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Phytotherapy of Djallonke Lambs Co-infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Trichostrongylidae) Using Methanol Extracts of Two Medicinal Plants in Menoua Division, West Region of Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Author NG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FAF and CY managed the analyses of the study. Author JWP took part in the designing of the study and managed literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

This study was performed on the West African Dwarf sheep which were experimentally infected with *Teladorsagia circumcincta* and *Trichostromgylus colubriformis*. We intended to investigate the anthelmintic potential of two plants: *Harungana madagascariensis* and *Momordica foetida*. During this experiment, 24 male lambs (Age: 3-5 months) were divided into 8 groups: An untreated control (Group 1), Albendazole 7.5 mg/kg (positive control Group); *Harungana. madagascariensis* 125 mg/ kg (Group 3); *Harungana madagascariensis* 250 mg/ kg (Group 4); *Harungana madagascariensis*

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500 mg/ kg (Group 5); *M. foetida* 125 mg/ kg (Group 6); *M. foetida* 250 mg/kg (Group 7); *M. foetida* 500 mg/kg (Group 8).
Treatment with albendazole 7.5 mg/kg, *M. foetida* (250 and 500 mg/kg) and *H. madagascariensis* (500 mg/kg) significantly (P<0.05) reduced egg per gram of faeces, adult worm load and diarrhoea scores, while improving feed intake, food conversion efficiency and weight gain of lambs when compared with untreated controls The highest EPG reduction rate was recorded in albendazole treatment group (98.64%) followed by *M. foetida* 500 mg/kg (77.78%), then *H. madagascariensis* with a reduction rate of 52.03%. There was a strong positive correlation between food conversion efficiency and weight gain (r = 0.99**, P=0.00); while adult worm burden and food conversion efficiency were negatively correlated (*r* = -0.68, *P* = 0.00).
Methanol extracts of *M. foetida* possessed significant anthelmintic potential at dose 500 and 250 mg/kg while *H. madagsacariensis*. exhibited only mild anthelmintic activity at 500 mg/kg. However, *H. madagascariensis* was more efficient in preventing or treating diarrhoea at 250 mg/kg than *M. foetida* at the same dose.

Keywords: Teladorsagia circumcincta; Trichostrongylus colubriformis; Harungana madagascariensis; Momordica foetida; phytotherapy.

1. BACKGROUND

Gastrointestinal nematode infection adversely affect productivity of small ruminants all over the world, especially in tropical and sub-tropical countries [1,2]. Cameroon is an agricultural country and livestock plays a vital role in its economy. Small ruminants are among the major economically important livestock in Cameroon, and provide 17% of protein needs of the people [3]. According to a Project Information Document (PID) on Livestock Development Project in Cameroon by [4] there is an estimated 5,805,297 heads of cattle; 6,298,059 goats and only 2,952,624 sheep. In the same report by the World Bank, livestock sub-sector's production does not meet the domestic needs despite its seemingly large size; and the domestic demand gap has increased from 2008 to 2015. Prominent among identified constraints as has been reported by [5] and even more recently by the [4], were infectious, parasitic and reproductive diseases which plague small ruminants leading to low productivity and high death rate especially among young animals. This is in agreement with the report of [6] who said gastrointestinal helminth parasites are widespread in Cameroon and limit livestock production in many areas of the country. *Teladorsagia sp*, *Trichostrongylus spp* and *Heamonchus conturtus* are the key species involved in gastrointestinal nematodiosis in Cameroon [6,7]

Ovine trichostrongyliasis and teladorsagiasis reduce productivity by depressing appetite, cause villous atrophy (or stunting of villi) which results in impaired digestion and malabsorption; and severe protein loss occurs across the damaged mucosa [8]. Diarrhoea is one of the most severe clinical feature faced by grazing sheep causing morbidity and mortality worldwide [9,10]. Quite often, ovine diarrhoea has been associated to infections with *Trichostrongylus* or *Teladorsagia* [9,11]. When both parasites are present in mixed infection, the effect is more dramatic [12,13].

Livestock producers in Cameroon and worldwide as well have derived substantial benefits from the use of conventional drugs in combating nematode infections [14,15,16,17]. However, the high cost of these drugs, has made veterinary services difficult for resource-poor farmers [18]. Again, with the widespread use or misuse of anthelmintic drugs, there is development of resistance of this nematode species to most drugs introduced [19]. An additional constraint in the use of chemotherapeutic drugs comes from consumers' ever-increasing need for drug free products, since drug residues in animal products may be potentially hazardous to consumers [20]. Thus, alternative strategies which are equally effective but safer are being sought for [21]. Moreover, there is the need to discover new therapeutic substances of natural origin with low toxicity to man and animals [22] as well as overcoming the rapid escalation in anthelmintic resistance. The use of plants with anthelmintic properties is considered as one of the most viable alternative methods for the control of gastrointestinal nematode infection [23].

Momordica foetida (Cucurbitaceae) has been known for its ethnomedicinal significance ranging from spiritual and psychiatric conditions to physical diseases such as hypertension, ulcers,

diabetes mellitus, contraceptive, antimalaria, dysmenorrhea, eczema, jaundice, leprosy, piles, pneumonia, psoriasis, rheumatism and scabies [24].

On the other hand, *Harungana madagascariensis* (*Hypericaae*) has medicinal application in the cure for leprosy, jaundice, ulcers, asthma, and prevention of poultry illnesses. The bark and root decoctions are remedy for gastrointestinal disorders and piles. It relieves stomach-ache, painful menstruation, cough with bloody sputum and dysentery. It equally arrests haemorrhage and diarrhoea [25,26].

