DRUG DISCOVERY

Anthelmintic effects of two extracts of traditional medicinal plants (*Balanites aegyptica* and *Khaya senegalensis*) on gastrointestinal parasites in goats

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ABSTRACT

This study was conducted with the aim of enhancing plants for treatment of gastrointestinal parasites in goats, as an alternative to the chemical control already known. Plant extracts from the leaves of *Balanites aegyptica* and *Khaya Senegalensis* were used and animals were placed in 04 batches for three repetitions: An untreated control batch (Lb); one batch treated (1 mL/subject) with Ivomec D (Lg); one batch treated (40 mL/subject) with *Balanites aegyptica* (Lv) extract; and one batch treated (40 mL/subject) with *Khaya senegalensis* (Lr) extract. In general, the plant extracts used throughout the study lower the parasite load of batches 3 and 4. They have a significant effect on the intestinal parasites of goats and this over a limited period of time (two weeks after administration) and prove to be more effective than the chemical dewormer (Ivomec D). The administered doses of these extracts, despite their minimal quantities (40 mL/kg of body weight), seems to be effective against gastrointestinal parasites in goats.

Keywords: Gastrointestinal parasites, Plant extracts, Parasitic load, Chemical dewormer.

1. INTRODUCTION

Background and justification

Digestive parasitosis is one of the main causes of zootechnical performance and economic losses especially in small ruminant farms (Getachew et al., 2007; Kabore et al., 2006). In the Sudano-Sahelian zone, they can strongly contribute to nearly half of lamb mortality (Dorchies et al., 2003) and losses of potential production that can exceed 50% (Mahieu et al., 2009). The control of gastrointestinal nematodes of small ruminants is mainly based on the use of anthelmintics (Getachew et al., 2007). Nowadays, the farmers do not easily have access to the good quality of synthetic dewormers, partly because of the liberalization of the veterinary profession practice and in the other hand because of the low purchasing power of most breeders (Houzangbe-Adote et al., 2001; Kane, 2008).

Moreover, their large-scale use has led to the development of resistance that requires the use of other alternative control methods (Getachew et al., 2007). One of them could be the use of plants for the treatment of animal diseases. Because they are available and inexpensive (Houzangbe-Adote et al., 2001) and their use to treat animal diseases has remained in the habits of breeders. However, the small ruminant farms developed by these breeders are exposed to a multitude of pathologies from the beginning of rainy seasons, with a clear domination of parasitism. Indeed, the parasitic infestations in a farm have consequences on the whole herd and are especially omnipresent in ruminants (Pautric-Thomas, 2003), especially in the systems where grazing is privileged.

Among the responsible of these pathologies, the parasites of the digestive tract are the most represented. The consequences of this infestation are generally the mortality caused by diarrhea, growth retardation, weight losses and even anemia in the animal (Tabel et al., 2009). Therefore, control measures against these pathogens are very often essential in breeding so as not to alter their state of health and impact the zoo technical performance of the animals. These measures are based on the strategic use of anthelmintics (Gnoula, 2007).

For many years, chemical anthelmintics have been discovered for infestations' treatment as Cabaret, (2012) points out. Nowadays, there is an upsurge use of natural dewormers derived from several traditional medicinal plants for the treatment of animals infected with intestinal parasites. This preference is intended to be promising, if we increase efforts to looking for adequate formulations and doses with in order to eradicate, in a progressively and definitively the various infestations of which high livestock are victims.

Based on this observation, the present study therefore aims to evaluate the anthelmintic properties of extracts of two plants (*Balanites aegyptica* and *Khaya senegalensis*) on gastrointestinal parasites in goats. Specifically, the aim was to assess the prevalence of intestinal parasites within the herd, to evaluate the effectiveness of the anthelmintic properties of the extracts of each plant (*Balanites aegyptica* and *Khaya senegalensis*) on intestinal parasites and compared the effectiveness of the anthelmintic properties of the extracts of each plant with that of the combination of ivermectin molecules and clorsulon (Ivomec D).

2. MATERIALS AND METHODS

Study site

This study was conducted at the goat farm of the Multipurpose Station of Agricultural Research of Garoua (SPRA-IRAD-GAROUA), located in agro-ecological zone 1 (the Sudano-Sahelian zone of Cameroon) (Figure 1).

