EAS Journal of Parasitology and Infectious Diseases
Abbreviated Key Title: EAS J Parasitol Infect Dis
ISSN: 2663-0982 (Print) & ISSN: 2663-6727 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-8 | Issue-2 | Apr-Jun- 2026 | DOI: https://doi.org/10.36349/easjpid.2026.v08i02.004

Original Research Article

In vitro Anthelmintic Activity of *Callistemon rigidus* (Myrtaceae) Bark Extracts Against *Haemonchus contortus* Eggs, Larvae and Females Adults

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Article History
Received: 12.04.2026
Accepted: 03.06.2026
Published: 16.06.2026

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: Haemonchis is a major health and welfare problem for small ruminants responsible for economic losses through reduced productivity and increased mortality. The *in vitro* efficacy of *Callistemon rigidus* was determined against *Haemonchus contortus* the most important parasite involved in this animal disease. Fresh eggs, infectives larvae (L_3) and female's adult were incubated at room temperature in aqueous and ethanolic extracts of *C. rigidus* for 24 h; 24 and 48h for effectivity assessment. The PBS used as negative control did not affect the involved developmental stages. Aqueous and ethanolic extracts induced 100 % mortality of female's adults at 1 mg/mL with LC_{50} values of 0.067 ± 0.01 mg/mL and 0.067 ± 0.011 mg/mL respectively. The inhibition of eggs hatching rate varied from 12.33±2.08 % to 83.67±2.21 % with LC_{50} value of 0.351 ± 0.023 mg/mL. While the ethanolic extract inhibited 15.33±1.53% to 100±00% with LC_{50} value of 0.269 ± 0.005 mg/mL eggs hatching. The larval mortality rates increased from 62.5±9.57 % to 91.66±9.62% for aqueous extract with LC_{50} value 0.2±0.02 mg/mL and from 78.93±6.53% to 100±00% for ethanolic extract with a corresponding LC 50 value of 0.12±0.01mg/mL. Finaly, this study indicated that *C. rigidus* possess potential anthelmintic effect and further *in vivo* study is indispensable to validate its use as alternative anthelmintic against that GIN.

Keywords: *Callistemon Rigidus,* Ovicidal, Larvicidal, *Haemonchus Contortus*, Cameroon.

INTRODUCTION

Breeding of small ruminants is an important prospective sector which may contribute in solving problem of farmers and by such, help in poverty alleviation [1]. In Tropical Africa, this sector of activity is hampered by gastro-intestinal nematodes causing the reduction of the production potential [2]. Among the disease that hinders the survival and productivity of sheep and goats, gastrointestinal nematode infection ranks highest on a global scale, with *Haemonchus contortus* being of overwhelming importance [3]. This gastrointestinal nematode is the most important nematode parasite of small ruminants, causing severe anaemia and high mortality in all classes of livestock [4]. It is one of the nematode species that dominate the parasitic spectrum of small ruminants in Africa in general and Subsaharian Africa in particular [5]. The infestation rate of different species of *Haemonchus* ranged from 50 - 85% [6]. The principal diagnostic feature of haemonchosis is anaemia, induced by the blood feeding nature of adults and larval stage. It is noted that the average blood loss has been calculated as 0.05 mL/parasite/day [7]. Since many years, the control of haemonchosis is generally achieved by the use of synthetic anthelmintic. The frequent use of these anthelmintic over the years has inevitably led to the development of drug resistance. The emergence of resistance to anthelmintic drugs which is now a worldwide phenomenon [8], and the increasing awareness of consumers about drug residues that potentially enter the food chain have stimulated investigation into alternative anthelmintic such as