The use of these two plant extracts as de-wormers for humans and livestock has long been practiced in the Western High lands of Cameroon (personal communication). However scientific validation of these practices have been lacking. This study therefore assessed the *in vivo* anthelmintic effects of three different dose levels of methanol extract of the bark of *Harungana madagascariensis* andwhole aerial parts of*Momordica foetida* against adult *Teladorsagia circumcinta* and *Trichostrongylus colubriformis* in experimentally infected lambs, with the view of providing scientific basis for their use in ethno-veterinary practices.

2. MATERIALS AND METHODS

2.1 Selection of Plants

An ethnobotanic study permitted us to select the above two medicinal plants with anthelmintic activity. These plants are frequently seen throughout the Western Highlands of Cameroon in areas of high altitude, wastelands, agricultural lands and on open mountainous slopes. They were harvested in April 2016, were identified and authenticated at the National Herbarium of Cameroon in Yaounde by voucher specimen N° 46105 / HNC for *Harungana madagascariensis* and N° 24393 / HNC for *Momordica foetida.* The plants collected were shade dried at room temperature. The dried plant parts were powdered mechanically using a commercial electrical stainless steel blender, and stored in airtight plastic bags at 4℃ until extraction.

2.2 Preparation of Methanol Extracts

Four hundred and fifty grams (450 g) of stored powder were macerated in 3L each of methanol. This mixture was stored for 72 hours at room temperature and stirred for 10-20 minutes daily. This solution was then sieved through Whatman No 1 filter paper and divided into portions of about 200 ml each and evaporated in a rotavapor at 50℃. The dry extracts were then stored at 4℃ for subsequent use during *in vivo* studies. The extraction yields was calculated as follows:

$$\text{Extraction yield} = \frac{\text{Weight of dried extract}}{\text{Original weight of plant powder}} \times 100$$

2.3 Preparation of Parasite Donor Sheep

Adult female *T. circumcincta* and *T. colubriformis* were obtained following standard procedures as described by [7,18,27]. Sheep guts were obtained from the abattoir of 'Marche B' in Dschang town and transported to the laboratory of animal Biology and applied Ecology (LABAE) of the University of Dschang. The abomasums and intestines were ligated at the junctions of the omasum and the abomasum, the abomasums and the first 3 meters of the small intestine. The abomasums and part of the small intestines were removed, and opened up with a blunt-tipped pair of scissors and the contents emptied into separate open mouth basins. The mucosa of abomasums and small intestine were washed gently with running tap water and the parasites washed off into the basins. Their mucosa were scrapped with a lancet and mixed in about 1000 ml of tap water. These solutions were left to decant for one hour and the procedure repeated many times until the solution became clear. The supernatant was eliminated and the parasites present in the residue were collected into different Petri dishes. Female *Trichostrongylus* worms were further recovered by incubation of the intestinal mucosa in physiological saline for 7 hours. The incubating solution were divided into small portions and poured into Petri dishes of about 5 ml and viewed under a dissecting microscope at 4x magnification. Identification of female *T. colubriformis* depended on the presence of excretory bursa 1 mm away from the anterior region, in addition to other taxaonomic characteristics described by Soulsby et al. [28], Gibbons and Khalil [29] and Taylor et al. [30]. Female *T. circumcincta* were distinguished by their numerous longitudinal cuticular ridges, which were seen posteriorly, the presence of a genital cone with a single papilla which projects from the surface of the body, presence of small prominent cervical papillae, together with other identification keys as described by [28,29,30].

Once identified, a pipette was used to isolate the females, which were crushed gently to rupture their uteri and liberate fertilized eggs. These eggs were then cultured at 25℃ in a damp mixture of heat sterilized sheep faeces and charcoal particles to allow oxygenation. After 7 days in culture medium, the third stage larvae (L3) were harvested, counted and stored in water at 4℃. About 10.000 L3 in two divided doses were used to inoculate two nematode naive sheep for each parasite. Inoculation was achieved orally using plastic syringes. These sheep were kept indoors in separate room at the research farm of the University of Dschang until infection became patent in the experimental lambs 21 – 25 days post inoculation. The number of larvae was estimated by counting the number of larvae contained in 0.1ml of a well homogenized faecal solution of infective larvae using electronic photocapture microscope. After ten repetitions of counting, the mean number of larvae in 1 ml of solution was determined and the volume containing 4000 or 5000 larvae was then deduced.

2.4 Experimental Animal

A total of 24 male sheep (West African Dwarf species), ages between 3-5 months were used. Their ages were determined using dentition as described by [31]. The animals were acquired from two sheep farmers in FongoTongo village, 40 km away from Dschang town. Two weeks before the start of the experiment, the selected animals were tagged in their various farms and vaccinated against Small Ruminant Pests (SRP) using Capri-pestovax. Upon arrival, they were housed on slated floors in the Experimental Animal House of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang. The animals were pre-treated with anthelmintic of zero residual effects (Albendazole 10 mg/kg), acclimatized for 10 days and their faecal samples examined daily within this period to confirm that animals were helminth free by the use of the concentration salt flotation technique. Within this time, the animals were given antibiotics, (Oxytetracycline 1 ml/10kg body weight) anticoccodia (Toltrazuril, 10 mg/kg) multivitamin and anti-stress (Stress vita 2.5 ml/10 kg body weight). After 10 days and a negative stool confirmation, all animals were orally inoculated using plastic syriges with 14000 L_3 larvae in three doses; day 1: 5.000 L3 of *T. colubriformis* per animal using a syringe via the oral route. Day 2: 5.000 L3 of *T. colubriformis* and day 3: 4.000 L3 of *T. circumcincta*. Animals were randomly allocated to eight sub groups of 3 individuals each based on their body weight six days after arrival as follows: Group 1: Untreated control (received the vechicle 3.2% dimethyl sulphoxide); Group 2: Albendazole 7.5 mg / kg (positive control); Group 3: *Harungana. madagascariensis* 125 mg/ kg; Group 4: *Harungana. madagascariensis* 250 mg/ kg; Group 5: *Harungana madagascariensis* 500 mg/ kg; Group 6: *M. foetida* 125 mg/ kg; Group 7: *M. foetida* 250 mg/ kg; Group 8: *M. foetida* 500 mg/ kg. Each group was placed in a secured separate pen with a dimension of 7.5m^2 (3m x 2.5) on slatted floors. A solid partition separated adjacent pens and care was taken to avoid contamination of pens with nematode larvae from outside sources. Water was provided to the animals *ad libitum*. All animal groups were served 6 kg of fresh forage (*Tripsacum laxum* and *Leucaena leucocephala*) harvested at least 24 hours before feeding time. The forage was sanitized as follows: two drops of chlorine bleach (6% sodium hypochlorite) was put in each litter of water and forage allowed in chlorinated water for five minutes. After this, forage was removed, rinsed in portable water and allowed in the sun for two hours. Weighed food was served per group and food refusals were weighed and recorded daily.