Materials and treatments

Materials

To carry out this study, we used different materials ranging from the establishment of the experimental device, the collection, the housing, the security of the animals, treatment and analyzes of the data. To ensure the sanitary condition of the animals, we got a pharmacy kit supplied with veterinary products; to ensure animal feed, we had a sufficient quantity of cotton husk, corn bran and cotton cake associated with peanut and cowpea haulms.

In addition, to ensure dietary mineral needs, we have provided bone meal, salt and natron; to ensure the water supply of our animals, we used a source nearby for the supply of quality water and permanently accessible; and finally for data collection, we also had experimental genetic material (preferably adult male and female goats), a digital camera for taking illustrative photos of the study, a package of pairs of gloves and sanitary materials necessary collection boxes, preservatives for storing faeces samples, etc., marking loops for animal identification, a data collection sheet for taking the parameters of each animal.

Treatments

The dewormers used for this experimental trial are essentially consisting of: Extracts from the leaves of *Banalites aegyptica*; extracts from the leaves of *Khaya senegalensis*; and standard treatment with conventional molecules of ivermectin and clorsulon "Ivomec D". During the study, the animals were disposed in 04 batches for three repetitions.

Experimental design

The experimental design of this study is presented (Table 1).

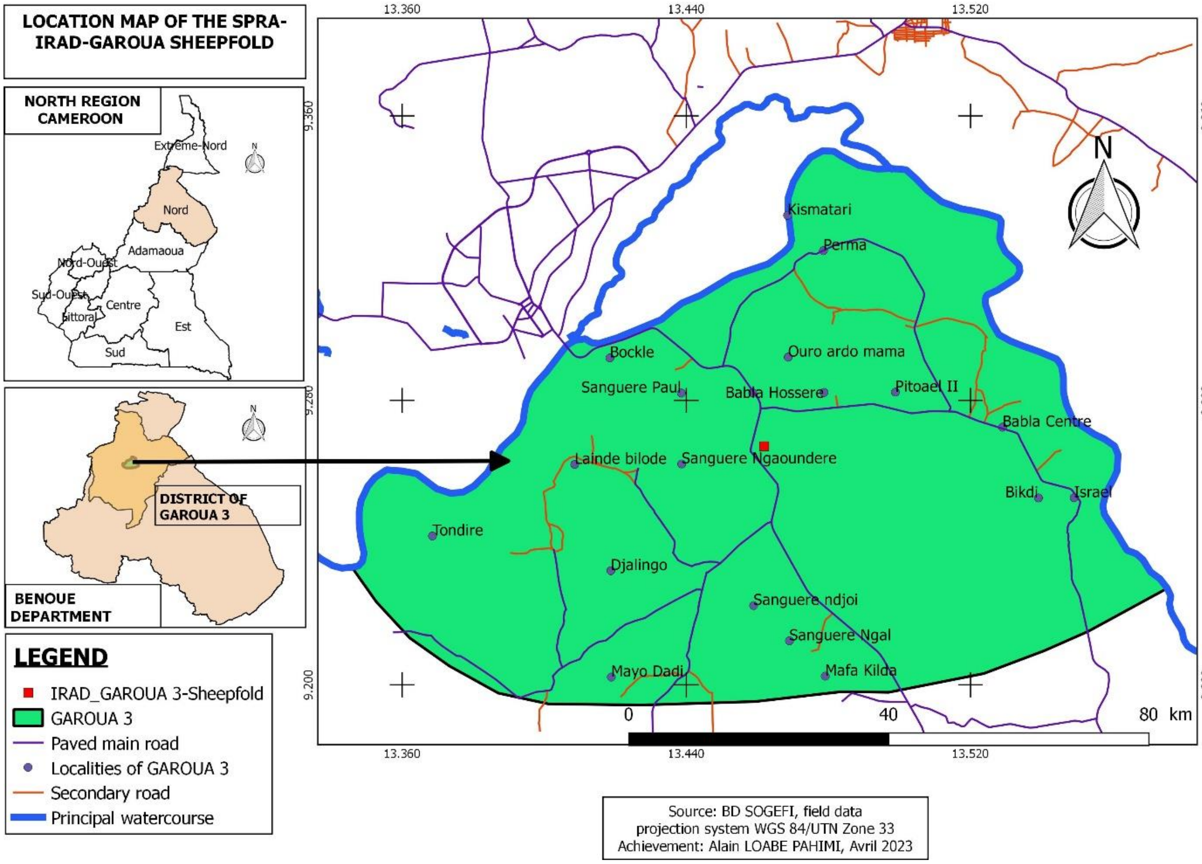

Figure 1 Location map of the SPRA-IRAD-GAROUA goat farm

Table 1 Experimental design of the study

Lots	Treatments	Dose in mL/kg de PV	Frequencies
Lot 1	Untreated control batch (Lb)	-	-
Lot 2	Treated batch with Ivomec D (Lg)	01	Once during the trial
Lot 3	Treated batch with extract of *Balanites aegyptica* (Lv)	40	Every two weeks
Lot 4	Treated batch with extract of *Khaya senegalensis* (Lr)	40	Every two weeks

Field operations

The field operation was structured around several axes.

Distribution of animals

The animals chosen for this test were selected according to age (03 months minimum to avoid mortalities who were too young and vulnerable) and left at random without taking gender into account. Each batch received 03 subjects and identifiable by the referenced label: "LOT1", "LOT2", "LOT3" and "LOT4". The animals of "LOT3" receive every two weeks a dose of 40 mL of *Balanites aegyptica* extract treatment and this during a period of 08 weeks (time of one repetition) and the animals of "LOT4" receive each two weeks a dose of 40 mL of *Khaya senegalensis* extract treatment, during the same period of 08 weeks. On the other hand, the animals of "LOT2" only received the chemical deworming Ivomec D (1 mL/subject) throughout the study.

