesis in parasites [11]. According to Tarnopolsky and Beal [12], alkaloids are competitive antagonists of muscarinic acetylcholine receptors preventing acetylcholine binding. They also have sedative properties leading to cell excitation and thus neurological dysfunction. Saponins could also affect P-glycoprotein (Pgp) function to inhibit egg hatching and mortality of free-living stages of gastrointestinal nematode [13].

However for acceptance of medicinal plants into scientific veterinary medicine, it is necessary that their effectiveness and safety be evaluated and confirmed through *in vitro* and *in vivo* testings [14]. Former studies revealed *in vitro* efficacy of *B. pilosa* against eggs and larva of *Heligmosomoides bakeri* a gastro-intestinal nematode of white mouse and *Haemonchus contortus*, a gastro-intestinal strongyle of small ruminants [15] [16]. The present study aimed to in-

vestigate *in situ* the anthelminthic activity of *Bidens pilosa* ethanolic leaf extract on small ruminants.

2. Methodology

2.1. Collection and Storage of Plant Material

Leaves of *Bidens pilosa* were collected at the teaching and research farm of the University of Dschang-Cameroon. An entire plant was taken to the National Herbarium of Cameroon where it was identified under the reference number 32987/HNC as *Bidens pilosa* Linn. The collected plant material was dried in shade, at ambient temperature for three weeks. Dried leaves were ground to powder and stored in airtight plastic bags in the Laboratory of Biology and Applied Ecology of the University of Dschang.

2.2. Ethanolic Extract Preparation

In this study, we used the ethanolic extract of *Bidens pilosa* because it was the most efficient during our previous *in vitro* trials on *Heligmosomoides bakeri* and *Haemonchus contortus* of white mouse and small ruminants respectively [15] [16]. Extraction was done according to the procedure described by Wabo Pone *et al.* [17] [18]. Briefly, five hundred (500) grams of stored powder were macerated in 06 l of ethanol 95%. The mixture was daily stirred to permit better extraction of the active ingredients. Seventy-two (72) hours later, the solution was sieved and filtered through a cotton layer and a filter paper of pore size 2.5 µm. The filtrate was evaporated in a rota vapor at 82˚C for 8 hours. The extract obtained was poured in a large Petri dish and allowed at room temperature for two days, at the end of which a completely dried ethanolic extract was obtained and stored in refrigerator (4˚C) for further use.

2.3. Preparation of Extract Concentration

The following formula (1) was applied to calculate the concentration of solution to prepare corresponding to each dose.

$$C = \frac{D \times M}{V \times 1000} \quad (1)$$

where C = Concentration; D = Dose of extract to administer (125 mg/kg, 250 mg/kg and 500 mg/kg); M = weight of the most heavy animal; V = Volume of extract solution to administer (3 ml).

2.4. Preparation of Reference Drug

The reference drug Albendazole 250 mg used in this study was bought from a Veterinary Clinic in the Divisional Delegation of Livestock, Fisheries and Animal Industries in Dschang. This drug was chosen due to its broad spectrum activity to prevent and treat gastrointestinal nematodes of domestic animals. Dosage recommends a single intake of one tablet of Albendazole for an animal of 50

kg. One tablet was weighed (7.25 g) and the algorithm (2) was applied to calculate the mass of albendazole needed for each animal in the positive control group.

$$malb = 0.145\ mc \tag{2}$$

where: malb = mass of Albendazole and mc = body mass of the animal.

Once this mass was calculated, the fraction of the product was weighed and diluted in 3 ml of distilled water before being administered to the animal.

2.5. Treatment and Grouping of Experimental Animals

For this study, Twenty (20) goats of both sexes were brought from local breeders while ensuring that they had not received any anthelminthic treatment for at least 4 weeks. Animals were kept for two weeks to get acclimatised with the environment before the start of the experiment. During that time, they were vaccinated against Small Ruminants Pest (SRP) using Capri-Pestovax. They also received a preventive antibiotic: phenoxyzone (1 ml/10kg) combined with Stress-Vita (1 ml/10kg), a multivitamin anti-stress, for six days. After two weeks, the experimental animals were divided into 05 groups (n = 4 regardless gender) according to their body weight and the number of eggs per gram of faeces (EPG) as followed:

- Animals of group 01 received Tween 80 at 2.8%;
- Animals of group 02 received Albendazole at the dosage of 5 mg/kg;
- Animals of group 03 received ethanolic extract of *B. pilosa* at the dosage of 125 mg/kg;
- Animals of group 04 received ethanolic extract of *B. pilosa* at the dosage of 250 mg/kg;
- Animals of group 05 received ethanolic extract of *B. pilosa* at the dosage of 500 mg/kg.

Each animal was weighed and treated with 3 ml of corresponding substance. The extract and Tween 80 were administered twice daily (morning at 8 a.m. and evening at 5p.m) for three days, while the Albendazole treatment was a single dose. The substances were administered orally using a syringe. Throughout the experiment, the animals were kept indoor.