2.5 Evaluation of Anthelmintic Activity *in vivo*

All the animals in the various experimental groups were monitored daily for signs of disease including anorexia, diarrhoea, live weight changes and death. Anthelmintic activity was determined by evaluating rate of EPG reduction, faecal consistency, feed intake and weight gain, and reduction of adult parasite load at necropsy.

2.5.1 Determination of faecal egg count reduction rate

Treatment was administered on day 26 post inoculation and twice daily for 4 days. EPG was determined on day 0, treatment day 3,5,7,15, and 30 post treatment using salt flotation and Mac Master technique as described by Thienpont et al. [32]. Egg Count percent Reduction (ECR) was calculated as below:

$$ECR\ (\%) = \frac{Pretreatment\ egg\ per\ gram\ (EPG) - Posttreament\ EPG}{Pretreatment\ EPG}\ x\ 100$$

2.5.2 Determination of adult worm reduction rate

On day 55 post inoculation (30 days post treatment), all 24 animals were humanely sacrificed and the adult worms collected as follows: the abomasums and small intestine were ligated at the junctions of the omasum to the abomasums, and the abomasum to the small intestine. Each organ was removed and processed as described in section 2.1.3. The contents of the each bucket were adjusted to make two litres, and the mixture thoroughly homogenized, and two aliquots of 250 ml each collected and counted subsequently to determine the numbers of adult *T. circumcinta* and *T colubriformis* in the aliquots. The total number of parasites in the abomasum and intestine respectively were calculated by multiplying by 4. The Rate of adult worm reduction was determined by the formula:

$$RR(\%) = \frac{Mean\ intensity\ of control\ group - mean\ intensity\ of treatment group}{Mean\ intensity\ of\ control\ group} X\ 100$$

2.5.3 Evaluation of faecal consistency

All the animals in the various experimental groups were monitored throughout the trial for signs of diarrhoea. The faecal consistency was judged immediately after individual collection according to a faecal score (FS) key ranging from 0 to 3**.** Each faecal sample was given a faecal score according to the consistency of the sample: normal pelleted faeces=0; soft pelleted faeces=1; paste-like faeces=2; liquid faeces=3 [33] Diarrhoea scores were calculated on day 0, 3, 5,7,15, and 30 before determination of faecal EPG. The daily diarrhoeal score for each pen was calculated as described by [34], by multiplying each lamb by the characterization scale of diarrhoea, and the results for all lambs within a pen was then added together and divided by number of lambs. Faeces with scores of 2 or 3 were designated 'Diarrhoeal feaces'.

2.6 Evaluation of Production Parameters

The extent to which infection with these parasites and treatment with methanol extracts of *H. madagascariensis* and *M. foetida* influenced production parameters was evaluated by determination of mean food intake, mean weight gain, and food conversion efficiency of treated and untreated lambs.

2.6.1 Mean food intake (MFI)

Mean food intake (MFI)which is the total amount of forage consumed by each lamb per group was evaluated from food refusals data.

$$Mean\ food\ intake\ (FI) = \frac{Total\ forage\ consumed\ per\ group}{number\ of\ animals\ per\ group} \quad (3)$$

2.6.2 Sheep live weight measurements

Animals were weighed weekly from the time of arrival to the end of the trial 10 weeks later. The effect of treatment with plant extracts on daily live weight gains was determined by subtracting the initial weight from the final weight per animal.

Weight gain (WG) =Final weight (W_{10}) - Initial weight (W_0)

2.6.3 Food conversion efficiency (FCE)

Food was served to animals as a group (per pen), so it wasn't possible to measure forage eaten by individual animals. Food conversion efficiency was therefore calculated from data of mean forage eaten per animal per day, with the assumption that each animal within the group ate the same amount of food [35].

$$Food\ conversion\ efficiency\ (FCE) = \frac{Weight\ gain}{mean\ for\ age\ consumed\ per\ animal}$$

2.7 Data Analysis

Statistical analysis was performed using SPSS version 20.0. To test the effects of treatment on studied parameters, one-way analysis of variance (ANOVA) was used. The Pearson correlation coefficient was used to establish the relationship among adult worm, weight gain and feed conversion efficiency. The post hoc test employed for all analysis was Duncan's Multiple Range Test. Results obtained were expressed as mean ± standard deviation. Probability values $P<0.05$, was considered significant.