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medicinal plants. In rural areas, the treatment of gastrointestinal parasites of small ruminants by medicinal plants is a common practice [9]. This calls into question the use of these anthelmintic. There is therefore an urgent need to seek innovative alternative solutions, in order to ensure a more sustainable control of this parasitism. Hence, new therapeutic approaches are necessary and might be find by the use of medicinal plants, since they are accessible at all times and inexpensive [10]. The therapeutic properties of plants are strongly linked to their phytochemical components. Among a number of phytochemical constituents present in plants, saponins, polyphenols, tannins and flavonoids are known for their anthelmintic activity [11]. Among the therapeutics' plants, *Callistemon rigidus* a Myrtaceae is known by traditional healer for its deworming virtues. This plant is used agains epilepsy, anxiety and candidiasis treatment as well as an insecticide (against mosquitoes) [12]. *C. rigidus* is used to facilitate healing and as an antiseptic. In Cameroon, the essential oil of *C. rigidus* is used in medicine to treat bronchitis, cough and respiratory infections [13]. As far as literature on this plant is concerned, there is no published work on *in vitro* anthelmintic activity of *C. rigidus* against *H. contortus*. This work aimed to screen for phytochemical properties and evaluate the *in vitro* activity of the aqueous and ethanolic extract of *C. rigidus* on *H. contortus*.

2. MATERIALS AND METHODS

2.1. Plant Material and Preparation of Extracts

The roots of *Callistemon rigidus* were collected from Dang/Adamawa region of Cameroon based on ethnopharmacological data. Voucher specimens were identified at the Department of Biological Sciences of the University of Ngaoundere. A voucher specimen was then deposited at 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*., [14]. The plant extract was further dissolved in dimethyl sulfoxide (DMSO) and phosphate buffer solution (PBS) to a final concentration of 100 mg/mL, mixted and aliquoted to determine their *in vivo* activity on *H. contortus*.

2.2. Tests on *Haemonchus Contortus*

2.2.1. Collection of Adults of *Haemonchus Contortus*

The abomasa of goats and sheep were sampled straight on the slaughtered animals in Ngaoundere. These abomasa were conducted to the laboratory of Zoology of the University of Ngaoundere. Using the protocol described by Kabore [9], female's adults of *H. contortus* were isolated based on the presence of vulva (compared to the males). The abomasa were incised in order to collect all worms and transferred in PBS solution. Females of *H. contortus* were isolated under binocular microscope at objective ×10.

2.2.2. Anthelmintic Bioassay on Adult Worms

The ethanolic and aqueous extracts of roots of *C. rigidus* were diluted in PBS into 05 concentrations (0.1, 0.3, 0.5, 0.7 and 1mg/mL). Healthy and highly motile worms were used for the test. Using the method described by Yongwa *et al*., [15a], one worm was introduced in a well (of 96 wells plate) containing 500 µL of solution for 03 columns of 06 wells. PBS was used as negative control and a positive control consisted of albendazole. The test solutions were concomitantly prepared and incubated with the subjects at 37 °C for 24h. Mortality was checked by visually under the binocular microscope. After shaking worm, immotile and fully elongated worms were considered to be dead. The mortality rate was determined after 24 h. The mortality rate was calculated using the formula below:

$$\text{Mortality rate} = ND/NT \times 100 \quad (1)$$

Where ND is the number of dead worms in each well and each concentration. NT is the total number of worms at each concentration.

2.3. Tests on Eggs of *Haemonchus Contortus*

2.3.1. Parasite Donor Goat

Female *H. contortus* collected from abomasum of goats and crushed to release eggs [16]. The obtained ova were cultured in petri dishes at room temperature for seven days [17]. The culture media constituted of 3 ml of sterile liquid of feaces prepared from 3 g of faeces removed from the rectum of parasites-free goat, to which was added charcoal. At the end of the 8th day, infective larvae were harvested. About 4500 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 4500 larvae were deduced, measured and inoculated into a worm-free goat. This goat served as *H. contortus* egg donor for the *in vitro* trials.

