



In vivo anthelmintic activity of *Callistemon rigidus* ethanolic extract on *Haemonchus contortus* in goats

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Abstract

The present study was conducted in order to evaluate the *in vivo* anthelmintic activity of ethanolic extract of *Callistemon rigidus* on goat and define the probable involved phytochemical compounds. Phytochemical contents were determined by chemical comparison using gallic acid and rutin as standards. Twenty goats were artificially infected with 3,000 larvae of *Haemonchus contortus*. There were divided in five groups of four animals. The first three groups were treated with the extract of the plant at 125; 250 and 500 mg/kg respectively. The fourth group was treated with albendazole at 5 mg/kg. The fifth group received distilled water. Weight, blood and fecal samples were collected before treatment and once a week after treatment in order to evaluate the body weight gain; the Packed Cell Volume (PCV) and the percentage of reduction of fecal eggs. On the day 43, animals were slaughtered and the total worm count was evaluated. The fertility of females *H. contortus* was evaluated by counting the number of eggs *in utero*. The ethanol extracted polyphenols (56.801 ± 0.40 mg GAE/g), flavonoids (8.628 ± 0.07 mg RE/g), tannins (20.93 ± 0.28 mg CE/g) and saponins (548 ± 13.54 mg/g). Oral administration of this extract of *C. rigidus* has induced a reduction of the fecal egg excretion; parasite load and fertility of female's parasite. The PCV and the body weight gain reveal the efficacy of the extract plant on *H. contortus*. *C. rigidus* could be used as alternative anthelmintic for control of *H. contortus*.

Keywords: *Callistemon rigidus*, Anthelminthic, Ethanolic extract, *Haemonchus contortus*, goats

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1. Introduction

Breeding of small ruminants occupies an important place in the economy of many households in Cameroon, contributing for about \$7.244 bn to the livestock product income with a growth rate of 4.8% in 2013 (INS, 2015). In fact, livestock farming makes it possible both to ensure their food security and to establish an essential source of income. Parasitic infections by gastrointestinal helminths in general and *H. contortus* in particular are a major impediment to animal health and result in a significant decrease in productivity. *H. contortus* is the

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most important nematode parasite of small ruminants, causing severe anemia and high mortality in all classes of livestock (Allonby and Urquhart, 1975). The percentage of infestation in different species of *Haemonchus* ranged from 50-85% (Qadir, 1967). The principal feature of haemonchosis is anemia, induced by the blood feeding of adults and larval stage. The average blood loss has been calculated as 0.05 mL/parasite/day (Clark et al., 1962).

Control of haemonchosis is generally achieved by use of synthetic anthelmintic. The frequent use of these anthelmintic over years has inevitably led to the development of drug resistance. The emergence of resistance to anthelmintic drugs which is now a worldwide phenomenon (Jackson and Coop, 2000) and the increased awareness of consumers about drug residues that potentially enter the food chain have stimulated investigation into alternative anthelmintic such as medicinal plants. The treatment of gastrointestinal parasites of small ruminants by medicinal plants is common practice in rural areas (Kabore, 2009). Indeed, the use of traditional medicinal plants for the treatment of helminthiasis is an alternative, since accessible at all times and inexpensive (Terriil et al., 2009). According to Lawal et al. (2005); Magaji and Yaro (2006) as well as Kawo et al. (2011), phytochemical components are responsible for both pharmacological and toxic activities in plants. These metabolites are said to be useful to the plant itself but can be toxic to animals including man. Among the therapeutics' plant, *C. rigidus* (Bottle Brush) family Myrtaceae is known by traditional healers for the virtues of deworming animals. It is widely distributed and used to treat various diseases. Essential oil from the plant is used in traditional medicine for the treatment of cough, bronchitis and respiratory tract infections (Jirovetz et al., 1997). Anti- Staphylococcal potential activities of *C. rigidus* have been evaluated by Gomber and Saxena (2007). Paradoxically, no scientific study has evaluated there *in vivo* anthelmintic effect. The present study is carried out to screen for phytochemical properties and evaluates the *in vivo* activity of the ethanolic extract of *C. rigidus* on goat's haemonchosis.

2. Materials and methods

2.1. Plant material and preparation of extract

Bark of *C. rigidus* were collected in Bini/Dang village (Sudano-Guinean zone: Adamawa Region) in Cameroon, based on ethnopharmacological data. Voucher specimens were identified in the Department of Biological Sciences of the University of Ngaoundere. A voucher specimen was then deposited in the National Herbarium of Yaounde/Cameroon with the N° 18564/SRF. The collected material was washed, dried and mashed in order to obtain a powder. Plant extracts were prepared as described by Ndjonka et al. (2012) Briefly 100 g of powder of the plant were macerated into 1 L of ethanol at 95 °C for 48 h at room temperature. The macerate was centrifuged at 3,500 rpm/min for 10 min and then filtered over filter papers No. 413 (VWR International, Darmstadt, Germany). The filtrate was then concentrated under reduced pressure by rotary evaporation (BUCHI Rotavapor R-200, Switzerland) at 40 °C. Residual solvent was removed by drying in a sweating-room at 35 °C and the extract was weighed and stored at +4 °C. The plant extract was further dissolved in dimethyl sulfoxide (DMSO) and PBS to a final concentration of 100 mg/ml, centrifuged and aliquoted to determine their *in vivo* activity on *H. contortus*.

