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Therapeutic Effects of Methanol and Aqueous Extract of *Terminalia glaucescens* (Combretaceae) on Gastro-intestinal Nematodes of Djallonke Sheep

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Abstract: Background: Gastrointestinal nematodes (GIN) are a major threat to sheep productivity and endangers animal welfare worldwide particularly in developing countries. They cause loss of production through mortality, weight loss, reduced milk, meat and wool production. Thus, GIN infection is an important limiting factor or constraint in livestock production. **Aim:** To evaluate the anthelmintic activity of organic and aqueous extracts of stem bark of *Terminalia glaucescens* against gastrointestinal nematodes of sheep using varieties of *in vivo* tests. **Materials and Methods:** Fourty (40) Djallonke sheep acquired natural infection with gastrointestinal nematodes of both sexes, aged 6-10 months old and weighing between 8-15kg, used in bioassay were distributed into 5 groups (n=8). Two experiments using methanol (Groups A to E) and hot water extracts (Groups A' to E') were simultaneously carried out. Groups A & A' received 1.25% dimethyl sulfoxide (DMSO) and distilled water 1ml/10 kg bodyweight (bwt) respectively, Groups B & B' received Fenbendazole at 6.25mg/kg bwt, Groups C & C', D & D' and E & E' received doses of 125, 250 and 500 mg/kg bwt of each extract. Sheep were subjected to different treatment with single dose of synthetic drug and double doses of plant extracts. **Results:** Methanol extract for all the doses tested was active *in vivo* on the adults of GIN, and reduced significantly ($p<0.05$) the faecal egg count (FEC) and total warm count (TWC) of the nematodes. The dose rate 500mg/kg showed the highest nematocidal activity of 77, 6% FEC and 73, 5% TWC reduction 14 days post-treatment. For hot water extract, these numbers were 65,3% and 62,1% for FEC and TWC respectively at the same dose for the same period of treatment. **Conclusion:** These results suggest the possible use of this medicinal plant in the control of gastrointestinal nematodes in sheep and justify their use in traditional veterinary practices.

Keywords: *Terminalia glaucescens*, gastrointestinal nematodes, sheep, Dschang, Cameroon

INTRODUCTION

Sheep and goats have been selected and bred for multiple purposes, including acquisition of secondary products such as milk, wool/hair and manure, which can be collected during the life of the animal, and final products such as skin, meat, horn and bones (Gillis *et al.*, 2019). Sheep and goat raising is attractive in rural households because of the relatively smaller resources and effort required to maintain them (Pollott *et al.*,2009). They can subsist on unpalatable low-quality fodder and browse and still be prolific, owing to the early sexual maturity, brief gestation duration, and short birth intervals. In Cameroon, sheep farming is practiced throughout the national territory with undeniable socio-economic importance, with a herd-size of 3,931,917 heads in 2021 (MINEPIA 2021), and estimated to be 10 202 369 animals in2018 (World Bank, 2016, MINEPIA, 2021) contributing its own way to the livelihoods of rural masses. Several factors constraint sheep and goat production in Cameroon.

Gastrointestinal nematodes mostly infect small ruminants. Their epidemiological patterns rely on the factors related to the parasite-host such as inadequate host nutrition, poor hygiene, and sanitation (Tesfaye *et al.,* 2021). Among these gastrointestinal nematodes (GIN) species, the most common infecting sheep are *Hemonchus contortus*, *Trichostrongylus colubriformis, T. axei Ostertagia ostertagi, Chabertia ovina, Trichuris ovis* and *Oesphagostomum columbianum* (Mekonen 2021). Infection usually occurs primarily through contaminated feed and water, enhanced by poor hygiene (Inuwa *et al.,* 2021). The problem of nematode parasitism is of particular importance throughout the developing world since nutritional resources available to small ruminant livestock are often inadequate and as a consequence, natural immunity is compromised resulting in low productivity and high mortality (Perry *et al.,* 2002). Thus, parasitism is an important limiting factor or constraint in livestock production (Marcelo 2009). In rural areas in North West Region of Cameroon, enough attention has not yet been given to traditional herbal medicines even though remedies based on locally available herbs are still prevalent. In the rural areas where veterinary services are absent, irregular and expensive, people are still aware of usage of plants for curing domestic animal diseases through generations because they are more accessible, easy to prepare and administer, less toxic to man, animals and the environment (Wabo Pone *et al.,* 2010). Under such circumstances natural plant products can be promoted as an alternative to synthetic anthelmintic drugs and this could help in poverty alleviation by empowering the people to use their own resources for the prevention and treatment of livestock diseases.