2.6. Goat Faecal Sample

Faecal samples were collected directly from the rectum of each goat before treatment (day 0) and after treatment (day 3, 7, 10, 14, 17 and 21). The specimens were transported to the laboratory for coprological analysis using the McMaster technique as described by Thienpont *et al.* [19] to determine the number of egg per gram of faeces (EPG). The percentage of faecal egg count reduction (FECR) was calculated using the following formula (3) [20]:

$$\text{FECR} = \frac{\text{initial EPG} - \text{EPG at} \times \text{time}}{\text{initial EPG}} \times 100 \tag{3}$$

2.7. Worm Recovery

On the twentieth day post treatment, the animals of each group were slaughtered and their abdominal cavity opened. The gastro-intestinal tract was sectioned, removed and placed in a 10 l container containing 5 l of water. This organ was opened longitudinally, the content was collected and inner wall was scratched to detach the worms. The solution obtained was homogenised then left to settle for 30 min. After three washing by successive decantation, worms were collected and counted under a binocular magnifying glass. The percentage of total worm count reduction (TWCR) was calculated as follows (4) [21]:

$$\text{TWCR} = \frac{\text{total worm count in control group} - \text{total worm count in treated group}}{\text{total worm count in control group}} \times 100 \quad (4)$$

2.8. Packed Cell Volume (PCV)

Blood samples were collected EDTA coated tube from the jugular vein of animals in each group before treatment and eighty days after treatment. Blood of each tube was introduced into micro-haematocrit tubes and centrifuged using a micro-haematocrit centrifuge at 12,000 rpm for 6 min and the Packed Cell Volume (PCV) was determined using the following formula (5):

$$\text{PCV} = \frac{\text{Height of red blood cell}}{\text{Total height of blood}} \times 100 \quad (5)$$

3. Statistical Analysis

Animal body weight, haematocrit and mean percentages of reduction in faecal egg density before and after the different treatments were compared using a two-factor analysis of variance (time and dose factors), supplemented by the Bonferroni post-test, while mean percentages of reduction in parasite load were compared using a one-factor analysis of variance followed by the Tukey multiple comparison post-test. Differences were considered significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$.

4. Results

4.1. Effect of Ethanolic Extract of *Bidens pilosa* on Fecal Eggs Excretion

Table 1 shows the variation of mean strongyle eggs density and their mean percentage of reduction after administration of albendazole and ethanol extracts of *B. pilosa* at the doses of 125 mg/kg, 250 mg/kg and 500 mg/kg. Ethanolic extract of *B.pilosa* did not show anthelminthic activity. The density of eggs excreted by treated animals in all doses remained high even 21 days after treatment. Hence negative values of FECR on almost all days after treatment and doses were obtained. On the contrary, a single dose of Albendazole induced 80.3% FECR 3 days after treatment which reached 100% 14 days after treatment.

Table 1. Variation of mean strongyle eggs density (mean ± standard deviation) and their mean percentage of reduction (%) after administration of Albendazole and ethanolic extract of *Bidens pilosa* (n = 4).

Treatments	Doses	Pre-traitment	Post-traitment					
	(mg/kg)	D_0	D_3	D_7	D_{10}	D_{14}	D_{17}	D_{21}
2.8% Tween 80	/	3400 ± 430.1	3625 ± 497.5 (−8.1)	4475 ± 466.5 (−35.3)	4313 ± 621 (−26.7)	4288 ± 494 (−28.2)	4813 ± 356.8 (−45.4)	5200 ± 837.7 (−55.2)
Albendazole	5	6425 ± 556	1250 ± 341.6 (80.3)^C	100 ± 115.5 (98.5)^C	50 ± 57.7 (99.2)^C	0 ± 0 (100)^C	0 ± 0^c (100)^C	0 ± 0 (100)^C
Bidens pilosa	125	4300 ± 559.8	4525 ± 704.2 (−5.1)	4650 ± 568.6 (−8.3)	5075 ± 537.7 (−20.6)	4575 ± 567.9 (−7.6)	5550 ± 776.7 (−30.9)	5475 ± 991.2 (−28.9)
	250	3225 ± 639.7	3150 ± 191.5 (−1.2)	3225 ± 221.7 (−3.5)	4025 ± 298.6 (−28.6)	4425 ± 543.9 (−41)	3175 ± 512.3 (0.4)^A	4650 ± 822.6 (−49.4)
	500	9900 ± 1314	8500 ± 535.4 (−1.2)	10,350 ± 2485.3 (−3.5)	10,850 ± 1112.1 (−28.6)	11,725 ± 1175.8 (−41)	14,675 ± 2734.2 (0.4)^A	16,075 ± 3646.3 (−49.4)

$^{A, B, C}$ indicate significant difference in the same column at $P < 0.05$; $P < 0.01$ and $P < 0.001$ respectively.