3. RESULTS

The crude methanol extract yield of 25% for *H. madagascariensis*; and 13.1% for *M. foetida* were obtained during this study. In this experiment we recorded an establishment rate of 45.85% for *T. circumcincta* and 68.61% establishment for *T. colubriformis.*

Mean ± SEM of faecal EPG counts before and after treatment with crude methanol extracts of *H. madagascariensis* and *M. foetida*, and albendazole 7.5 mg/kg as a reference drug are shown in table 1. There was no physical clinical sign of toxicity in all groups of sheep treated with plant extracts within the first four hours of administration. Both plant extracts reduced egg count in a dose dependent manner. The effect of plant extracts on worm laying ability was evident only from day 3 post treatment, with *H. madagascariensis* 500 mg/kg (group 5), *M. foetida* 250 mg/kg (group 7), and *M. foetida* 500 mg/kg (group 8) treatment groups recording significantly lower EPG of feaces. On the same day, there was significant increase in faecal egg count as detected in the untreated lambs (group 1) and the lower dose treatment group 3 (*H. madagascariensis* 125 mg/kg), group 4 (*H. madagascariensis* 250 mg/kg) and group 6 (*M. foetida* 125 mg/kg. This trend was maintained on day 7, 15 and day 30 post treatment. Albendazole was administered on day 4 and 24 hours later, it was noted that treatment with albendazole caused a significantly higher EPG reduction percentage (P<0.05) when compared to the untreated lambs. By the third day post administration of albendazole, there was a 97% faecal EPG reduction (Table 1). The maximum efficacy observed was 98.64 % for albendazole on day 30, while for plant extracts the maximum efficacy recorded was 77.78 % *for M. foetida* 500 mg/kg and 52.03% for *H. madagascariensis* on the same day.

The effect of treatment with albendazole and methanol extracts of *Harungana. madagascariensis* and *Momordica. foetida* on adult worm load of lambs is represented in Fig. 1.

The number of adult worms recovered at necropsy varied significantly between the different treatment groups. It was noticed that *M. foetida* 500 mg/kg and albendazole 7.5 mg/kg had similar anthelmintic potential in the abomasum, and caused the highest significant deparasitization against *T. circumcinta*, followed by *H. madagascariensis* at 500 mg/kg dose level and *M. foetida* at 250 mg/kg dose level (Fig. 1). Generally, *H. madagascariensis* recorded significant adult worm reduction (P<0.05) in the intestines only at 500 mg/kg dose level while *M. foetida* caused deparasitization at dose rate of M250 and M500 mg/kg (group 7 and 8 respectively. The lower dose levels of *H. madagascariensis* (H125 mg/kg, H250 mg/Kg) and *M. foetida* (M125 mg/kg) resulted in non-significant reduction (p<0.05) when compared with the untreated control (group 1).

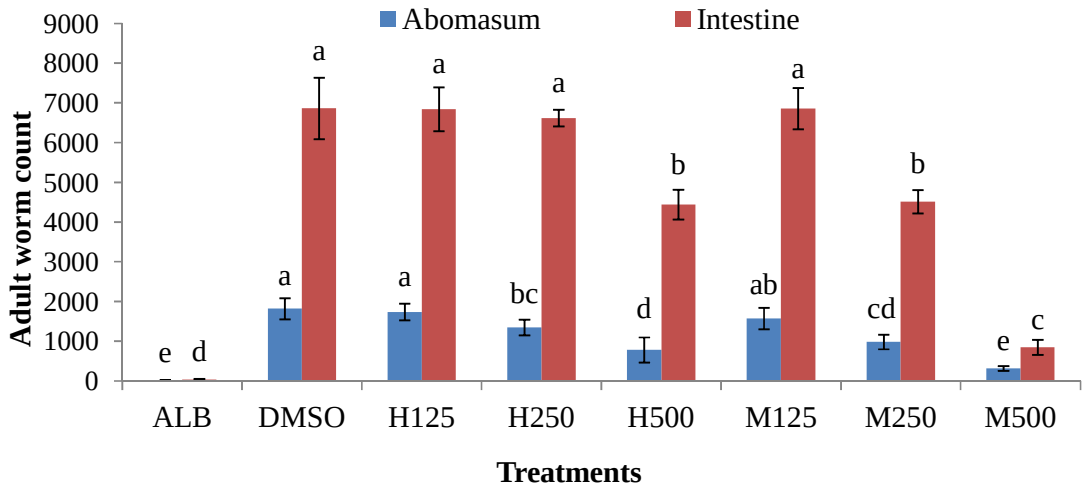

Fig. 1. Variation in adult worm burden recovered at necropsy from the abomasums and intestines of lambs infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* 30 days post treatment with albendazole and methanol extracts of *Harungana madagascariensis and Momordica foetida*

a,b,c,d,e on bars of the same colour: bars with the same letters are not different significantly (P>0.05). DMSO= Dimethyl sulphoxide (3.2%); ALB=Albendazole7.5 mg/kg; H125= Harungana madagascariensis 125 mg/kg; H250= Harungana madagascariensis 250 mg/kg; H500= Harungana madagascariensis 500 mg/kg; M125= M.omordica foetida 125 mg/kg; M250= M.omordica foetida; M500= M.omordica foetida 500 mg/kg.

Table 1. Mean faecal EPG counts (means ± standard deviation) and faecal egg count reduction percentage (%) of lambs co-infected with *Teladorsagia circumcinta* and *Trichostrongylus colubriformis* L3 larvae before and after treatment with albendazole and methanol extracts of *Harungana madagascariensis* and *Momordica foetida*