Faeces collection

The faeces were taken directly from the rectum of animal in order to limit contamination from the external environment (free nematodes, Diptera larvae, etc.). Samples were taken after every 14 days. About 05 to 10, droppings were collected using the gloves and stored in an appropriate cooler. This glove will then be returned and will serve as a sampling bag. Each sample was identified by a reference code and was each time sent to the National Veterinary Laboratory (LANAVET) for coproscopic analyses.

Extraction of traditional medicinal plant extracts

We performed a simple aqueous extraction. The leaves of the two harvested plants (*B. aegyptica* and *K. senegalensis*) were dried for 2 weeks away from dust and sunlight, then transformed into powder using a traditional wooden mortar and preserved in glass boxes at room temperature in an enclosure protected from water. Part of each vegetable powder obtained (50 g) is macerated in 1 liter of distilled water. The macerates are then homogenized for 24 hours and then filtered twice in succession on absorbent cotton and once on Whatman 3 mm filter paper. The filtrates are evaporated at 50°C for 3 to 4 days to give the aqueous extracts (Guédé-Guina et al., 1996).

Administration of plant extracts

The animals of batches 3 and 4 only received these plant extracts for a quantity of 40 ml/animal and this after every two (02) weeks.

Laboratory operations

The extraction of aqueous substances from the chosen plants was done as mentioned in a simple way. The counting of eggs contained in the faeces was done using the McMaster method. The McMaster method originally developed in Australia is the most widely used for counting nematode eggs (Bosco et al., 2014).

It is a technique that is easy to implement, inexpensive and relatively quick and sensitive. This quantitative method is based on the principle of flotation and consists of counting the number of parasitic elements contained in 0.30 ml of suspension of faecal matter diluted to 1/15th. This measuring tool requires the use of a Mc Master slide which includes two analysis chambers. The coprological analysis allowed us to highlight the quantities of parasite eggs in the faeces of animals, expressed in Eggs per Gram of faeces (OPG). We can note that the minimum detection threshold of this method is 50 OPG. Flotation enrichment was done in saturated sodium chloride solution. The number of eggs per gram of faeces used (OPG) was determined using this formula:

$$OPG = \frac{n1+n2}{2} \text{ X } 100$$

With n1 the number of eggs counted in cell 1 and n2 the number of eggs counted in cell 2

When the OPG is between 0 and 500, the infestation is low; when it is above 500 and below 1000, the infestation is moderate; and when it is above 1000, the infestation is strong.