2.2. Quantitative phytochemical evaluation

The total phenolic was assessed using Folin-Ciocalteu reagent (Awah et al., 2012). The absorbance was measured at 765 nm using a spectrophotometer and the results were expressed as mg of gallic acid equivalents (GAE) per gram of extract (mg GAE/g). The flavonoid content was determined applying aluminum chloride colorimetric method described by Barros et al. (2011) and expressed as mg of rutin equivalents (RE) per gram of extract (mg RE/g). The condensed tannin content was analyzed by using the vanillin assay described by Ba et al. (2011). The results were expressed as mg of catechin equivalents (CE) per gram of extract (mg CE/g). The saponin constituent was quantified according to the modified method described by Jamuna et al. (2014). 0.01 g of extract was added to 10 ml of distilled water and the mixture was vigorously shaken for 30 min. The height of moss was measured by using a graduated ruler and quantified according to the following formula:

$$\text{Saponin (mg/g)} = [(0.432) * (\text{height of moss in cm after 15 s}) + 0.008] / (\text{weight of extract in gram})$$

2.3. In vivo assay on *H. contortus*

This work was carried out in accordance with the Animal Ethical Committee of the Ngaoundere Regional Delegation of livestock; Fisheries and Animal Industries Authority, Cameroon . Number 075/16/L/RA/DREPIA.

Twenty goats of 6-8, month-old, were selected for the study. They were divided into five balanced groups ($n = 4$), according to the bodyweight (10 ± 2 kg live weight). The breeding was done indoors, to avoid any natural nematode infection. Goats were fed daily with forage (*Pennisetum* sp) beforehand disinfected with sodium hypochloride 24 h before and they had free access to water. The grass was suspended from the diet when the trial started.

2.3.1. Artificial infestation of goats

Goats' abomasal were obtained from municipal slaughterhouse of Yagoua/Cameroon after necropsy of animals. Females *H. contortus* were collected and crushed to free eggs. They were then cultured *in vitro* in petri dishes at room temperature for nine days. At the end of the culture, infective larvae were harvested. About 3,000 third-stage larvae were estimated by counting the number of larvae contained in 0.1 ml of well homogenized solution of infective larvae. After five repetitions of counting, the mean number of larvae in 0.1 ml of solution was determined and the volume containing 3,000 third-stage larvae (L_3) were measured and inoculated to animals.

2.3.2. Anthelmintic assay

The trial lasted for two months and consisted of three successive periods. A 7-day period for adaptation to the diet (D-7 to D-1).

A first experimental period (D_0 to D_{20}). D_0 correspond to the day when the 20 goats were experimentally infected with 3,000 third-stage larvae (L_3) of *H. contortus* each. The one lasted for 21-days (D_{21} - D_{42}) corresponding to a period of goats 'treatment and parasitological analyses. During this experimental period, the animals were divided into five groups. The treated group (group A; B and C) received orally the ethanolic extract of *C. rigidus* during three (03) days and twice a day at doses of 125; 250 and 500 mg/kg respectively. Group D and E constituted the control group. Group D received albendazole orally at 5 mg/kg and the group E received distilled water and DMSO.

Blood samples were collected in EDTA coated tube once a week from D_{21} to D_{42} for all groups. Blood of each tube were introduced into micro-haematocrit tubes and centrifuged using a micro-haematocrit centrifuge at 12,000 rpm for 5 min and the Packed Cell Volume (PCV) was determined using a hematocrit reader.

Fecal egg counts were determined by the modified McMaster technique using saturated sodium chloride solution as floating medium and eggs per gram (EPG) of faeces was determined by the following formula: $EPG = \text{Total number of eggs in 2 chambers} \times 50$, where. The fecal samples of the animals were collected per rectum and examined for EPG on day 21 (pre-treatment) and days 25, 28, 35 and 42 (post-treatment). The fecal egg count reduction test (FECRT%) was determined according to the formula of Bauer *et al.* (1986).

All animals were weighed before the treatment; and once a week after treatment in order to monitor the body weight variation.

All subjects were slaughtered at the end of the second experimental period (D_{21} - D_{42}). All parasites present in the abomasa were counted in a 10% aliquot of the abomasa contents. The percentage of reduction of the number of parasites was determined using the formula of Enriquez (1994). Fertility of females was evaluated by counting the number of eggs *in utero* according to the technique of Kloosterman *et al.* (1978).