Members of the family Combretaceae are used by traditional healers for many medicinal purposes such as abdominal disorders, cough, colds, conjunctivitis, diarrhea, dysmenorrhoea, worms, gastric ulcers, dysentery and heart diseases (Dongmo *et al.*, 2006). The genus Terminalia is among some of the plants most widely used for medicinal purposes in Africa (Masoko *et al.,* 2006; Kamtchouing *et al.,* 2006). In Cameroon, it is traditionally used in the treatment of diabetes (Njomen *et al.,* 2009). It is an important drug in folk medicine. The objective of this study was to evaluate the anthelmintic activity of organic and aqueous extracts of stem bark of *T. glaucescens* against gastrointestinal nematodes of sheep using varieties of *in vivo* tests.

MATERIALS AND METHODS

Study Area, Site of Plant Collection

This study took place from November 2022 to May 2023. Stem bark of *Terminalia glaucescens* was collected from its natural habitat in Menoua Division (5°27'0" Latitude North and 10°4'0" Longitude East), located in the Western highlands of Cameroon. With a population size of about 372, 244 inhabitants. This zone covers a surface area of 1, 380 km^2, leading to a population density of 270 inhabitants/km^2 (BURCREP, 2010). Menoua Division comprises six Sub-divisions out of which three were selected for this study based on the availability of *T. glauscence* plant and sheep population size namely: Dschang, Fokoue and Fongo- Tongo (Figure 1). The average altitude of Menoua Division is 1, 382 m. It is limited to the North by the Mifi Division, to the South by the Mungo Division and the Nkam River, to the East by the Haut-Nkam Division and to the West by the Bamboutos mountain range. This Division is a peculiar zone as far as topography and climate are concerned. It is located in a savannah landscape within the Guineo-Congolese bioclimatic domain, on the Cameroon Volcanic Line. Two seasons can be distinguished as follows: the rainy season (March to October) and the dry season (November to February). Annual precipitation ranges from 1, 200 mm to 1, 800 mm. The maximum precipitation is in August and September. The average annual temperature is 20.2 °C and fluctuates during the day between 13.4 °C and 27.5 °C. The daily humidity varies from 33 to 98% (Tazen *et al.,* 2013). These characteristics create favorable conditions for maintaining a high density of gastrointestinal parasitic diseases. Agriculture and animal husbandry are the main economic activities; no industrial activity is observed in the area.