Data collection methods

Data collection was facilitated by the use of data collection sheets designed taking into account the conduct of trial and the parameters to be assessed.

Data processing and analysis

Data management methods

The data collected on the designed sheets were then entered into an Excel version 2013 spreadsheet, on a bi-weekly basis in order to carrying out future analyses.

Data management software

For data management, Excel software version 2013 and JMP 8 software version 8.0 were used.

3. RESULTS

Prevalence of intestinal parasites within the herd

Figure 2, shows the prevalence of intestinal parasites within the herd. We observe that the parasite load of the animals in batch 1 (Lb) was the highest (OPG t0 = 3725, OPG t1 = 2234, OPG t2 = 300; OPG t3 = 392 and OPG t4 = 1009); followed by the parasite load of the animals of batch 2 (Lg) (OPG t0 = 2059, OPG t1 = 0, OPG t2 = 100; OPG t3 = 442 and OPG t4 = 1300); animals from batch 3 (Lv) (OPG t0 = 792, OPG t1= 0, OPG t2= 75; OPG t3= 117 and OPG t4= 600 and animals from batch 4 (Lr) (OPG t0 = 142, OPG t1 = 0, OPG t2= 67; OPG t3= 150 and OPG t4= 425).

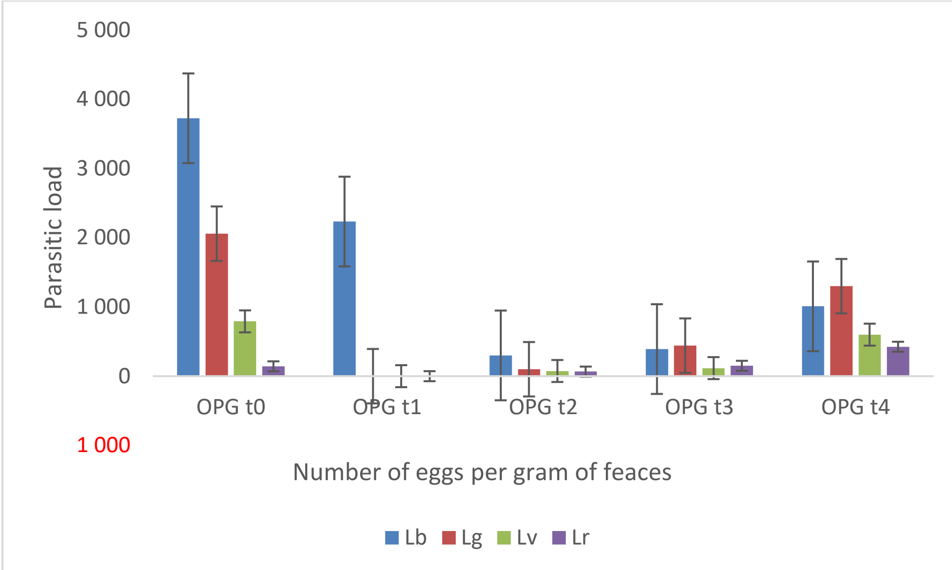

Figure 2 Prevalence of intestinal parasites within the herd

Effectiveness of the anthelmintic properties of the extracts of each plant (*Balanites aegyptica* and *Khaya senegalensis*) on intestinal parasites

Figure 3 compares the effectiveness of the anthelmintic properties of the extracts of each plant (*Balanites aegyptica* and *Khaya senegalensis*) on the intestinal parasites of goats divided into batches. We observe that the animals of batch 3 (Lv) have a moderate parasite load for OPG t0 and t4 and low for OPG t1, t2 and t3; on the other hand, the animals of batch 4 (Lr), a low parasitic load is generally observed for the OPGs t0, t1, t2, t3 and t4.