2.4. Statistical analyze

The results expressed are the mean \pm standard error of the mean. Statistical analysis was performed by using the software SPSS version 17.0. The post hoc statistical significance test employed was Duncan, differences between the means were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Phytochemical compounds from ethanolic extract of *C. rigidus*

The ethanolic extract of *C. rigidus* gave 16, 8% of dry powder. The phytochemical screening of the *C. rigidus* barks revealed the presence of alkaloids, total polyphenol; tannins; flavonoids; saponins, and triterpens (Table 1). Results of the quantitative phytochemical study revealed that ethanolic bark's extract of *C. rigidus* contents 56.801 ± 0.40 mg GAE/g of polyphenols; 8.628 ± 0.07 mg RE/g of flavonoids; 20.93 ± 0.28 mg CE/g of tannins and 548 ± 13.54 mg/g of saponins. In a study performed by Danga *et al.* (2014) the same compounds have been found. Recently in a study, Rajesh *et al.* (2009) mentioned that an activity of a plant extract depends on the availability of secondary metabolites like tannins, saponins, triterpens and flavonoids. The ethanolic

Table 1: Quantitative phytochemical content of ethanolic extract of *C. rigidus* bark

	Polyphenols (mg GAE/g)	Flavonoids (mg RE/g)	Tannins (mg CE/g)	Saponins (mg/g)
EEBC	56.801 ± 0.40	8.628 ± 0.07	20.93 ± 0.28	548 ± 13.54

Note: EEBC: ethanolic extract of bark of *Callistemon rigidus* GAE: Gallic Acid Equivalent; RE: Rutin Equivalent; and CE: Catechin Equivalent.

extract of barks of *C. rigidus* contains almost the same secondary metabolites namely tannins, saponins and flavonoids which might be toxic for the worms and responsible of the observed anthelmintic activity. According to Hoste *et al.* (2009), tannins are able to bind to glycoproteins on the cuticle of parasites. Similar mode of action of tannins on nematodes was also reported with synthetic phenolic anthelmintics (oxyclozanide, niclosamide, and nitroxylin) (Martin, 1999) as these drugs interfere with energy generation in helminth parasites by uncoupling oxidative phosphoryl consequently leading to depletion of parasite ATP (Martin, 1999).

3.2. Effect of the ethanolic extract of *C. rigidus* on the PCV

PCV is a measurement of the proportion of red cells in the blood used to diagnose anemia caused by hematophagous parasites. It is more often considered as a measurement of resilience, especially in infection coming predominantly from *H. contortus* (Baker, 1997). The Table 2 presented the result of the values of PCV of animals during the experimental period. In general, the PCV has increase in all animals treated with albendazole from 30 ± 1 to 18.66 ± 0.96 and those treated with ethanolic extract of *C. rigidus* (21.33 ± 4.16 to 27 ± 4.36); (23.67 ± 6.11 to 28.5 ± 2.12) and (28.67 ± 1.53 to 31 ± 1.73) respectively in doses of 125; 250 and 500 mg/kg. In contrary, PCV of animals of the control group has decreased form 30 ± 1 to 18.66 ± 0.96 during the experiment. Analysis of variance shows that there is a significant difference ($p < 0.05$) between the control group and all treated group except groups treated at doses of 125 and 250 mg/kg on D₄₂.

According to Nemi (1993) the PCV in a healthy goat is in the range 19-32%. Thus, a decrease in this hematological parameter would indicate an anemia. For this purpose, PCV make possible to judge the presence of hematophagous parasites and in this case that of *H. contortus*. The average values of PCV as observed in Table 2 are in the normal range 19-32% in all groups except the control group at D₄₂ where the value of the PCV is less than 19%. This decline in PCV can be explained by the action of *H. contortus*. These results highlight are not different ($p > 0.05$) from those obtained by other authors (Kabore, 2009; and Ndoutamia and Ganda, 2005). The PCV values recorded during the treatment of both albendazole treated group and the different doses of ethanolic extract of *C. rigidus* barks would indicate an anthelmintic activity on *H. contortus*.

Table 2: Effect of ethanolic extract on PCV

Days	Pre-treatments	Post-treatments			
	D ₂₁	D ₂₅	D ₂₈	D ₃₅	D ₄₂
Control	30 ± 1 ^a	27.33 ± 2.51 ^a	26.33 ± 3.79 ^a	20.33 ± 3.05 ^a	18.66 ± 0.96 ^a
Albendazole	25.67 ± 4.04 ^a	26.33 ± 7.5 ^a	27 ± 7.55 ^a	29.33 ± 5.13 ^a	30.67 ± 4.73 ^b
125 mg/kg	21.33 ± 4.16 ^a	23.5 ± 2.12 ^a	24.5 ± 2.12 ^a	25.5 ± 0.71 ^a	27 ± 4.36 ^{ab}
250 mg/kg	23.67 ± 6.11 ^a	24.67 ± 5.86 ^a	25.67 ± 5.13 ^a	25.33 ± 4.73 ^a	28.5 ± 2.12 ^{ab}
500 mg/kg	28.67 ± 1.53 ^a	29 ± 1 ^a	30 ± 2 ^a	29.67 ± 1.15 ^a	31 ± 1.73 ^b

Note: Values are means ± SEM, n = 4, * $p < 0.05$, a significant difference compared to the control. SEM: Standard Error of Mean. Small letters compare means in a column and different letters indicate significant difference at $p = 0.05$

3.3. Effect of the ethanolic extract of *C. rigidus* on body's weight

Oral administration of ethanolic extract of *C. rigidus* shows a variation in body weight gain during post-treatment periods (Table 3). Overall, there is a fluctuation in the average body weight gain between groups. A