4.2. Effect of Ethanolic Extract of *Bidens pilosa* on Packed Cell Volume and Body Weight

From Table 2 presenting the effect Albendazole and ethanolic extract of *B. pilosa* on PCV and body weight, it can be noticed that the substances have not significantly influence the two parameters 19 days after treatment.

5. Discussion

In situ studies are more relevant in evaluation of anthelminthic properties of plant extract than *in vitro* studies since parasites are put in contact with the plant extract in their natural habitat. During this study, treatment of naturally infested goats with all the doses of ethanolic extract of *B. pilosa* did not induce the reduction of faecal eggs excretion. Former studies revealed *In vitro* ovicidal and larvicidal activity of ethanoloc extract of *B. pilosa* on *Heligmosomoides bakeri* and *Haemonchus contortus* [15] [16]. In fact on *Haemonchus contortus*, a gastro-intestinal strongyle of small ruminants, the ethanolic extract of B. pilosa inhibited 92.5% ± 7.5% and 67.4% ± 7.4% egg embryonation and egg hatch at 5 mg/ml, with IC50 values of 2.1 mg/ml and 3.3 mg/ml respectively and induced 100% ± 0% and 89.8% ± 3.2% L1 and L2 larvae mortality at 5 mg/ml with LC50 values of 1.8 and 1.96 mg/ml respectively [16]. Several authors also observed in their studies that some plant extracts are efficient *in vitro* on eggs and larva while *in vivo* on adults no anthelminthic activity was registered. Oleiveira *et al.* [22] recorded *in vitro* 100% and 99.77% inhibition of egg hatch and larvae development of *H. contortus* at 5 mg/ml and 80 mg/ml respectively with ethyl acetate extract of *Cocus nucifera* fruit and *in vivo*, no activity on gastro-intestinal nematodes of sheep. In vitro, the ethyl acetate/ethanol (3:1) extract of *Piper tuberculatum* resulted in an IC_{50} for hatching and an LC_{50} for larval development of *H. contortus* of 0.031 mg/ml and 0.02 mg/ml respectively while *in vivo*, none

Table 2. Effect of ethanolic extract of *Bidens pilosa* on packed cell volume and bodyweight (mean ± SD) 19 days after treatment. (n = 4).

Treatments	Doses	PCV (%)		Bodyweight (kg)	
	(mg/kg)	Pre-traitment	Post-traitment	Pre-traitment	Post-traitment
2.8% Tween 80	/	29.8 ± 1.8	27.2 ± 2.4	6.8 ± 0.5	6.8 ± 0.7
Albendazole	5	26.7 ± 1.4	29.5 ± 1.9	10.6 ± 1.1	11.1 ± 1.6
Bidens pilosa	125	27.8 ± 1.7	26.8 ± 0.9	8.6 ± 1.1	8.5 ± 1.3
	250	29.1 ± 1.2	28.6 ± 3.8	7.1 ± 1.3	7.3 ± 0.6
	500	26.4 ± 0.8	25.9 ± 1	7.3 ± 0.6	7 ± 0.7

of the doses tested (150 and 250 mg/kg) caused a significant reduction in faecal egg excretion and parasite load in R. *norvegicus* experimentally infested with *Strongyloides venezuelensis* [23]. Thus, the ovicidal and larvicidal activity of plant extracts among others, *B. pilosa* does not automatically imply a similar action on adult worms because *In situ* anthelmintic activity is influenced by the physiology and availability of plant extract preparations in the host [24]. As with *B. pilosa*, several studies have experimentally demonstrated the ineffectiveness of traditionally used medicinal plants presumed to be active against gastrointestinal nematodes [25]-[30]. Several hypotheses can explain the lack of activity in situ, including biotransformation or destruction of active compounds by microorganisms or rumen pH and the different conditions observed *in vitro* versus *in situ*. According to **Vandamne and Ellis** [31], ruminal microflora can alter the metabolism and mechanism of action of certain nutrients, drugs and bioactive compounds when administered orally. *In vitro*, the extracts are in direct contact with the organism and the contact time is fixed, which would increase the probability of observing activity. *In vivo*, inactivity could also be related to the dose and reduced contact time. Increasing the dose or administering it for a longer period of time could increase the contact time with the parasite and thus produce the desired effect of decreasing worm fecundity or elimination.

6. Conclusion

This study intended to scientifically validate the anthelminthic effectiveness of *B. pilosa* leaves extracts. Infused and ethanolic extracts exhibited evidence of *in vitro* ovicidal and larvicidal activities on *H. contortus* while *in situ*, ethanolic extract showed no anthelminthic activity. However, PCV values and bodyweight remain almost stable after treatment, indicating that the ethanolic extract could help animals to tolerate adverse effects of parasitism, particularly the anemia effect. However, further optimization and standardization of the ethanolic extract of *B. pilosa* could enhance its anthelminthic activity.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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