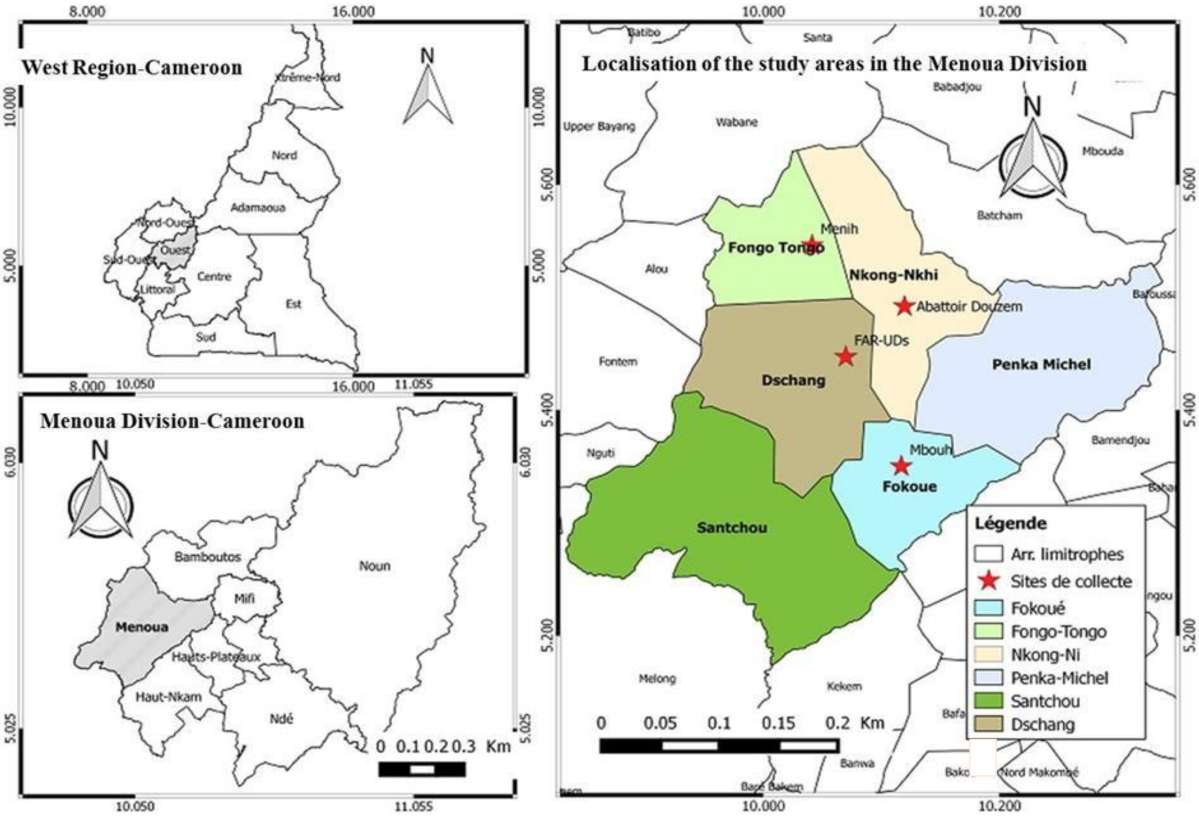

Figure 1: Location of Menoua Division in the West Region of Cameroon.

Source: Michel *et al.*, 2021

Identification of Plant

Identification of this plant was done in the National Herbarium in Yaounde with the following voucher number: No 32194/ HNC. Stem bark of *T. glaucescens* were cut into small pieces (2cm x 5cm), spread on cardboard and place in a well aerated room (24-25ºC), for seven days to allow plant parts to get well dried. Later it was ground using blender devices, drilled, powdered and stored in air-tight plastic bags at room temperature (25ºC) and relative humidity of about 67% for subsequent use in the laboratory Wabo Pone *et al.,* 2011).

Preparation of Plant Extracts

Organic and aqueous extracts i.e methanol and hot water (Decoction) extracts (100ºC) respectively were prepared to compare their effects and to increase the chances of detecting anthelmintic activity on gastrointestinal nematodes in sheep.

Methanol Extract

Four hundred and fifty (450) grams of the dried powder were mixed in five L of methanol (95%). This mixture was stirred manually in the morning (8.00-9.00 am) for 72 hours, later filtered through a Wattman filter paper of pore size 2.5µm. The methanol extract was obtained using the protocol described by Abdullahi *et al.,* 2020) and subjected to solvent extraction using Soxhlet apparatus for 12 hours. The extract obtained was concentrated using vacuum rotatory evaporator and the solvent free extract was stored in refrigerator (4ºC) for further use.

Hot Water Extract

Four hundred and fifty (450) grams of powdered plant material was mixed with 5L of distilled water in a pot and boiled for 15 minutes. It was allowed to cool down to room temperature and filtered through muslin gauze and Wattman filter paper. The decocted solution was evaporated in an oven at 40ºC for three days.