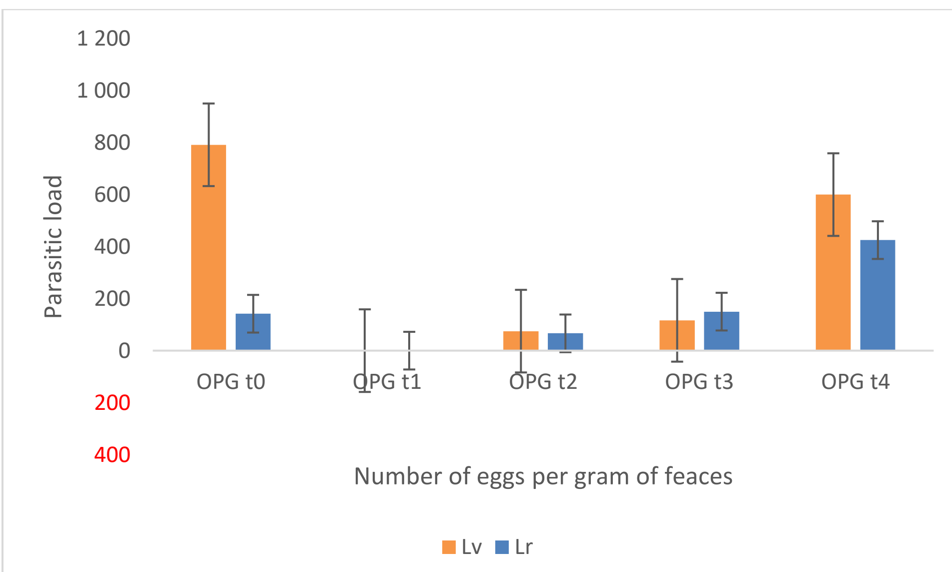

Figure 3 The effectiveness of the anthelmintic properties of the extracts of each plant

Effectiveness of the anthelmintic properties of the extracts of each plant to that of Ivomec D

Figure 4 compares the effectiveness of the anthelmintic properties of the extracts of each plant compared to that of Ivomec D. The animals of batch 2 (Lg) having received ivomec D, compared to the animals of batches 3 (Lv) and 4 (Lr), have a high parasite load for OPG t4 and low for OPG t1, t2 and t4. For all the parasite load data, the animals that received ivomec D had parasite loads that were clearly higher than those of the animals in batch 3 and batch 4.

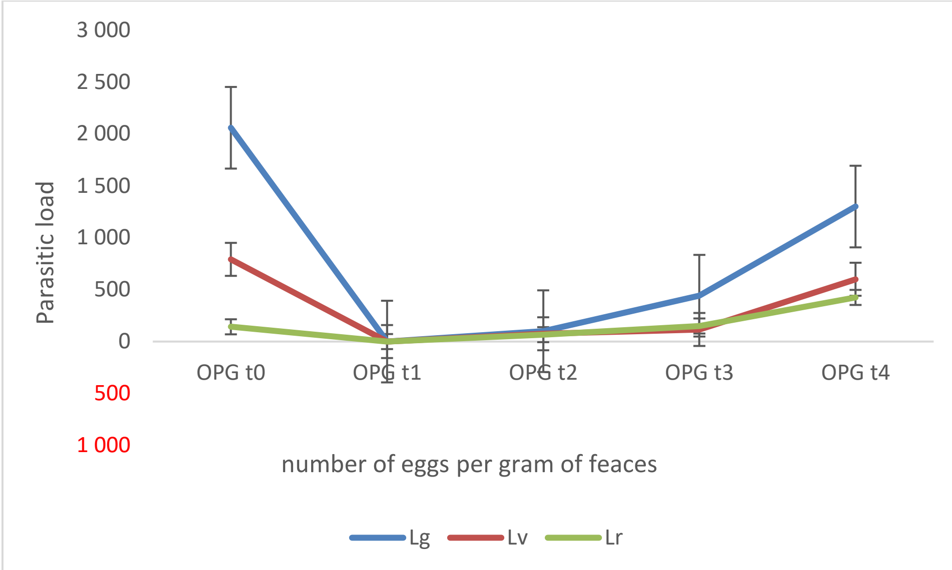

Figure 4 Effectiveness of the anthelmintic properties of the extracts of each plant compared to the effectiveness of Ivomec D

Principal component analysis

Figure 5 shows the result of the principal component analysis of all the parasitic loads in this study. From this Figure, we observe a very strong and negative correlation between the parasitic loads OPG t1 and OPG t2 and an equally strong and positive correlation between the parasitic loads OPG t3 and OPG t4. On the other hand, we observe a correlation that is not enough strong between the parasitic load OPG t0 and the parasitic load OPG t1 and very strong with the parasitic load OPG t2.