2.3.2. Recovery of Nematode Eggs

After the pre-patent period of 21 days, 3 g of faeces were collected directly collected from the rectum of the donor goat. According to the procedure described by Wabo *et al.,* [18], faeces were homogenized in a mortar by adding 60 mL of salt (Na Cl 40% W/V). The solution was cleaned of organic debris by filtration through a 250 µm mesh-size sieve into a beaker and finally poured into four conical tubes until the formation of a meniscus at the top. Three minutes later, slides and cover slides containing the eggs were rinsed with distilled water into 100 mL beaker. The beaker was allowed to stand for 30 minutes for the sedimentation of the eggs at the bottom. To completely remove the salt solution, eggs were washed three times by siphoning out 90 mL of solution and replacing with the same amount of distilled water each 30 minutes. Finally, the

supernatant was removed and the remaining 10 mL solution containing eggs was used in the assay.

2.3.3. Egg Hatch Assay (EHA)

The *in vitro* EHA was based on the method described by Coles *et al.,* [19]. Fresh eggs of *H. contortus* were used to evaluate the ovicidal activity of aqueous and ethanolic extract of *C. rigidus.* To do this, 30 eggs of *H. contortus* were distributed into each well of a 24-flat-bottomed microtitre plate and incubated in different concentration (0.1, 0.3, 0.5, 0.7 and 1 mg/mL) of aqueous and ethanolic extract of *C. rigidus*. Albendazole was used as positive control and evaluated at various concentrations (0.1, 0.3, 0.5, 0.7 and 1 mg/mL) and PBS as negative control. The plates were incubated for 48 h at 27 °C. The experiment was replicated three times for each extract on the same plate. After incubation, the hatched larvae and unhatched eggs were counted using an inverted microscope under 20 × magnifications. The percentage Egg Hatching Inhibition (%EHI) was calculated using the formula above:

$$\%EHI = 100 - (\frac{Number\ of\ L1\ larvae}{Number\ of\ fresh\ eggs\ in\ culture}) \times 100 \quad (2)$$

2.4. Tests on Infective Larvae of *Haemonchus Contortus*

2.4.1. Recovery of Nematode Larvae

The infective larvae were obtained by stool culture from feces of goats (goat donor) previously infested by *H. contortus* larvae. The feces were collected directly from the goat's rectum. The eggs contained in the fecal matter were subsequently placed in stool culture at room temperature for 7 days. The larvae were subsequently extracted from the faecal mass using the Baermann apparatus, the principle of which is based on the hygrotropism of the larvae.

2.4.2. Evaluation of Larvicidal Activity of *Callistemon Rigidus*

Larval mortality assay using L_3 larvae was performed according to the method described by Wabo pone *et al.* [18]. Aqueous and ethanolic extract of *C. rigidus* and positive control made by albendazole were dissolved in PBS. Twenty larvae were distributed into each well of a 24-flat-bottomed microtitre plate at different concentration (0.1, 0.3, 0.5, 0.7 and 1 mg/mL). PBS was used as negative control. The plates were incubated for 24 and 48 h at 27 °C. The experiment was replicated three times for each extract on the same plate. After the incubation's period, the number of dead larvae was counted under the microscope based on their straight shaped, their immobility and the presence of holes on their tegument. The percentage of mortality (Mt%) was determined using the following formula:

$$Mt\ (\%) = (\frac{Number\ of\ dead\ larvae}{Number\ of\ larvae\ in\ culture}) \times 100 \quad (3)$$

2.5. Phytochemical Tests

The concentrated residues from the aqueous and ethanolic extracts of *C. rigidus* were screened for secondary metabolites according to the method describe by Ndam *et al*., [20].

2.6. Statistical Analysis

The 50% inhibitory concentrations (IC_{50}) for eggs hatching rates were calculated using linear regression equations drawn after transformation of the eggs hatching inhibition rate to probit according to the decimal logarithm of concentrations. While the 50% lethal concentrations (LC_{50}) for L_3 larvae was determined using linear regression equations drawn after transformation of larval mortality rate to Probit according to the decimal logarithm of concentrations. Comparison of the mean inhibition percentage of eggs hatching and mean percentage of larval mortality at different concentrations with control was performed by two-way analysis of variance (ANOVA). Statistical analyses were performed using the software SPSS version 17.0 software. The post hoc statistical significance test employed was LSD, differences between the means were considered significant at $P < 0.05$. The lethal concentrations (LC_{50}) were determined.