Reference Drug

The reference drug Fenbendazole used in this study was bought from a Veterinary Clinic in the Sub Divisional Delegation of Livestock, Fisheries and Animal Industries in Fongo-Tongo Menoua. This drug was chosen due to its broad-spectrum activity to prevent and treat gastrointestinal nematodes of domestic animals. Controls for the bioassay were 1.25% DMSO (maximum concentration of DMSO in test dishes) and distilled water (DW) (Wabo Poné *et al.,* 2011).

Experimental Animals

40 Sheep were bought from Mbororo herdsmen in Fokoue village, about 35km from Dschang Menoua Division. Before the start of the experiment, all animals were identified using ear tags, vaccinated against Small Ruminant Pest (SRP) using Capri-pestovac, four weeks before they were transported and lodged in the experimental school farm of the Faculty of Agronomy and Agricultural Sciences (FASA), University of Dschang.

A preventive antibiotic treatment with Oxytetracycline 1ml/ 20 kg body weight was given to these animals for three days through intramuscular route. An anti-infectious complex multivitamin antistress (2.5g/10kg body weight for 5 days) was also given to the sheep. Early in the morning, a thermometer was cleaned and gently inserted in the rectum of each animal for about five minutes to get the readings and record the temperature.

Experimental Design: Group Size and Treatment

Animals were kept for 14 days to get acclimatized with the environment before the start of the experiment. The experimental sheep (n=40) were distributed into 5 groups (n=8) at random using age, sex, body weight, and parasitic load as the blocking factor. The ages of the sheep were determined by their dentition. These animals were aged between 6 and 10 months, weighing between 8 and 15kg bodyweight (bwt). Hosts were grazed on natural pasture during the day and housed in pens at night. Water was given *ad libitum*. The sheep had naturally acquired mixed infections with gastrointestinal nematodes. Infection was confirmed before the beginning of the experiment through preliminary fecal sampling of sheep and subsequent coprological examination of the samples (Thienpont *et al.,* 1086**).** Only hosts, not having been treated during the last 10 weeks and showing an egg per gram (EPG) of 2000 or more were selected for the trial. Actually, two experiments were simultaneously carried out: one for methanol (Groups A to E) and the other for hot water extracts (Groups A' to E'). Groups A & A' were given 1.25% DMSO and DW (1ml/10kg bwt) and serve as negative controls. Groups B & B' were treated with a commercial anthelmintic (Fenbendazole), 6.25mg/kg bwt as positive control. Groups C & C', D & D' and E & E' were treated with plant extracts using three doses 125, 250 and 500 mg/kg bwt respectively. Sheep were subjected to different treatment with single dose of synthetic drug and double doses of plant extracts.

Sheep Fecal Samples

Prior to treatment, which took four days, each sheep was weighed and fecal samples were collected between 8-10 am through the rectum using hand gloves. The specimens were immediately transported to the laboratory of Applied Biology and Ecology, University of Dschang for coprological analysis from 0 day pre-treatment and at 6^{th}, 10^{th} and 14^{th} day post treatment. The gastrointestinal nematode eggs were identified using the identification key for gastrointestinal helminths of ruminants described by Thienpont *et al*. 1986.The percent fecal egg count reduction (FECR) was calculated using the following formula (Cringoli *et al.,* 2017).

$$FECR\ (\%) = \frac{a-b}{a} \times 100$$

Where a= Egg per gram of feces (EPG) Pretreatment and b= EPG Post treatment

Worm Recovery

Fifteen days post treatment, six sheep per group (75.0%) were slaughtered and the body cavity opened to remove the gastrointestinal tract. This organ was placed in 10L bucket containing 8 L of distilled water and opened longitudinally with a small pair of scissors. The intestine was scratched and the exudates containing parasites were successively washed 3 times in the water. The percentage of total worm count reduction (TWCR) was calculated as described by (Wabo Poné *et al.,* 2009).

$$\text{TWCR (\%)} = \left(\frac{Total\ worm\ count\ in\ control\ group - Total\ worm\ count\ in\ treated\ group}{Total\ worm\ count\ in\ control\ group}\right) x\ 100$$

Statistical Analysis

Results were expressed in terms of mean ± SEM (Standard error on the mean). Two ways analysis of variance (ANOVA) followed by post-hoc Turkey HSD was performed using the program Stat Soft. Inc (2008). Values of the probability $p<0.05$, $p<0.\ 01$ and $p<0.001$ were considered significant.