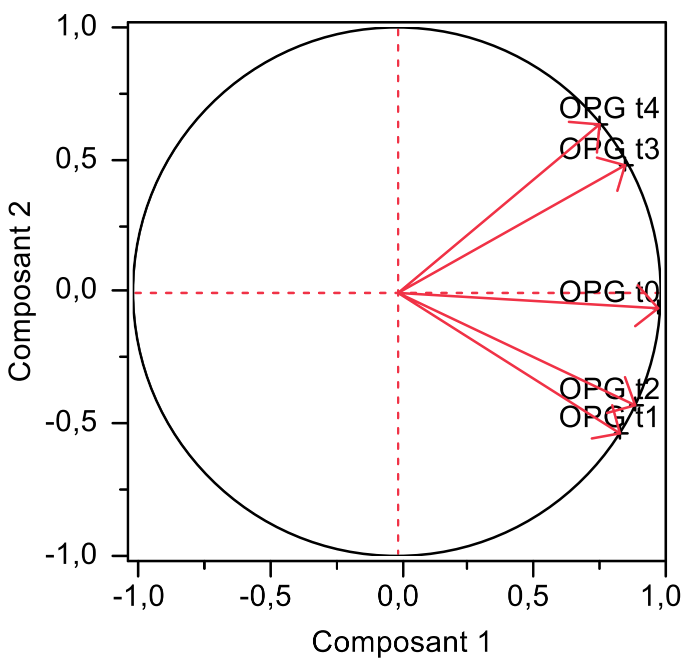

Figure 5 Principal component analysis of all parasitic loads

Table of correlations

Table 2, presents the existing correlations between the different parasitic loads obtained throughout our study. From this table, we observe that the OPGs with values materialized in bold are those with a very strong correlation.

Table 2 Correlations between the different parasitic loads obtained

	OPG t0	OPG t1	OPG t2	OPG t3	OPG t4
OPG t0	1,0000	0,8636	*0,9204*	0,8101	0,7315
OPG t1	0,8636	1,0000	*0,9919*	0,4703	0,2957
OPG t2	*0,9204*	*0,9919*	1,0000	0,5727	0,4146
OPG t3	0,8101	0,4703	0,5727	1,0000	*0,9487*
OPG t4	0,7315	0,2957	0,4146	*0,9487*	1,0000

4. DISCUSSION

At the beginning of our trial, we noted in the faeces the presence of strongyle eggs (Strongyloid, *Trichostrongylus* sp) in all batches of animals, explaining the high OPG t0 values of almost the majority of batches of animals (Lb, Lg, Lv and Lr). This result explains the fact that the parasitism of small ruminants (goats) is largely caused by gastrointestinal nematodes such as strongyles. This result corroborates the work of Achi et al., (2003). Moreover, these parasite loads represent the initial values (OPG t0) for the batches of animals before administration of the treatments. Figure 1 reveals that the parasite load decreases from OPG t1 for the animals of batches 2, 3 and 4.

The animals of batch 1 did not in fact undergo any treatment throughout the test; on the other hand, the animals of batch 2 underwent treatment with a chemical deworming agent; in addition, the animals of lot 3 and 4, respectively, underwent treatment with extracts of *Balanites aegyptica* and *Khaya senegalensis*. This result shows that the infestation varies from a high threshold to a moderate threshold for the animals of batch 1; from a low threshold to a high threshold for the animals of batch 2; low to moderate for the animals in batches 3 and 4.

The leaf-based plant extracts of *Balanites aegyptica* (40 mL/kg bodyweight) and *Khaya senegalensis* (40 mL/kg bodyweight) lower the parasite load of animals 3 and 4 batches, throughout of the study (OPG t1, t2, t3 and t4). Plant extracts based on the leaves of *Balanites aegyptica* and *Khaya senegalensis* have a significant effect on intestinal parasites in goats over a period of two weeks after administration of the extracts. Leaves are the main site of biosynthesis and storage of the active principles responsible for the biological properties of plants (Bitsindou, 1996). This result corroborates the work of Alawa et al., (2003), Fall et al., (2008) and Okhale et al., (2016).