3. RESULTS

3.1. Effect of *Callistemon Rigidus* Barks Extract on Female *Haemonchus Contortus*

The aqueous and ethanolic extracts of bark of C. *rigidus* were tested for anthelmintic activity against female's adults *H. contortus*. No mortality was observed after 24 h of incubation for the negative control made by PBS (table 1).

Table 1: Effect of *Callistemon rigidus* extract on female *Haemonchus contortus* after 24 h

	Concentrations (mg/mL)	Mortality of females *H. contortus*
PBS	0	0
EEC	0.1	77.78±9.62
	0.3	88.89±9.62
	0.5	94.44±9.62
	0.7	100±00
	1	100±00
AEC	0.1	77.78±9.62
	0.3	88.89±9.62

	Concentrations (mg/mL)	Mortality of females *H. contortus*
	0.5	88.89±9.63
	0.7	100±00
	1	100±00
Albendazole	0.1	77.78±9.16
	0.3	94.44±9.65
	0.5	100±00
	0.7	100±00
	1	100±00

AEC: Aqueous Extract of *Callistemon rigidus*; EEC: Ethanolic Extract of *Callistemon rigidus*

3.2. Effect of *Callistemon Rigidus* Root Extract on *Haemonchus Contortus* Eggs Hatching Inhibition

The efficacy of aqueous and ethanolic extracts of bark of *C. rigidus* in eggs hatching inhibition of *H. contortus* at different concentrations is presented in table 2. From this table, one can observe that negative control (PBS) had no effect on egg hatching inhibition while albendazole used as positive control inhibited the egg hatching in a concentration dependent manner.The mortality was between 12.33±2.08 and 100±00.

Table 2: Effect of *Callistemon rigidus* barks extract on *Haemonchus contortus* eggs hatching inhibition

	Concentrations (mg/mL)	Egg hatching inhibition (EHI)
PBS	0	0
EEC	0.1	15.33±1.53
	0.3	55±1.73
	0.5	57±1
	0.7	97.67±2.08
	1	100±00
AEC	0.1	12.33±2.08
	0.3	49±4.58
	0.5	51±2.64
	0.7	81±4.58
	1	83.67±2.21
Albendazole	0.1	65.67±1.15
	0.3	27.68±2.52
	0.5	63.67±4.16
	0.7	86±2.65
	1	100±00

AEC: Aqueous Extract of *Callistemon rigidus*; **EEC**: Ethanolic Extract of *Callistemon rigidus*

3.3. Comparison of the LC_{50} Values of Albendazole to Those of Aqueous Extract of *Callistemon Rigidus* and Ethanolic Extract of *Callistemon Rigidus*

According to the table 3 below, the results indicat that albendazole had induced egg hatching inhibition with a LC_{50} of 0.25 ± 0.012. Ethanolic extract of bark of *C. rigidus* was efficient with a LC_{50} of 0,269 ± 0,005 mg/mL. The aqueous extract of *C. rigidus* also inhibited eggs hatching with a LC_{50} value of 0.351 ± 0.023 mg/mL.

Table 3: Comparison of LC_{50}

	LC_{50} (mg/mL)			
	Adults females mortality (24h)	Egg hatching inhibition (24h)	Larval mortality	
			24 h	48 h
Albendazole	0.059 ± 0.025[a]	0.25 9± 0.012[a]	0.22±0.02[a]	0.10±0.01[a]
AEC	0.067 ± 0.01[a]	0.351 ± 0.023[a]	0.73±0.07[d]	0.2±0.02[a]
EEC	0.067 ± 0.011[a]	0.269 ± 0.005[a]	0.42±0.08[e]	0.12±0.01[a]

Letters compare means in the columns. Different letters indicate significant difference ($p < 0.05$) **AEC:** Aqueous Extract of *Callistemon rigidus*; **EEC:** Ethanolic Extract of *Callistemon rigidus*