	Sampling days					
Treatment	**Day 0**	**Day 3**	**Day 5**	**Day 7**	**Day 15**	**Day 30**
DMSO (3.2%)	10200.00±1113.55^{a}	11733.33±416.33^{b} **(-15.03%)**	12666.67±2100.79^{c} **(-24.18)**	14266.67±2100.79^{e} **(-39.87)**	15533.33±3411.74^{d} **(-52.28)**	18400.00±3411.74^{e} **(-80.39)**
Alb7.5 mg/kg	9800.00± 400.00^{a}	11200.00± 346.41^{b} **(-14.29)**	5733.33±503.32^{a} **(41.50)**	200.00±200.00^{a} **(97.96)**	200.00±200.00^{a} **(97.96)**	133.33±115.47^{a} **(98.64)**
H 125	9666.67±832.67^{a}	11133.33±503.32^{b} **(-15.17)**	11800.0±1000.00^{c} **(-22.07)**	13200.0±871.78^{de} **(-36.55)**	14400.00±1200.00^{d} **(-48.97)**	15666.67±305.51^{de} **(-62.07)**
H 250	9933.33± 642.91^{a}	11266.67±832.67^{b} **(-13.42)**	11466.67±611.01^{c} **(-15.44)**	11800.0±916.51^{d} **(-18.79)**	13733.33±3288.36^{d} **(-38.26)**	15266.67±2343.79^{d} **(-53.69)**
H 500	9866.67±305.51^{a}	8866.67±757.19^{a} **(10.14)**	7066.67± 529.15^{ab} **(28.38)**	4800.00±529.15^{b} **(51.35)**	4066.67± 416.33^{bc} **(58.79)**	4733.33±230.94^{bc} **(52.03)**
M 125	9800.0±871.78^{a}	11266.67±901.85^{b} **(-14.97)**	11533.33±1301.281^{c} **(-17.6871)**	12333.33±611.01^{d} **(-25.85)**	12733.33±611.01^{d} **(-29.93)**	15000.00±916.52^{d} **(-53.06)**
M 250	9600.00±529.15^{a}	8933.33±115.47^{a} **(6.94)**	8466.67±416.33^{b} **(11.81)**	6666.67±1331.67^{c} **(30.56)**	5600.± 1442.22^{c} **(41.67)**	5266.67±1474.22^{c} **(45.14)**
M 500	9600.00± 400.0^{a}	8400.00± 400.00^{a} **(12.5)**	6666.67±416.33^{ab} **(30.56)**	3333.33±461.88 ^{b} **(65.28)**	2466.67±642.91^{ab} **(74.31)**	2133.33±901.85^{ab} **(77.78)**
P -value	0.98	0.014	0.02	0.02	0.01	0.01

^{a,b,c,d,e}: Means with the same letters on the same columns are not different significantly (P>0.05).

DMSO= Dimethyl sulphoxide 3.2%; ALB=Albendazole7.5mg/kg; H125= Harungana madagascariensis125mg/kg; H250=Harungana madagascareinsis 250mg/kg; H500=Harungana madagascariensis500mg/kg; M125= Momordica foetida125mg/kg; M250= Momordica foetida 250mg/kg; M500= Momordica foetida 500mg/kg

Table 2 shows the mean faecal score per group and prevalence of diarrhoeal faeces on different sampling days. Mean faecal scours >1 registered on any sampling day implies at least one animal in the group had diarrhoea. Occureance of soft faeces was seen only from day 30 post inoculation. Albendazole 7.5 mg/kg completely prevented diarrhoea (Group 2) due to Teladorsagiasis/Trichostrongyliasis with 0% prevalence throughout the study The untreated lambs (Group 1); treatment with *H. madagsacariensis* 125 mg/kg (Group 3), *M. foetida* 125 mg/kg (Group 6) and *M. foetida* 250mg/kg (Group 7) recorded higher diarrhoea scores with at least one animal developing diarrhoea on one or more of the six sampling days (Table 2). In contrast to this, treatment with *H. madagsacariensis* 250 mg/kg (Group 4) and 500 mg/kg (Group 5), *M. foetida* 500 mg/kg (Group 8) seemed to be more effective in preventing the development of diarrhoeal faeces, even though these lambs had slightly softer faeces when compared to albendazole treated lambs.

Fig. 2 represents the variation in food intake of lambs co-infected with *Teladorsagia circumcinta* and *Trichostrongylus colubriformis* L3 and treated with albendazole as reference drug, *Harungana madagascariensis* and *Momordica. foetida* methanol extracts.

All animal groups experienced a decrease in amount of forage consumed within the first week of arrival. From the second week, there was improvement in the amount of forage eaten in all groups, until week 4 and 5 (three weeks post inoculation). Treatment was administered during week 5, and it could be seen that treatment with albendazole 7.5 mg/kg (group 2), *H. madagsacariensis* 500 mg/kg (group 5), *M. foetida* 250 mg/kg and *M. foetida* 500 mg/kg (group 7 and 8 respectively) significantly improved food consumption of lambs during the last 5 weeks post treatment (Fig. 2).

The effect of co-infection with *T. circumcincta* and *T. colubriformis*, and treatment using these plants extracts on production parameters was highlighted more when the efficiency of food conversion and subsequent weight gains between the different groups during the trial period were compared (Table 3).

There was a variation in total food consumed per animal per group during the trial. Lambs treated with albendazole, *H. madagascariensis* and *M. foetida* at their highest doses (500 mg/kg) consumed the most that is, >125 kg of forage (Table 3) while the untreated control lambs and those that received plant treatment at the lower doses consumed less. This variation in food consumed was translated into an overall significant effect on mean weight gain ($F = 6.205$. $df = 7$; $p = 0.002$). Untreated lambs and those treated with both plant extracts at dose level 125 mg/kg had similar weight gains, which was significantly lower ($P < 0.05$) than weight gains of lambs treated with both plants at 250 mg/kg. However, the highest significant weight gains

Table 2. Mean faecal score per group and prevalence of diarrhoeal faeces (%) of lambs co-infected with *Teladorsagia circumcinta* and *Trichostrongylus colubriformis* L3 larvae before and after treatment with albendazole as reference drug and methanol extracts of *Harungana madagascariensis* and *Momordica foetida*