The plant extracts administered (40 mL/kg body weight for each extract) prove to be more effective than the chemical deworming agent (Ivomec D, 1mL/subject). However, their effectiveness seems to be limited in time. They reduce the parasite load over only a few days, although the infestation observed in batches 3 and 4 varies from low to moderate. On the other hand, the effectiveness of the chemical deworming which is on the long term, present in batch 2 an infestation which varies from low to high.

The results of this study also support the work of Githiori et al., (2006), who claimed that herbal remedies have in most cases the lower-level reductions in parasitism than synthetic anthelmintics in vivo control tests. It should nevertheless be noted that several authors have obtained much lower parasite loads with larger quantities of plant extracts: 100 mg/kg of live weight; 160 to 242.5 mg/kg body weight. These are respectively Sacromento et al., (2010) with papaya seeds; Kabore, (2009), with *Anogeissus leiocarpus* and *Daniellia oliveri*.

The parasitic load OPG t0 is very different from the other parasitic loads (OPG t1, t2, t3 and t4). This is simply because the OPG t0 corresponds to the initial parasite load of the animals, before administration of the extracts and the chemical deworming. On the other hand, a very strong correlation is observed between the OPG t1 and t2 and between the OPG t3 and t4, because they correspond to the loads recorded after administration of the extracts and the chemical deworming. The effectiveness of the extracts is no longer constant when observing the different parasitic loads. The egg load increases (from OPG t1 to OPG t4) as the number of weeks evolves, without having significant effects on the level of infestation. This result can be explained by the fact that the different batches of animals were not in strict housing. In free grazing, they would have consumed contaminated fodder that is why OPG t1 differs from the other OPGs (OPG t2, t3 and t4) throughout this study.

The very strong existing correlations between the parasite load OPG t1 and OPG t2, OPG t3 and OPG t4, is justified by the proven efficiency of the extracts and the chemical dewormer (Ivomec D) on the gastrointestinal parasites of goats. The doses of the two extracts administered, despite their minimal quantities (40 mL/kg body weight) compared to that administered by Sacromento et al., (2010) and Kabore, (2009), prove to be effective on these parasites gastrointestinal.

5. CONCLUSION

This study permitted to show the anthelmintic activity of plant extracts made from the leaves of *Balanites aegyptica* and *Khaya senegalensis* in goats, in particular on gastrointestinal nematodes. It therefore confirms that the use of these plant extracts as anthelmintics for small ruminants is justified. We observe that the infestation varies from high to moderate for the animals of batch 1; low to high for batch 2 animals; from low to moderate for the animals of batches 3 and 4. On the other hand, the effectiveness of the two plant extracts being proven, compared to the chemical deworming, but all things considered, their action is beautiful and well limited in time. The extract based on *Khaya senegalensis* seems to be slightly more effective than that based on *Balanites aegyptica*, given the reduction in the parasite load recorded at OPG t1, t2, t3 and t4.

All of these results obtained constitute a basis for future research, with the aim of contributing to the development of a therapeutic approach based on plant extracts. This is why additional studies must a priori be carried out, such as: The determination of the acute toxicity of the extracts of these two plants (*Balanites aegyptica* and *Khaya senegalensis*) by calculating the LD50 on batches of mice placed in experimental conditions; the determination of the duration of the treatment and the persistence of the product in the organism of goats and small ruminants in general; finally the determination of the best dose offering promising results for a definitive vulgarization/dissemination in peasant environment.

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Author contributions

This study was possible by the contribution of all its authors. Indeed, Alain Loabe Pahimi developed the research protocol, carried out the collection, analysis and processing of data but also wrote the first version of the manuscript. Christophe Wang-Baa Temoa, Pierre Wandjoumbe and Christine Soukbo contributed to the collection, analysis of data and proofreading of the manuscript. Finally, as for Souley Bagari Iya and Yannick Mole Zogo, they participated in the development of the research protocol and the proofreading of the latest version of the manuscript.

Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for sample collection. The Animal ethical guidelines are followed in the study for experimentation.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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