3.3. Larvicidal Activity of *Callistemon Rigidus*

The larvicidal activity of ethanolic and aqueous extracts of barks of *C. rigidus* was evaluated on infective larvae of *H. contortus* and the results of this study are presented in table 4. From this table, it is noticeable that efficacy of extracts and positive control had been

concentration dependent. The negative control had no effect on L_3 while positive control was efficient inducing 52.20±9.86% average mortality rate value at 0.1 mg/mL and 100±00% at 1 mg/mL after 24 h of incubation, and a LC_{50} of 0.22±0.02. This activity was more important after 48 h of incubation by inducing 80.43±3.99 % average mortality rate at 0.1mg/mL to 100±00 % at 0.5 mg/mL, and a LC_{50} of 0.10±0.01. The ethanolic extract of *C. rigidus* occasioned after 24 h of incubation, an average mortality rate ranked from 44.28±6.52% to 91.67±11.05% at a concentration of 0.1 mg/mL and 1 mg/mL respectively with a LC_{50} of 0.42±0.08. After 48 h of incubation, the same extract has been more active inducing an average mortality rate of 78.93±6.53 % at 0.1 mg/mL and 100±00% at 0.7 mg/mL and a LC_{50} value of 0.44±0,06mg/mL. The aqueous extract of *C. rigidus* was efficient as well by inducing an average mortality rate ranked from 27.5±5% to 56.25±6.29% after 24h of incubation with a LC_{50} value of 0.73±0.07 mg/mL. Meanwhile, after 48 h, it induced average mortality rate ranked from 62.5±9.57% to 91.66±9.62% with a LC_{50} value of 0.2±0.02 mg/mL.

Table 4: Larvicidal activity of *Callistemon rigidus*

	Concentrations (mg/mL)	24 h	48 h
PBS	0	0	0
EEC	0.1	44.28±6.52	78.93±6.53
	0.3	54.37±5.15	86.87±8.51
	0.5	58.24±5.15	91.67±5.27
	0.7	61.67±8.82	100±00
	1	91.67±11.05	100±00
AEC	0.1	27.5±5	62.5±9.57
	0.3	35.42±9.75	75±9.13
	0.5	50±2.45	75±11.55
	0.7	50±8.16	87.5±8.66
	1	56.25±6.29	91.66±9.62
Albendazole	0.1	52.20±9.86	80.43±3.99
	0.3	54.37±5.15	89.37±5.91
	0.5	78.03±5.14	100±00
	0.7	92.25±9.67	100±00
	1	100±00	100±00

AEC: Aqueous Extract of *Callistemon rigidus*; EEC: Ethanolic Extract of *Callistemon rigidus*

3.4. Phytochemical Screens

Phytochemical analysis of aqueous and ethanolic extracts of barks of *C. rigidus* reveal the presence of alkaloids, tannins, flavonoids, saponins, triterpenes, polyphenol and anthraquinons in both extract of the plants (Table 5).

Table 5: Phytochemical screening

Extracts	polyphenol	Flavonoid	tannin	alkaloid	triterpen	saponin	Anthraquinon
AEC	+	++	++	+	+	++	+
EEC	++	+	+++	+	+	+++	+

+: present. AEC: Aqueous Extract of *Callistemon rigidus*; EEC: Ethanolic Extract of *Callistemon rigidus*

4. DISCUSSION

In the present study, aqueous and ethanolic extracts of *C. rigidus* were undertaken to assess their anthelmintic activity against the eggs, larvae and female adults of *H. contortus.* The major finding of this study was the high efficacy of the extracts.

In fact, the female's adult's mortality essay revealed a significant efficacy of the aqueous and ethanolic extracts of *C. rigidus* with 100% at the concentrations 0.7 mg/ml and 1 mg/ml. These findings corroborated those of Hounzangbe-Adote *et al*., [21], with the alcoholic extracts of four Benin plants, Zanthoxylum zanthoxyloides, *Newbouldia laevis*, *Morinda lucida* and *Carica papaya*. In addition, Dedehou *et al.,* [22], obtained a maximum mortality rate of 100% after 24 h of incubation in the hydro-acetonic extract of *Erocarpus erinaceus* at the concentration of 1200 µg/mL. The efficacy anthelminthic of aqueous and ethanolic extracts of *C. rigidus* is due to the presence of phytochemical compounds. In a study performed by Yongwa *et al.,* [23b], they confirmed that the anthelminthic activity of *C. rigidus* depend not only on the availability of secondary metabolites like tannins, saponins, triterpens and flavonoids but also on the proportion in the extract.