Treatments	Body weight gain post-treatment			
	(D ₂₁ -D ₂₅)	(D ₂₁ -D ₂₈)	(D ₂₁ -D ₃₅)	(D ₂₁ -D ₄₂)
Control	1 ± 0.41 ^a	0.83 ± 0.04 ^a	-0.67 ± 0.05 ^a	-1 ± 0.01 ^a
Albendazole	0.67 ± 0.01 ^a	0.67 ± 0.08 ^a	1.03 ± 0.06 ^a	1 ± 0.01 ^{ab}
125 mg/kg	0.75 ± 0.03 ^a	0.67 ± 0.06 ^a	1 ± 00 ^a	1.75 ± 0.03 ^b
250 mg/kg	1.01 ± 0.09 ^a	1 ± 0 ^a	0.97 ± 0.05 ^a	1.37 ± 0.32 ^b
500 mg/kg	0.67 ± 0.07 ^a	0.77 ± 0.07 ^a	1 ± 00 ^a	1 ± 0.01 ^{ab}

Note: Values are means ± SEM, n = 4, *p < 0.05, a significant difference compared to the control. SEM: Standard Error of Mean. Small letters compare means in a column different letters indicate significant difference at p = 0.05.

non-significant difference ($p > 0.05$) between the post-treatment periods (D₂₁-D₂₅); (D₂₁-D₂₈) and (D₂₁-D₃₅) with the exception of the post-treatment period (D₂₁-D₄₂) where a significant difference ($p < 0.05$) was observed between the animals of the control group and the animals treated with the ethanolic extract of *C. rigidus* at a dose of 125 and 250 mg/kg. Similarly, a significant difference ($p < 0.05$) was also noted in the post-treatment period (D₂₁-D₄₂). Animals' body weight values in the treatment groups at the end of the experiment showed a significant difference ($p < 0.05$) at 125 and 250 mg/kg. This result should be due to the fact that the administration of plant extract at low doses does not induce any harmful effects to the body weight of animals. Indeed, it is recognized that tannins can lead to high levels of rumen protein misused and induce inhibition of gastrointestinal enzyme activity (Delaveau, 1988; and Terrill et al., 1992). The ethanolic extracts of *C. rigidus* administered at the maximum dose of 500 mg/kg in our study did not induce any change in body's weight. This result corroborates that of Terril et al. (1992) who observed that the consumption by ruminants of rations containing large amounts of tannins results in stunting compared with control animals.

3.4. Effect of the ethanolic extract of *C. rigidus* on the fecal eggs excretion

After treatment of animals with ethanolic extract of *C. rigidus*'s bark, the major result outcome of this study relative to the fecal eggs excretion is the reduction of the level of fecal eggs excretion of *H. contortus* (Table 4). Albendazole has significantly reduced eggs excretion during the study. Administration of ethanolic extract of

Treatments	Pre-treatments	Post-treatments			
	D ₂₁	D ₂₅	D ₂₈	D ₃₅	D ₄₂
Control	1150 ± 50	1164.93 ± 30.411 (-1.36) ^a	1183.33 ± 28.87 (-2.96) ^a	1133.33 ± 115.47 (1.58) ^a	1257.33 ± 124.34 (-9.22) ^a
Albendazole	1783.3 ± 28.87	95.17 ± 8.80 (94.67) ^b	0 ± 0 (100) ^b	0 ± 0 (100) ^b	0 ± 0 (100) ^b
125 mg/kg	2083.3 ± 104.08	1165 ± 75.66 (44.06) ^{ba}	940 ± 14.14 (54.85) ^{ba}	878.5 ± 34.65 (57.87) ^{ba}	863.7 ± 51.26 (58.5) ^{ba}
250 mg/kg	1100 ± 100	479.25 ± 13.79 (56.41) ^b	719.2 ± 27.23 (34.61) ^{ba}	578.3 ± 25.66 (47.43) ^{ba}	408 ± 13.86 (62.91) ^b
500 mg/kg	4850 ± 229.13	1896.7 ± 95.04 (60.9) ^b	1745 ± 28.28 (64.05) ^b	2100 ± 70.71 (56.75) ^{ba}	1350 ± 70.72 (72.14) ^b

Note: Values are means ± SEM, n = 4, *p < 0.05, a significant difference compared to the control. SEM: Standard Error of Mean. Small letters compare means in a column and different letters indicate significant difference at p = 0.05, R_{EPG}: Reduction of eggs per gram.

C. rigidus to animals has not induced significant reduction of the fecal eggs excretion at the dose 125 mg/kg. On the other hand, administration of the ethanolic extract of *C. rigidus* has significantly increased the percentage of reduction of the fecal eggs excretion at the dose 250 mg/kg for days 25 and 42 and at the dose of 500 mg/kg for all days of observation.

