South Asian Journal of Parasitology

6(4): 88-98, 2022; Article no.SAJP.91981

Anthelminthic Activities of Drinkable Suspension of *Alafia barteri*'s Extracts on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae)

Yondo Jeannette [a*], D. Gangueu Djape Clotilde [b], G. Mbogning Tayo [b], M. Ngangout Alidou [b], Djam Chefor Alain [a], Wabo Pone Josué [b] and Mpoame Mbida [b]

[a] Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, P.O. Box: 93 Dschang, Cameroon.
[b] Department of Animal Biology, Faculty of Sciences, University of Dschang, P.O. Box: 67 Dschang, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Author YJ computed the differents doses of plant extracts and revised the manuscript. Author DGDC performed data collection in the labs. Author GMT and Author late WPJ did statistical analyses. Author MNA raised the mice at the pet store. Author DCA revised the manuscript. Authors MM and CGDC designed the experimental protocol. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/91981

Original Research Article

Received 20 August 2022
Accepted 01 October 2022
Published 14 December 2022

ABSTRACT

Resistance of helminths parasites to synthetic medicine leaded the International community to look for news methods of controlling gastrointestinal helminths like the use of medicinal plants with anthelminthic properties. The aim of this study was to evaluate *in situ* the anthelmintic activity of the drinkable solution of *Alafia barteri* (aqueous and ethanolic extracts) on *Heligmosomoides bakeri*. Mice were orally infected with L_3 infective larva. After the prepatent period, animals whose qualitative analysis revealed the presence of *H. bakeri* eggs in their feces were randomly divided into 5 groups of 5 animals each. Group 0 served as negative control and received distilled water or DMSO 1.24% depending on the test. Group 1 was the positive control and received a single dose of 15 mg / kg bwt of Albendazole (0.28 mg/ml). The three others groups (2, 3 and 4) received the treatment with plant products at the dose of 980, 1960 and 3920 mg / kg bwt respectively during 7

**Corresponding author: E-mail: yondojanet@yahoo.fr;*

days. The efficacy of *A. barteri* was evaluated through the fecal egg count reduction (FECR), the total worm count reduction (TWCR) and through the development of eggs taken from treated animals. Results showed that, effects of treatments were non-dose dependent. Ethanolic extract exhibited the highest anthelminthic activity in the reduction of the fecal eggs (91.36 % reduction) during the second week after treatment with 1960 mg / kg bwt. With the aqueous extract, we observed the best FECR of 87.41 % in the same dose. The best TWCR (89.89%) was observed at 980 mg / kg bwt with the aqueous extract. The minimum larval development rate (LDR) was exhibited with ethanolic extract (51.48 %). From the global results obtained, we can say that *A. barteri* has compounds with vermicidal anthelminthic properties, particularly with ethanolic extract.

Keywords: Anthelminthic activity; Alafia barteri; Heligmosomoides bakeri.

1. INTRODUCTION

Parasitic infections constitute a major health problem in tropical and subtropical zones [1]. Among these infections, helminthes infections remain a serious public health problem [2]. They are diseases caused by worm parasites which obligatory live in a host organism for at least one stage of their life cycle. Their transmission is favored by the lack of hygienic condition, poverty and the lack of general assessment [3]. Because they are rarely pattern of consultation, helminthiasis are classified amount tropical neglected diseases in favor of acquired immunodeficiency syndrome (AIDS), malaria and tuberculosis; while these diseases affect more than 2 billion people worldwide, and the greatest number of infections occurring in sub-Saharan Africa, America and Asia [4]. Apart the fact that they have chronic and insidious symptoms, helminthiasis are able to breakdown the health status of the host, particularly in infants and children. By so doing, they can cause detrimental effects on human growth, nutrition, cognition, school performance, work productivity and pregnancy, which may severely impair the quality of life. They can even increase the level of sero-prevalence of AIDS and malaria [5]. Moreover, helminthiases also represent the most important problem affecting the productivity of livestock with important economic losses like the death of highly infected animals [6]. Anthelminthic control is done by the used of synthetic anthelminthic drugs, but resistance, toxicity, secondary effect in the host and the increasing concern about the presence of drug residues in animal products has led to a renewal of interest in the use of plant based drugs [7]. Somewhere else, the main portion of the population infected in rural area does not have access to these drugs. In this way, it becomes very important to look for others methods for helminths control that can be less aggressive and more accessible like the used of medicinal plant. Medicinal plants with anthelminthic properties are cheaper and effective against helminthes and they have the advantage to be less toxic and largely biodegradable [7]. According to traditional practitioners of the Menoua Division, West Region of Cameroon, *Alafia barteri* used in this study has many therapeutic properties like anthelminthic and antimalarial properties. Consequently, the aim of this work was to evaluate *in vivo* anthelminthic activities of *A. barteri* (Apocynaceae) on *H. bakeri* through the fecal egg count reduction (FECR), total worm count reduction (TWCR) and through the inhibition of *H. bakeri's* eggs taken from treated animals.