Table 1 presents the variation of the mean EHI of *H. contortus* at different concentrations. It appears from these results that extracts of *C. rigidus* were efficient against eggs hatching of *H. contortus.* In fact, aqueous and ethanolic extract of *C. rigidus* inhibited significantly ($p < 0.05$). 83.67±2.21% and 100±00% eggs hatch at 1 mg/mL respectively after 24 h of incubation. The ethanolic extract of *C. rigidus* was highly efficient compared to aqueous extract against eggs hatching and larvae. These findings corroborated those of Hounzangbe-Adote *et al*. [21] with the alcoholic extracts of four Benin plants, Zanthoxylum zanthoxyloides, *Newbouldia laevis*, *Morinda lucida* and *Carica papaya*. In addition, Dedehou *et al.,* [22], obtained a maximum mortality rate of 100% after 24 h of incubation in the hydro-acetonic extract of *Erocarpus erinaceus* at the concentration of 1200 µg/mL.

The comparison of LC_{50} value revealed that, ethanolic extract of *C. rigidus* was highly efficient compared to aqueous extract against eggs hatching ($p < 0.05$). These results are similar to those of Okombe [24], who obtained 87.53 ± 3.1 and 90.36 ± 2.4% respectively for aqueous and ethanolic extract of *Vitex thomasii* at the concentration of 2 mg/mL. Almost similars observations have been noted by Payne *et al.,* [25], where water extract of *Dichrocephala intergrifolia* presented a weak activity on eggs hatching of *Heligmosomides bakeri* as compared to ethanolic extract. Mbogning *et al.,* [26], also noted a high efficacy of the ethanolic extract of *Bidens pilosa* compared to the aqueous extract. The variation in the activity of the extracts types of the plants may be due to difference in proportion of the active components responsible of the anthelmintic activity observed resulting from the difference of solubility in solvent as presented in table 5. According to Eguale [27], and Yongwa [15 and 23] the variation of the anthelmintic activity of plant extracts is due to the different proportion of components present in plant extracts. It can also relate to the different chemical ingredients extracted in different solvents and their different biological effects on parasite [28]. According to Alvarez *et al.,* [29], as compared to albendazole, the phytochemical components may diffuse through the outer surfaces such as the eggshell and inhibited egg hatching.

The larval activity essay presented in table 3 revealed that the activity of the extracts was dependent on concentration and also time of exposure. The higher the concentration of the extract, the higher the inhibition of the extracts. Also, the longer the time of exposure of the extract to the larvae, the more effective the inhibition will be. The higher concentration of aqueous and ethanolic extracts induced more effective eggs inhibition after 24h and 48 h of incubation. Almost similar results have been validated by Olukotun [30], with a L_3 paralysis inhibition of 59 %; 60 % and 98 % respectively after 24 h; 48 h and 72 h of incubation with aqueous extract of *Terminalia Catappa.* Data in table 3 also revealed the high efficacy of the ethanolic extract compare to the aqueous extract with 100±00% and 91.66±9.62% of mortality respectively. These finding results are similar to Wabo *et al*., [18], and Payne *et al.,* [25], where aqueous extract of *Erythrina sigmoidea* and *Dichrocephala integrifolia* presented low activity on larvae of *Heligmosomides bakeri* as compare to ethanolic extract. Same observations have been noted by Mbogning *et al.,* [26], with *Bidens pilosa* extracts. Similar results have been validated by Okombe [24], with a L_3 paralysis inhibition of 85.61% at 2 mg / mL concentration with ethanolic extracts of *Vitex thomasii* root bark powder and 71.11% with aqueous extracts from the same plant. These different may be due to the variation of proportion of phytochemical components.