RESULTS

Yield of methanol and hot water extracts were 16,0% and 26.9% respectively. There were no visible clinical signs nor any abnormal behavior observed in all animals treated either with methanolic or aqueous extracts of *T. glaucescens*. The mean rectal temperature of each animal was within normal range (38.5-39.5°C). The species of nematode eggs recovered from the experimental animals were identified by fecal culture as *Haemonchus contortus, Trichostrongylus axei, Oesophagostomum columbianum, Chabatia ovina* and *Trichuris ovis* and the prevalence calculated as seen in Table 1.

Table 1: Prevalence of different species of parasites in experimental sheep

Species of parasites	Number of sheep infected	Prevalence (%)
Haemonchus contortus	40	100.0
Trichostrongylus axei	36	90.0
Oesophagostomum columbianum	22	55,0
Chabatia ovina	24	60
Trichuris ovis	23	57.5

Effect of the Methanol and Aqueous Extracts of *Terminalia glaucescens* on the Evolution of Total Faecal Egg Load of Gastrointestinal Nematodes in Experimental Sheep

Terminalia glaucescens extracts administered orally gradually reduced the faecal egg count (FEC) in experimental sheep. The multiple comparison post treatment reveals the relative sensitivity of nematodes and level of significance of the effect of different treatments (doses), relative to the control (positive and negative) groups. At day 6, there were reductions of FEC and progressively to day 10 and 14 post treatment (Table 2). The effect of methanol and hot water extracts of *T. glaucescens* on the parasitic load at dose of 500mg/kg were maximal (800.0 ± 236.6 and 700.0±198.3 respectively) 14 days post treatment, $p<0.001$ significantly different compared to distilled water and compared to day 0. Hot water extract (HWE) at dose of 500mg/kg had FEC reduction rates from days 6, 10 and 14 post treatments (Table 2). At 125 and 250mg/kg, decoction reduced the FEC from days 6 to 14 progressively. At 500mg/kg, HWE significantly reduced FEC (800.0±73.0), $p<0.001$ compared to DW at day 14 (Table 2). All doses of the plant extracts showed dose – dependent ($P<0.05$) effects on reduction of FEC of experimental animals. The effect of doses between days and interactions with control groups are feasibly significant on 6, 10 and 14 days post treatment.

Table 2: Effect of methanol and hot water extracts of *Terminalia glaucescens* on the parasitic load in term of egg per gram of feces

Treatment	Doses	Day			
	(ml or mg/kg Bwt)	0	6	10	14
Distilled water	1ml/10kg	4450,0±827,3	4150,0±1697,9	4300,0±929,5	4200,0±734,4
1.25% DMSO	1ml/10kg	5350,0±201,3	5100,00±321,5	4950,0±516,9	5200,0±229,5
Albendazole	6,25mg/kg	5450,0±246,0	$0,0±0,0^{***γ}$	$0,0±0,0^{***γ}$	$0,0±0,0^{***γ}$
Methanol extract	125mg/kg	5300,0±491,9	$4700,0±529,8^{c}$	2250,0±88,5***cγ	$1700,0±152,8^{***cγ}$
	250mg/kg	5850,0±1140,4	$2750,0±421,7^{***cα}$	$800,0±236,6^{***γ}$	$700,0±198,3^{***γ}$
	500mg/kg	6400,0±44,7	$2000,0±288,7^{***bγ}$	$1400,0±178,9^{***bγ}$	$900,0±196,6^{***aγ}$
Hot water extract	125mg/kg	$4250,0±424,9^{b}$	$3850,0±1105,9^{c}$	$3200,0±559,2^{b}$	$1350,0±233,5^{\#\#\#γ}$
	250mg/kg	5150,0±246,0	$2450,0±714,8^{\#bγ}$	$2050,0±117,6^{\#γ}$	$1150,0±194,5^{\#\#\#γ}$
	500mg/kg	$4150,0±156,5^{b}$	$1950,0±589, 1^{\#\#aγ}$	$1600,0±258,2^{\#\#γ}$	$800,0±73,0^{\#\#\#γ}$