The reduction of the fecal eggs excretion registered in this study is similar to those of Al-Shaibani et al. (2009) on the anthelmintic activity of *Fumaria parviflora* against gastro-intestinal parasites of sheep. These results are also similar to those of Paolini et al. (2003) on the activities of tannins on goats artificially infested with *H. contortus*. Elsewhere, Olounladé et al. (2017) show that *Newbouldia laevis* at the dose of 1.6 g/kg induced a significant effect on gastrointestinal parasites of sheep.

3.5. Effect of the ethanolic extract of *C. rigidus* on the parasite load

The determination of the parasite load is an important parameter for the evaluation of the *in vivo* effect of a drug. Indeed, the use of increasing doses of plant extracts in order to achieve an expected anthelmintic efficacy *in situ* is due to the fact that the targeted parasite could develop escape strategies to the administered drug. The Figure 1 presents the evaluation of the percentage of reduction of the parasite load of the animals subjected to the experiment; Results of this study show that the different treatments administered to animals' groups have induced effectively parasite load. Indeed, as expected, albendazole resulted in a 100% reduction in parasite load at the end of the study. The ethanolic extract of *C. rigidus* induced a gradual reduction of the parasite load in the order of 37.62%; 50% and 64.87% at the respective doses of 125; 250 and 500 mg/kg respectively. Analyze of variances shows that there is no significant difference ($p > 0.05$) between animals of doses 125 and 250 mg/kg and the control group. On the other hand, a significant difference ($p < 0.05$) has been observed between the ethanolic extract *C. rigidus* at the dose 500 mg/kg and the control group. According to our study, after the post-mortem examination of animals, the major result of this study on the evaluation of the parasite load is a reduction in the number of adult worms in goats that received the ethanolic extract of bark of *C. rigidus* (Figure 1). The ethanolic extracts of *C. rigidus* at the highest dose induced more than 50% parasite load reduction Mbafor et al. (2014) also achieved a reduction efficiency of more than 50% of the parasite load after a dose of 500 mg/kg of the methanolic extract of *Terminalia glausescens* in naturally infested sheep. A non-significant difference ($p > 0.05$) was observed in the animals treated with the ethanolic extract at doses of 125 and 250 mg/kg compared to non-treated animals. In contrast, albendazole and ethanolic extract of *C. rigidus* barks at the 500 mg/kg dose induced a significant reduction ($p < 0.05$) compared to the control. The reduction in parasite load obtained during this study corroborates the results of numerous studies (Niezen et al., 1998; Kahiya et al., 2003; and Paolini et al., 2003). The administration of tannins to animals leads to a reduction in the parasite load (Brunet et al., 2008). Polyethylene glycol (PEG) the main reagent used in experiments especially in the field of nutrition for their ability to deactivate the condensed tannins contained in forages in order to