The anthelmintic activity of *C. rigidus* extracts could be attributed to the polyphenol, flavonoid, tannin, alkaloid, triterpenes, saponin and Anthraquinones. These metabolites may have worked in combination or singly to cause eggs hatching inhibition; larval mortality and female's adult mortality. According to Schoenian, [31] albendazole kills the parasite by binding to the beta-tubulin and prevent its incorporation into micro-tubules which are very important for the metabolism of energy. Paralysis of parasites tissues makes them unable to feed, leading to death cause by lack of energy metabolism. Thus, may be our phytochemical components caused their effect through the same mechanism. According to Al-Shaibani *et al.*, [32], since transcuticular diffusion is a common mean of entry of non-nutrient and non-electrolytes substances into helminth parasite, the possible explanation for better anthelmintic activity of ethanolic extract compare to aqueous extract could be due to the easier transcuticular absorption of the ethanolic extract into body of parasite than aqueous extract. According to Cala *et al.,* [33], tannins connect to free proteins or to larval cuticul, reducing the availability of nutrients causing the death of the larvae due to starvation. The anthelmintic activity of extracts plant would be also due to alkaloids which have ability to intercale with DNA synthesis of parasites [32]. Anthelmintic efficacy would also be the action of saponnins present in extracts of the plant. According to Nalule *et al.,* [28], saponnins cause feed refusal and starvation of the parasite leading to their death due to lack of energy.

Analysis of the phytochemical assay of *C. rigidus* presented in Table 5 revealed the presence of the Polyphenols; Flavonoids; Tannins; Anthraquinones; Alkaloids; Triterpenes and Saponins both in the aqueous extract and the ethanolic extract of the said plant. Danga *et al.,* [34], highlighted the presence of these same

phytochemicals in the methanolic extract of the leaves of *C. rigidus*. Furthermore, Praveen *et al*., [35], carried out a study on the phytochemical composition of plants of the genus *Callistemon* and proved that these plants are rich in triterpenes; flavonoids; steroids and saponins. In the reccent study perform by Yongwa *et al.,* [23], on the phytochimical quantification of ethanolic extract of *Callistemon rigidus* reveal that, saponin, polyphenolic tannin and flavonoid are the most concentrated metabolic in the ethanolic extract of the plant.

5. CONCLUSION

In conclusion, this work focused on the *in vitro* activity of the aqueous and ethanolic extracts of *Callistemon rigidus* on *Haemonchus contortus.* The results from this study show the potential values of *C. rigidus* bark extracts for the management of haemonchosis since these extracts are active against adult females, eggs and infective larvae. Particularly the later one which is main stage contaminating pasture. Thereby, the control of this parasite will reduce considerably contamination and help in the overall helminth control program. However, further studies are required to investigate the potential presence of toxic effects in order to determine the minimum non-lethal doses for the treatment of gastrointestinal helminths.

Acknowledgement

All materials used in this study were kindly donated to Prof. Dr. D. Ndjonka by the Alexander von Humboldt Foundation (AvH). This donation is greatly appreciated.

Author Contributions:

- Yongwa Gilbert conducted the research and wrote the first draft of the manuscript;
- Ndouwe TISSEBE MENGA Honoré, Belga François Ngondandi and Dedie Pierre, analyzed the data and corrected the manuscript ;
- Saotoing Pierre and Ndjonka Dieudonné supervised the research. All authors read and approved the final manuscript.

Conflict of Interest: Authors have declared that no competing interests exist

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Cite This Article: Yongwa Gilbert, Ndouwe Tissebe Menga Honore, Dedie Pierre, Belga François Ngnodandi François Ngnodandi, Saotoing Pierre, Ndjonka Dieudonné (2026). *In vitro* Anthelmintic Activity of *Callistemon rigidus* (Myrtaceae) Bark Extracts Against *Haemonchus contortus* Eggs, Larvae and Females Adults. *EAS J Parasitol Infect Dis, 8*(2), 61-68.