***: p<0.001 significantly different compared to distilled water and DMSO; #: p<0.05; ##: p<0.01; ###: p<0.001 significantly different compared to distilled water; a: p<0.05; b: p<0.01; c: p<0.001 significantly different compared to albendazole; α: p<0.05; γ: p<0.001 significantly different compared to day 0. Bwt: Body weight. DMSO: Dimethyl sulfoxide

Effect of *Terminalia glaucescens* Extracts on Total Worm Count of Gastrointestinal Nematodes in Experimental Sheep

The reduction in total worm counts (TWC) were 73,5 % and 62,1 % for methanol and decoction extracts respectively 14 days post treatment at 500 mg/kg (Figure 2). *Haemonchus contortus* was the predominant species and *Trichostrongylus axei* was the next in abundance.

Animals treated with Fenbendazole registered a 90,0 % reduction in TWC, 6 to 14 days post treatment. Methanol extract had the higher efficacy at doses of 125, 250 and 500 mg/kg, meanwhile decoction extract recorded a lower anthelmintic effect at same doses.

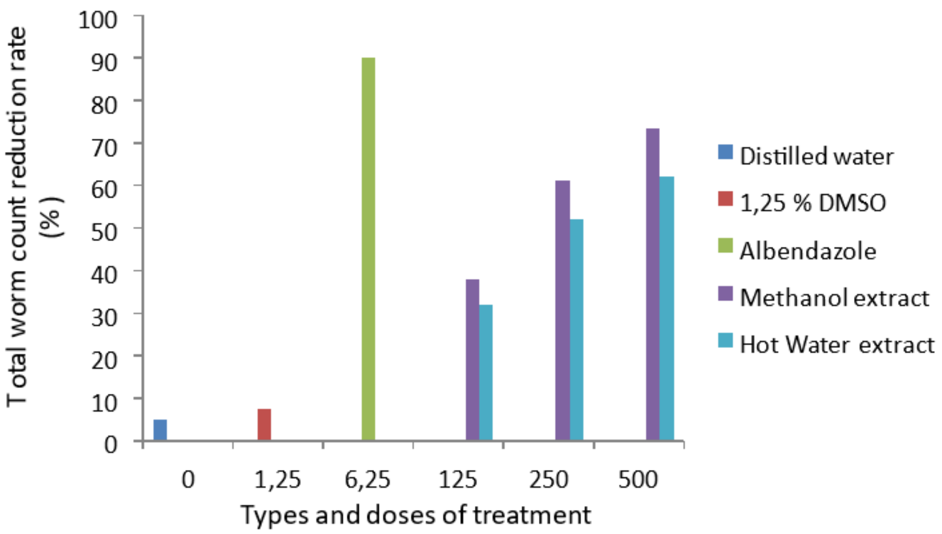

Figure 2: Total worm count reduction (%) with methanol and decoction extracts of *Terminalia glaucescens* compared to worm count in sheep in control groups.

DW: Distilled water; DMSO: Dimethylsulfoxide; ME: Methanolic extract; HWE: Hot water extract; ALB: Albendazole