Treatments	(Day 0)	(Day 3)	(Day 5)	(Day 7)	(Day 15)	(Day 30)
DMSO 3.2%	0.60.67(0)	0.67(0)	1.33(0)	1.33(0)	1.67(33.3)	2.33(66.7)
ALB 7.5	0.67(0)	0.67(0)	1(0)	0(0)	0(0)	0(0)
H125	0.33(0)	0.67(0)	1.67(66.7)	1.67(66.7)	2(100)	2(100)
H 250	0.33(0)	0.67(0)	0.67(0)	1(0)	0.67(0)	0.67(0)
H 500	0.67(0)	0.67(0)	0.67(33.3)	0.33(0)	0.33(0)	0.3(0)
M 125	0.67(0)	1(0)	1.33(33.3)	1.33(33.3)	1.33(33.3)	1.67(33.3)
M 250	0.33(0)	0.67(0)	1.33(33.3)	1.67(66.7)	1.33(33.3)	1.33(33.3)
M 500	0.67(0)	1(0)	1(0)	0.67(0)	0.33(0)	0.33(0)

DMSO= Dimethyl sulphoxide (3.2%); ALB=Albendazole 7.5 mg/kg; H125= Harungana madagascariensis 125 mg/kg; H250=Harungana madagascariensis 250 mg/kg; H500=Harungana madagascariensis 500 mg/kg; M125= Momordica foetida 125 mg/kg; M250= Momordica foetida 250 mg/kg; M500= Momordica. foetida 500 mg/kg.

Faecal consistency was classified as: normal pelleted = 0; soft pelleted = 1; paste-like = 2; liquid diarrhoea =3. Faeces with scores of 2 or 3 were designated 'Diarrhoeal faeces'

were recorded in albendazole treated lambs and those treated with *H. madagascariensis* and *M. foetida* at 500 mg/kg dose level.

This weight gain pattern was a reflection of the feed conversion efficiency between the different animal groups. Lambs treated with albendazole, 500 mg/kg of both *H. madagascariensis* and *M. foetida* (which recorded the highest weight gains) also showed the highest significant ability to convert forage to flesh. This was followed by lambs treated with both extracts at 250 mg/kg while untreated lambs,and those treated with *H. madagascariensis* and *M. foetida* at 125 mg/kg had the least significant ability to convert forage to flesh, .thus they had lower weight gains.

The above result was confirmed by carrying out a Pearson correlation between the production parameters. There was a strong positive correlation between food conversion efficiency and weight gain (r = 0.99**, P=0.00); while adult worm burden and food conversion efficiency were negatively correlated (*r* = -0.68, *P* = 0.00) as shown on Table 4.

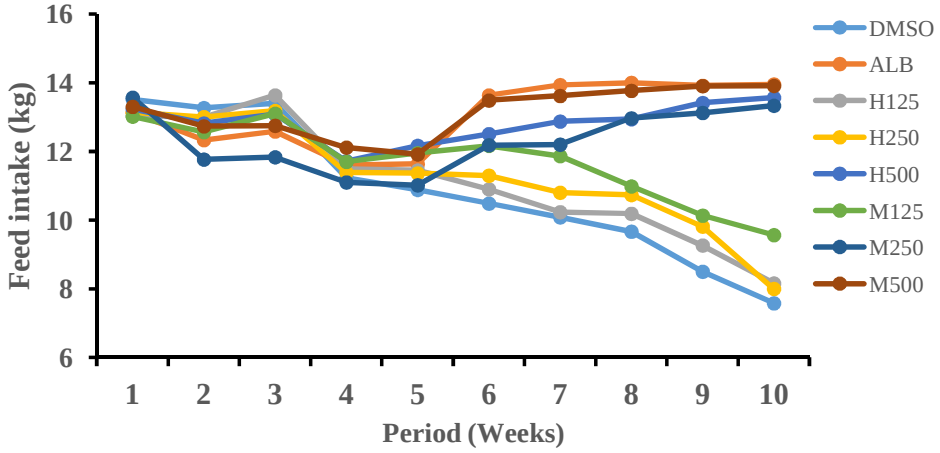

Fig. 2. Mean weekly forage eaten by lambs co-infected with *Teladorsagia. circumcinta* and *Trichostrongylus colubriformis, .*and treated with different doses of methanol extracts of *Harungana madagascariensis* and *Momordica foetida* and albendazole as a reference drug

DMSO= Dimethyl sulphoxide (3.2%); ALB=Albendazole 7.5 mg/kg; H125= Harungana madagascariensis 125 mg/kg; H250=Harungana madagascareinsis 250 mg/kg; H500=Harungana madagascariensis 500 mg/kg; M125= Momordica foetida 125 mg/kg; M250= Momordica foetida 250 mg/kg; M500= Momordica. foetida 500 mg/kg

Table 3. Mean weight gain, food intake and food conversion efficiency of sheep co-infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* and treated with methanol extracts of Harungana *madagascariensis* and *Momordica foetida*

Treatment	WG in kg (Mean ± SD)	FI (kg)	FCE
DMSO 3.2%	0.16 ± 0.72^{a}	112.71	.001 ± .006^{a}
Alb7.5 mg/kg	1.42 ± 0.12^{c}	130.85	.012± .001^{c}
H125 mg/kg	0.12 ± 0.81^{a}	111.34	0.0009 ± .007^{a}
H250 mg/kg	0.46 ± 0.16^{ab}	108.61	.004 ± .002^{ab}
H500 mg/kg	0.54 ± 0.35^{c}	128.32	.013 ± .003^{c}
M125 mg/kg	0.11±0.46^{a}	117.05	.0008 ± .0003^{a}
M250 mg/kg	1.14 ± 0.45^{bc}	123.09	.009 ± .004^{bc}
M500 mg/kg	1.43 ± 0.28^{c}	131.58	.011 ± .002^{c}
P-value	0.03	/	0.04