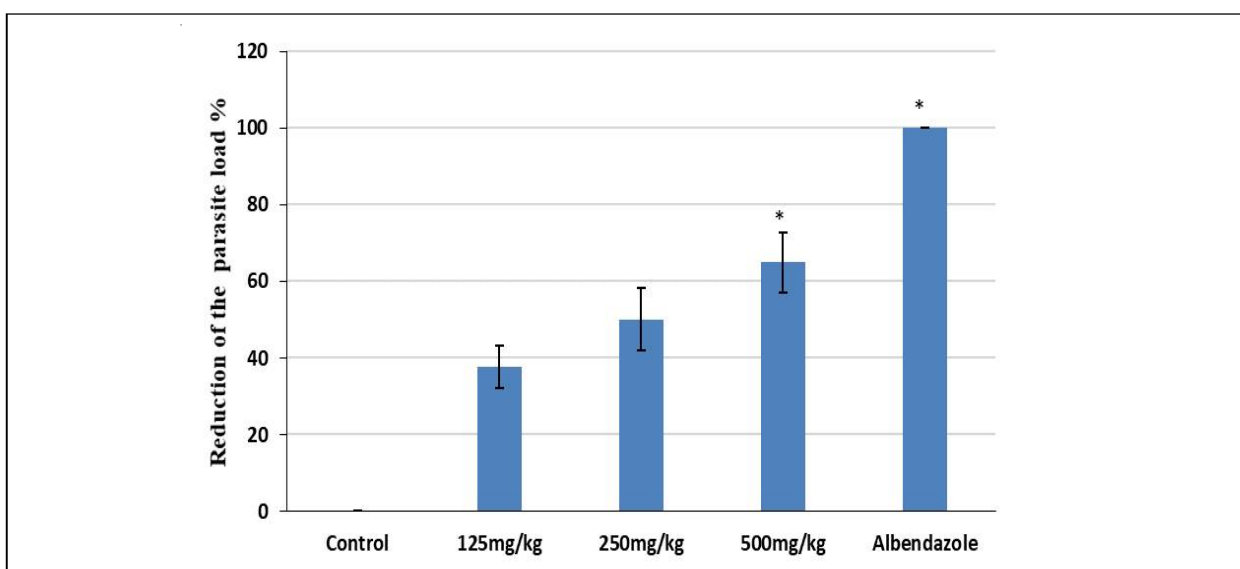


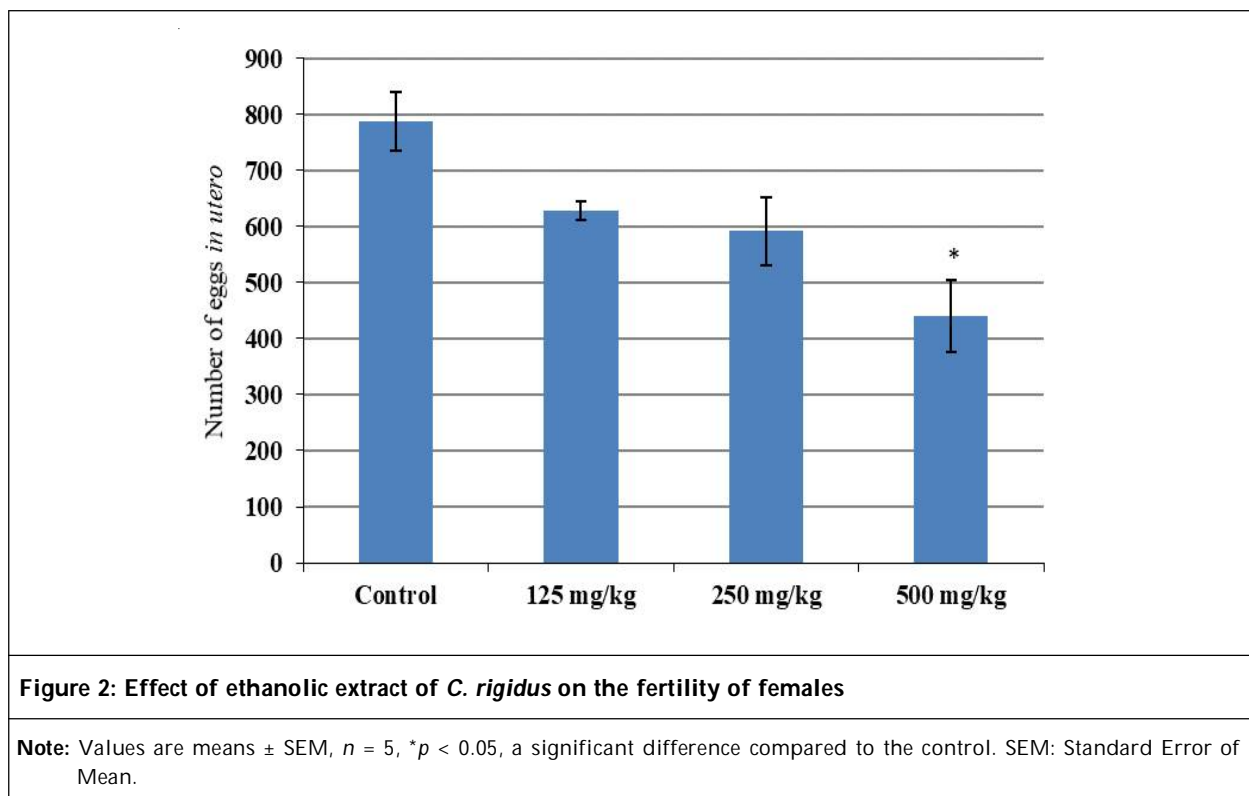
Figure 1: Anthelmintic activity of *C. rigidus* bark on parasitic load

Note: Values are means \pm SEM, $n = 5$, * $p < 0.05$, a significant difference compared to the control. SEM: Standard Error of Mean.

neutralize their potential negative effect (Makkar, 2003). Indeed, the combination of PEG with condensed tannins gives an inert complex (Silanikove et al., 2001). According to several authors (Brunet et al., 2008; Alonso-Diaz et al., 2008; Hoste et al., 2007 and Kabasa et al., 2000), the combination of Polyethylene glycol with tannins has no anthelmintic effect on nematodes. This suggests that condensed tannins are largely responsible for anthelmintic effects.

3.6. Effect of the ethanolic extract of *C. rigidus* on the fertility of females

The evaluation of the number of eggs *in utero* of females of *H. contortus* (Figure 2) shows that ethanolic extract of *C. rigidus* did not show important activity ($p > 0.05$) at the lower doses of 125 and 250 mg/kg but were found effective ($p < 0.05$) at the maximum dose 500 mg/kg compared to the group receiving distillate water. The fertility of adult worms can vary significantly from one animal to another (Olounladé et al., 2017). The main results of the experiment show that the administration of the ethanolic extracts of *C. rigidus* to the animals results to the reduction of the fertility of the female worms as illustrated in the above-mentioned figures. Indeed, the anthelmintic efficacy of a drug is also reflected in its ability to interact with fertility of female worms by reducing the number of eggs *in utero*. The results of our study highlight a significant statistical effect on the biology (precisely the fertility) of the populations of *H. contortus*, these consequences on the fertility of the female worms of *H. contortus* being especially present with the highest dose (500 mg/kg) of *C. rigidus*. These results corroborate those of Sabater (2012), in which the reduction of the fertility of female *H. contortus* worms was at the highest doses of quebracho compared to the control group. The anthelmintic efficacy of *C. rigidus* is due to the presence of certain secondary metabolites like polyphenols; flavonoids; saponins and tannins. According to some studies, administration of tannins to goats resulted in reduction of fertility of female worms of gastrointestinal parasites (Paolini et al., 2003; and Paolini et al., 2005).