DISCUSSION

In many developing countries, medicinal plants are the most commonly used method to control internal parasites due to their easy accessibility and affordability. Reasons why *in vivo* studies are more relevant in control practices of gastrointestinal nematodes in farm animals and thus considered more reliable than *in vitro* studies in evaluation of plant extract properties, although cost of large scale screening of plant extract is probably inhibitory (Githiori *et al.,* 2006). The results of the present study show that the two extracts of *T. glaucescens* have a broad spectrum of anthelmintic activity against gastrointestinal nematodes of sheep. The effect of methanol and hot water extracts of *T. glaucescens* on the parasitic load at dose of 500 mg/kg was (800.0±236.6; and 700.0±198.3) 14 days post treatment. The highest reduction rate in total worm count (TWC) was 73, 5 % and 62, 1% at 14 days post treatment at same dose for methanol and decoction extracts respectively. These results are similar to those obtained by (Ademola *et al.* 2007). with aqueous and ethanolic extracts of *Nauclea latifolia* on the adult worms of different gastrointestinal nematodes of sheep in Ibadan Nigeria. The variations in FEC and TWC reduction rates observed on the 6, 10 and 14 days post treatment with the different plant extracts could be due to anthelmintic properties. These values obtained from FEC reduction tests with the various doses of different extracts were significantly different ($p<0.05$; $p<0.01$; $p<0.001$) compared to the negative control. This observation was similar to the findings of Al-Shaibani *et al.* 2009 on the anthelmintic activity of *Fumaria parviflora* (*Fumariaceae*) against gastrointestinal nematodes of sheep at Tandojam area in Pakistan. The extracts of this plant had dose and time dependent effects on the parasites. Methanol extract significantly reduced the FEC and TWC of gastrointestinal nematodes of sheep. The activity was more visible at the dose rate of 250mg/kg by days 10 and 14 post treatment, and resulted in 74,8% and 61,06% respectively. This activity was dose and time dependent, and could affect the worm population in some cases. Aqueous extract (decoction) also had a remarkable effect. In comparison to the positive control, methanol and hot water extracts were able to reduce the parasite burden but none of the natural plant products presented an advantage to Fenbendazole. This could be related to the fact that Fenbendazole is metabolized in the liver to oxfendazole (fenbendazole sulfoxide) which has an anthelmintic effect (Jolie *et al.*, 2024).The genus *Terminalia* is known as a rich source of triterpenoids and their glycosides derivatives, flavonoides, tannins and other aromatic compounds with *T. glaucescens* as one of the species with medicinal properties. The mechanism of action of extracts of *T. glaucescens* is not fully understood but the anthelmintic activity could be attributed to its bioactive compounds jointly or separatedly. The phyto-chemical analysis of *T. glaucescens* revealed the presence of secondary metabolites like alkaloids, flavonoids, glycocises, tannins, saponins, steroids and Terpenoid (Bulama *et al.* 2014). Suhail (2016) suggested that anthelmintic activities of plant extracts on larvae and adults of gastrointestinal nematodes could be attributed to tannins capacity to bind to proteins membrane, meanwhile condensed tannins may bind to the cuticle of larvae, which is high in glycoproteins and cause their death. In adult worms, condensed tannins may create a hostile gut environment for the intestinal helminths (Al-Shaibani *et al.,* 2008) thus reducing their fecundity and consequently FEC in animals. In this study, the methanol extract was slightly more effective than the decoction. Trans-cuticular diffusion is a common means of entry in to helminths parasites for non-nutrients and non-electrolyte substances in nematodes (Al-Shaibani *et al.*, 2009). It has also been shown that this route is predominant for the uptake of major broad spectrum anthelmintic by different nematodes, cestodes and trematodes as opposes to oral ingestion. This slight difference between methanol and hot water extract could be due to easier trans-cuticular absorption of the methanolic extract into the body of parasites than the aqueous extract. Fenbendazole had a 100% efficacy probably because it acts as cholinergic agonist on the neuromuscular junction in nematode parasites, causing paralysis of the worm, leading to their death and subsequent expelling from the host (Martin *et al.,* 2015).

CONCLUSION

This study has proven that *T. glaucescens* extracts possess very strong potency in the treatment of various gastrointestinal infections. This is illustrated by the capability of their extracts to reduce fecal egg count and total worm count in infected animals.

These results can be used to design an effective parasite control program against gastro- intestinal nematode infections in sheep, which will be expected to have a greater impact on the sheep industry and as consequence, help in poverty alleviation by empowering rural breeders to use their own natural resources for the prevention and treatment of livestock diseases particularly infection with gastro-intestinal nematodes.

It equally highlights the necessity to test a wider range of doses to compare the phases of fecal egg count and total worm count reduction on nematodes of other species of livestock and its toxicity effects.

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