WG= Mean weight gain; FI=forage intake; FCE=Food conversion efficiency, Alb=Albendazole7.5 mg/kg; H125= Harungana madagascariensis125 mg/kg; H250=Harungana madagascariensis 250 mg/kg; H500=Harungana madagascariensis 500 mg/kg; M125= Momordica foetida 125 mg/kg; M250= Momordica foetida 250 mg/kg; M500= Momordica foetida 500 mg/kg;

Table 4. Pearson correlation between total adult worm count, food conversion efficiency and weight gain of lambs co-infected with *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*, and treated with albendazole as reference drug, *Harungana madagascariensis* and *Momordica foetida* methanol extracts

Parameters	Total adult worm	Weight gain	Feed conversion efficiency
Total adult worm	1.00		
Weight gain	-0.69**	1.00	
Feed conversion efficiency	-0.68**	0.99**	1.00

***Correlation is significant at the 0.01 level. (2-tailed)*

4. DISCUSSION

Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin [36]. The present study investigated the phytochemical and anthelmintic properties of *H. madagascariensis* and *M. foetida*. Most traditional healers use primarily water as the solvent but in our studies we found that plant extracts in organic solvent (methanol) provided more consistent anthelmintic activity compared to those extracted in water. This is because methanol has high polarity index thus, it is able to extract almost all the chemical components of plants which has anthelmintic qualities [37].

In this study, the number of L3 larvae used to inoculate experimental animals were 4000 L3 of *T. circumcincta* and 10.000 L3 larvae of *T. colubriformis* (that is, 14000 L_3 larvae in three doses). This number was chosen based on the number recommended for in vivo testing of anthelmintic products by Woods *et al.* [38]. These authors indicated that 3000-6000 L_3 larvae was significant for testing anthelmintic efficacy using *Teladorsagia sp* and *Trichostrongylus sp* in sheep. However, 14000 L_3 larvae were used to inoculate each lamb because one of our objectives in this study was to measure the effect of treatment on some productivity parameters.

The establishment of *T. circumcincta* and *T. colubriformis* L3 larvae of 45.85% and 68.61 % respectively was rather high. However, the infective dose was split across 3 days to reduce the possibility of rumen bypass and to maximize the establishment of larvae in the small intestine) [39]. This high establishment percentage suggested that the establishment of *T. colubriformis* did not interfere with that of *T. circumcincta* and vice versa. This could be attributed to the fact that these two nematode species occupy different locations in the gastrointestinal tract with predilection site for *T. circumcincta* being the abomasum and that of *T. colubriformis* being the anterior small intestine [28]. However, similar high establishment percentage has been reported by [39,40] who observed that higher establishment of gastrointestinal parasite larvae occurred when the number of infective larvae were reduced (< 15000 L3) and when the two infecting species occupied different locations in the gastrointestinal tract.

Harungana madagascariensis at its highest dose caused only 52.03% faecal egg count reduction and 39.86 % adult deparasitization. These values were significantly lower as compared to treatment with *M. foetida* (77.88 feacal egg count reduction and 86.6% deparasitization) at the same dose (500 mg/kg).The anthelmintic effect recorded in this study could be attributed to variations in the type and quantity of the various bioactive compounds present in each plant extract. Preliminary studies on the photochemistry of both plant extracts revealed the presence of active biochemicals such as such as alkaloids, glycocised, tannins, saponins, steroids and phlobatannins [41].Tannins and flavonoids which were present in these extracts have a detrimental effect on adult worms, some of which are able to bind to the macrotubules on the worm cuticle, causing their destruction [42] However, the finding that *H. madagascariensis* had the least deparasitization percentage (39.9%) at 500 mg/kg while *M foetida* was most effective (86.6%) at 500mg/kg is suggestive of the fact that *M. foetida* contains a greater proportion of these secondary metabolites than methanol extracts of *H. madagascariensis.*This is in agreement with [43] who observed the absence of flavonoids in *H. madagascariensis* extracts but was present in *M. foetida* extracts.

Infections with *T. circumcincta* and *T. colubriformis* nematode species can present a variety of clinical signs that range from no clinical manifestation at all to diarrhoea, anorexia and

weight loss [44,45,]. This is in line with Gjerde et al. [33] whoequally reported that softening of faeces may occur during the third and fourth week after infection with gastrointestinal parasite. We therefore investigated the effectiveness of plant extracts in preventing development of soft faeces by evaluating the faecal consistency (diarrhoea scores) of lambs in different treatment groups on different sampling days. It was observed that treatment with *H. madagascariensis* 250 mg/kg and 500 mg/kg, *M. foetida* 500 mg/kg prevented the incidence of diarrhoea when compared to the untreated control lambs. Many authors have reported the use of *H. madagascariensis* in treating diarrhoea [26,46] From our findings, *H. madagascariensis* at 250 mg/kg had weak anthelmintic effect, causing only 9.0% deparasitization, but was more potent in preventing diarrhoea than *M. foetida* at the same dose (250 mg/kg). This is suggestive of the fact that diarrhoea was not only as a function of the parasitic load. Although the lambs that received the effective doses of plant extracts (H500 mg/kg; M250 mg/kg and M500 mg/kg) had significantly lower adult worm burdens, *M. foetida* at 250 mg/kg did not prevent the advent of diarrhoea, neither was it effective in treating diarrhoea when it developed. There seems to be a threshold value for adult worms, above which infection may result to diarrhoea. Once the adult worms were killed by the extracts, clinical diarrhoea also diminished. Our results are in agreement with those of [33] who achieved an improvement in faecal consistency when adult nematodes and coccidia were killed using anthelmintic and anticoccidia drugs respectively. Several authors had indicated that ovine diarrhoea could occur as a result of a decreased absorptive capacity of the small intestines due to physical damage caused by large number of worms inhabiting the mucosa [11] or due to the increased abomasal pH, there by promoting rapid proliferation of bacteria in the abomasum, which can contribute to the diarrhoea [45] since *H. madagascariensis* induces anti-bacterial hence anti diarrhoel effects even at dose level 200 mg/kg body weight [46], it is suggestive that absence of diarrhoeao in lambs treated with *H. madagascariensis* was due to the bacteriocidal effect of this extract. Therefore, reducing parasitic load as well as preventing bacterial development could prevent advent of diarrhoea in lambs.