4. Conclusion

In summary, this work focused on the *in vivo* activity of the ethanolic extracts of *C. rigidus* (Myrtaceae) on the nematodes *H. contortus* the goat parasites. It appears from the results that ethanolic extracts of *C. rigidus* has anthelmintic effects on fecal eggs excretion; total worms count and the fertility of females. The extract did not impact the hematocrit and the body weight gain of animals.

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References

- Allonby, E. W. and Urquhart, G. M. (1975). The epidemiology and pathogenic significance of haemonchosis in a merino flock in East Africa. *Vet. Parasitol.* 1, 129-143.
- Alonso-Diaz, M. A., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Aguilar-Caballero, A. J. and Hoste, H. (2008). *In vitro* larval migration and kinetics of exsheathment of *Haemonchus contortus* exposed to four tropical tanniniferous plants. *Vet. Parasitol.* 153, 313-319.
- Al-Shaibani, I. R. M., Phulan, M. S. and Shiekh, M. (2009). Anthelmintic activity of *Fumaria parviflora* (Fumariaceae) against gastrointestinal nematodes of sheep. *International Journal of Agriculture and Biology.* 11, 431-436.
- Awah, F. M., Uzoegwu, P. N., Ifeonu, P., Oyugi, J. O., Rutherford, J., Yao, X. J., Fowke, K. R. and Eze, M. O. (2012). Free radical scavenging activity, phenolic contents and cytotoxicity of selected Nigerian medicinal plants, *Food Chemistry.* 131, 1279-1286.
- Ba, K., Tine, E., Destain, J., Cisse, N. and Thonart, P. (2010). Étude comparative des composés phénoliques, du pouvoir antioxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt, *Biotechnol. Agro. Soc. Environ.* 14, 131-139.
- Baker, R. L., (1997). Résistance génétique des petits ruminants aux helminthes en Afrique. *INRA Productions Animal.* 10, 99-110.
- Barros, L., Cabrita, L., Baos, M. V., Carvalho, A. M. and Ferreira, I. C. F. R. (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants, *Food Chemistry.* 127, 1600-1608.
- Bauer, C., Merkt, J. C., Janke-Grimm, G. and Burger, H. J. (1986). Prevalence and control of benzimidazole-resistant small strongyles on German thoroughbred studs. *Vet. Parasitol.* 21, 189-203.
- Brunet, S., Martinez-Ortiz de Montellano, C., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Aguilr-Caballero, A. J., Capetillo-Leal, C. and Hoste H. (2008). Effect of the consumption of *Lysiloma latiliquum* on the larval establishment of gastrointestinal nematodes in goats. *Veterinary Parasitology.* 157, 81-88.
- Clark, C. H., Kiesel, G. K. and Goby, C. H. (1962). Measurement of blood loss caused by the *Haemonchus contortus* in sheep. *American Journal of Veterinary Research.* 23, 977-980.
- Danga, Y. S. P., Nukenine, E. N., Esimone, C. O. and Younoussa, L. (2014). Larvicidal and pupicidal toxicities of *Plectranthus glandulosus* and *Callistemon rigidus* leaf essential oils against three mosquito species. *J Mosq Res.* 4 (2), 5-14.
- Delaveau, P., (1988). Polyphénols et tanins dans l'alimentation. *Aliments,* 137-139.
- Enriquez, (1994). Role of immunoglobulin A monoclonal antibodies against P23 in controlling murine *Cryptosporidium parvum* infection. *Infection and Immunity.* 66, 4469-4473.
- Gomber, C. and Saxena, S. (2007). Anti-staphylococcal potential of *Callistemon rigidus*. *Central European Journal of Medicine.* 2(1), 79-88.
- Hoste, H., Brunet, S., Paolini, V., Bahaud, D., Chauveau, S., Fouraste, I. and Lefrileux, Y. (2009). Compared *in vitro* anthelmintic effects of eight tannin-rich plants browsed by goats in the Southern part of France. *Option Méditerranéennes.* 431-436.
- Institut national des statistiques (2015). *Annuaire statistique du Cameroun, Chapitre 15: élevage et pêche.* pp. 257-268.
- Jackson, F. and Coop, R. L. (2000). Development of anthelmintic resistance in sheep nematodes. *Parasitol.* 20, 95-107.
- Jamuna, S., Subramaniam, P. and Krishnamoorthy, K. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochoeris radicata* L. for *in vitro* antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine.* 359-367.
- Jirovetz, L., Fleischhacker, W., Buchbauer, G. and Ngassoum, M. B. (1997). Analysis of the essential oils of *Callistemon rigidus* (Myrtaceae) from Cameroon by GC/FID and GC/MS, *Sci. Pharm.,* 65, 315-319.