Assessing stock performance is a key component of improving sheep production and profitability. One of the major production traits of interest to sheep farmers in Cameroon is lamb growth rate, as it determines how quickly the farmers can make returns on their investment. In this study, the influence of co-infection with *T. circumcinta* and *T. colubriformis* on sheep performance was evaluated by measuring the mean feed intake and weight gain throughout the trial period. Information obtained also permitted us to evaluate the gain in production by treatment with plant extracts. All animal groups experienced a decrease in amount of forage consumed within the third and fourth week post inoculation. This could be attributed to the pathological consequences of parasitism. [47] had described the time course of infection with *Trichostrongylus colubriformis* in lambs. They observed that the course of this gastrointestinal nematode infection typically follows three distinct phases. First, a hypo responsive phase characterized by a patent infection being established without a detrimental effect on animal performance. Secondly, the acquisition phase during which protective immunity is developed and the host displays neither complete resistance nor resilience. During this time, .animal performance is reduced as immune mechanisms begin to regulate the parasite population through reducing female worm fecundity, expulsion of established worm populations and limiting the establishment of incoming parasite larvae. Finally, the expression phase during which time a mature immune response prevents further infection and growth returns to rates comparable with uninfected controls. From this time, both resistance and resilience are displayed. They equally added that the most important of these phases for animal production is the acquisition of immunity, during which live weight may plateau or even drop. largely as a result of reduced voluntary feed intake and reduced nutrient utilization even in the order of 20% to 50% This is suggestive of the fact that the lower weight gains seen in untreated lambs and those treated with *H. madagascariensis* at doses 125 mg/kg and 250 mg/kg could be as a result of reduced feed intake which was a consequence of co-infection with *T. cricumcincta* and *T. colubriformis.* This was further confirmed by the significant negative correlation which existed between parasitic load and weight gain (r =.-0.69.P = 0.00).

The increase in feed intake hence live weight observed in lambs treated with *H. madagascariensis* at doses 500 mg/kg, *M. foetida* at dose rate 250 mg/kg and 500 mg/kg from third week post treatment could be

attributed to the reduced parasitic load after treatment. Our findings are in accordance with the observations of [48,49] who observed that sheep parasitized with higher intensity of gastrointestinal nematodes had reduced appetite. In the same light, [50] made a systematic review of the current knowledge on the influence of gastrointestinal nematode infections on sheep production by summarizing the results obtained from of 94 trials. In 78 of the 94 trials, a negative effect of parasitism on feed consumed hence live weight gain was reported. He also observed that anorexia is commonly associated with *Trichostrongylus* and *Teladorsagia,* infections. Several hypotheses have been suggested to explain the reduction in food ingestion. On the one hand, the lesions produced by larval migration or adult feeding could produce certain pain and digestive discomfort, which would have a negative intake impact [49]. Again, the rapid restoration of appetite following anthelmintic treatment suggests that nematode themselves may influence appetite directly. This has been supported by [48,49] who suggested that nematode infection will depress appetite of the host through secretion of cytokine-like molecules. Further, amongst the gastrointestinal hormones cholecystokinine (CCk) is considered one of the most important factors in the alimentary behaviour This hormone is produced by the cells that cover the first part of the duodeneum. Once released, it causes contraction of the gall bladder and a plenitude feeling at the central nervous system [49]. This is true with parasites like *T. colubriformis* which inhabit the first half of small intestine. The injuries in the stomach produced by *T*. *circumcincta* alter the normal functioning of the stomach glands [51]. This decreases the production of hydrochloric acid (HCl), increasing gastric pH and not allowing the activation of pepsinogen to pepsin.

5. CONCLUSION

The valorization of anthelmintics of plant origin in the control of parasitic infestations can protect livestock and promote production. Based on this, we took the initiative to evaluate the anthelmintic properties of bark of *H. madagascariensis* and whole aerial parts of *M. foetida*, two Cameroonian plants commonly used in the Western Highlands as anthelmintics. *H. madagascareiensis* possesses some anthelmintic activity against *T. circuncuncta and T. colubriformis*. However, this effect is not good enough when compared to *M. foetida* and albendazle, which recorded a significantly higher anthelmintic activity Overall, *M. foetida* was almost as twice as strong as the most effective dose of *H. madagascariensis* (500 mg/kg), in reducing EPG and adult worm burden. However, *H. madagascariensis* was more efficient in preventing or treating diarrhoea at 250 mg/kg than *M. foetida*. Again, both plant extracts at 500 mg/kg prevented clinical features associated with nematodiasis such as development of, anorexia and reduced food conversion efficiency. These anthelmintic potentials are important in epidemiological terms because they help to reduce contamination of pasturage, hence reducing incidence of teladorsagiasis / trichostrongyliasis in particular and reducing economic losses due to gastrointestinal nematode infection. The present study is a preliminary work on the use of *H. madagascariensis and M. foetida* against gastrointestinal nematode species of lambs in Cameroon. However, further studies on the pharmacokinetics and the quantitative phytochemistry of methanol extracts of both plants and their toxic effects if any should be carried out before their extensive use in Cameroon.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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