- Kabasa, J. D., Opuda-Asibo, J. and Meulen, U. T. (2000). The effect of oral administration of polyethylene glycol on faecal helminth egg counts in pregnant goats grazed on browse containing condensed tannins. *Trop. Anim. Health Product.* 32(2), 73-86.
- Kabore, A., (2009). *Activité anthelminthique de deux plantes tropicales testées in vitro et in vivo sur les Strongles gastro-intestinaux des Ovins de races Mossi du Burkina Faso.* Thèse de Doctorat, Université Polytechnique de Bobo-Dioulasso, 167.
- Kahiya, C., Mukaratirwa, S. and Thamsborg, S. M., (2003). Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. *Vet. Parasitol.* 115, 265-274.
- Kawo, A.H., Suleiman, Z.A. and Yusha'u M. (2011). Studies on the antibacterial activity and chemical constituents of *Khaya senegalensis* and *Ximenia americana* leaf extracts. *Afr. J. Microbiol. Res.*, 5(26), 4562-4568.
- Kloosterman, A., Albers, G. A. A., and Van Den Brink, R. (1978). Genetic variations among calves in resistance to nematode parasites. *Veterinary Parasitology.* 4, 353-368.
- Lawal, M., Wasagu, R. S. U. and Ladan, M. J. (2005). Hepatotoxicity risk assessment of neem (*Azadirachta indica*) seed extract using albino rats. *Biol. Environ. Sci. J. Trop.* 2(2), 36-38.
- Magaji, R. A. and Yaro, A. H. (2006). Acute toxicity evaluation of the aqueous extract of *Syzygium aromticum* in mice. *Biol. Environ. Sci. J. Trop.* 3 (1), 1-3.
- Makkar, H. P. S. (2003). Review. Effects and fate of tannins in ruminants' animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin rich feeds. *Small Ruminant Research.* 49, 241-256.
- Martin, R. J. (1999). Mode of action of Anthelmintic drugs. *Vet J.* 154, 11-34.
- Mbafor, F. T., Payne, V. K., Wabo Poné, J., Mbogning Tayo, G. and Tchoumboue, J. (2014). *In vivo* anthelmintic activity of *Terminalia glaucescens* (Combretaceae) extract against gastrointestinal nematodes of sheep. *British Journal of Pharmaceutic Research*, 4(18), 2136-2145.
- Ndjonka, D., Ajonina-Ekoti, I., Djafsia, B., Luersen, K., Abladam, E. and Liebau, E. (2012). *Anogeissus leiocarpus* extract on the parasite nematode *Onchocerca ochengi* and on drug resistant mutant strains of the free-living nematode *Caenorhabditis elegans*. *Vet Parasitol.* 190, 136-142.
- Ndoutamia, G. and Ganda, K. (2005). Détermination des paramètres hématologiques des petits ruminants du Tchad. *Revue Méd. Vét.*, 156(4), 202-206.
- Nemi, J. (1993). *Essential of Veterinary Haematology.* 1st Ed., P. 159.
- Niezen, J. H., Robertson, H. A., Waghorn, G. C. and Charleston, W. A. G. (1998). Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. *Veterinary Parasitology.* 80, 15-27.
- Olounladé, A. P., Attakpa, Y. E., Azando, E. V. B., Hounzangbé – Adoté, M. S. and Hoste, H. (2017). Effet *in vivo* De *Newbouldia laevis* (Bignoniaceae) Sur Des Strongles Gastro-Intestinaux Des Moutons. *European Scientific Journal*, 13, 335-351.
- Paolini, V., DE LA Farge, F., Prevot, F., Dorchies, P. and Hoste, H. (2005). Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. *Vet. Parasitol.*, 127, 277-283.
- Paolini, V., Frayssines, A., De La Farge, F., Dorchies, P. and Hoste, H. (2003). Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. *Veterinary Research.* 34, 331-339.
- Qadir, A. (1967). Investigation on the incidence of gastro-intestinal parasites of goats in the East Pakistan Agricultural University Campus. *Ceylon Veterinary Journal.* 15, 58-61.
- Rajesh, K. V., Sumathi, C. S., Balasubramanian, V. and Ramesh, N. (2009). Elementary chemical profiling and antifungal properties of cashew (*Anacardium occidentale* L.) nuts. *Botan. Res. Internation.* 2(4), 253-257.
- Sabater, F. (2012). Détermination d'une dose efficace et d'une dose toxique de tanins condensés dans le contrôle des strongyloses digestives chez les caprins. Thèse d'exercice, Médecine vétérinaire, Ecole Nationale Vétérinaire de Toulouse - ENVT, 135.

- Silanikove, N., Perevolotsky, A. and Provenza, F. D. (2001). Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. *Anim. Feed Sci. Technol.* 91, 69-81.
- Terrill, T. H., Douglas, G. B., Foote, A. G., Purchas, R. W. Wilson, G. I. and Barry, T. N. (1992). Effects of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. *J. Agric. Sci.* 119, 265-273.
- Terrill, T. H., Dykes, G. S., Shaik, S. A., Miller, J. E., Kouakou, B., Kannan, G., Burke, J. M. and Mosjidis, J. A. (2009). Efficacy of sericea lespedeza hay as a natural dewormer in goats: Dose titration study. *Vet. Parasitol.* 163, 52-56.

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