2. MATERIALS AND METHODS

2.1 Plant Material

Alafia barteri is a climber plant belonging to the Apocynaceae family and widely distributed in Central and West Africa. In Nigerian traditional medicine, this plant is used against rheumatism, toothache, sickle cell anemia and fever [8,9]. In Cameroon, stem bark of this plant are chewing associated with groundnut as vermicide. The plant has been collected in Santchou Reserve Forest, in the Menoua Sub-Division, Cameroon West Region. It has been identified in the Cameroon National Herbier by comparing to the reference specimen N°30575CNH. Once in the labs, bark was isolated from the stem and dry in shade for 2 weeks. After that, the dry bark was crushed and the powder obtain was conserved in the laboratory inside a plastic bag for further used [10].

2.2 Preparation of Extracts

2.2.1 Ethanolic extract

Inside a 10 L glass, 5 L of ethanol 95% was added to 400g of plant powder and the mixture was stirred twice a day. Past 3 days, the mixture

was filtered using filter paper and the solution obtained was introduced inside an evaporator heated at 79°C to evaporate ethanol. After that, the extract inside the plat was placed in an oven heated at 50°C for 2 days to evaporate the remaining ethanol. This drying ethanolic extract obtain was weight and conserved [11].

2.2.2 Aqueous extract

Five Litter of boiled water was introduced inside a glass containing 400g of plant powder. The mixture was stirred and after 3 hours, it was filtered using sieve and filter paper. The solution obtained was introduced in the plate and put inside an oven heated at 50°c for 4 days to allow the complete evaporation of water. The dry aqueous extract obtained was weight and conserved in the labs according to usual procedure in the LABEA [12].

2.3 Doses Preparation

Based on the therapeutic dose, doses 980 mg/kg, 1960 mg/kg and 3920 mg/kg was retained in this work. Since the treatment had to be administrated in the drinkable water, concentrations of 6. 125 mg/ml, 12. 25 mg/ml and 24. 5 mg/ml were obtained respectively. In fact, 3 grams of dry extract was weight and dissolved in the mortar with 1. 5 ml dimethyl sulfoxide (DMSO) (for ethanolic extract) to facilitate the solubility of extract in the water. Water was then added to obtain a volume of 122. 4 ml solution. For doses 3920 mg / kg, 1960 mg / kg and 980 mg / kg, 4 ml, 2 ml and 1 ml of this solution were respectively taken and introduced inside the mice's drinking bottles. When necessary, water was added to avoid animal dehydration. To be sure that all animal would take his dose of product, the disposition was one animal per cage and the treatment lasted for 7 days. The standard dose of Albendazol used was 15 mg/kg corresonding to 0.28 mg/ml concentration.

2.4 Animal Material

The animal used consisted of laboratory white mouse of the Swiss strain (*Mus musculus*) and the gastrointestinal parasite of mouse (*H. bakeri*). *H. bakeri* is a popular laboratory model providing a tractable experimental system that is easy to maintain in the laboratory and far more cost-effective than other laboratory nematode – rodent model systems [13]. Sixty white mice both male and female, of 6 weeks and weighing between 18-25g were used. Mice have been breeding in standard condition in the RUBAE laboratory. Food and water was given *ad libitum*.

2.4.1 Obtaining of L_3 infective larva of *H. bakeri*

H. bakeri L_3 infective larva was obtained from experimentally infected mice in the RUBAE laboratory. Methods describe by Smyth [14] was used. Three grams of infected mice's fresh feces has been collected and crush in the mortar. Nine milliliters water was added before spread the paste inside the petri-dish. After that, this device was left in the lab for 7 days. Relative humidity in the lab was 65-67%. Past this period, L_3 infective larva was collected using Baermann device.

2.4.2 Infestation of mice with larvea

L_3 larva obtain was used to infest sixty mice. For the preparation of the inoculum, 30 ml of larval solution was collected and put inside a bottle with conic base. Then 0.1 ml of this solution was withdrawn and placed inside the cover of a Petri dish. The Petri dish was placed on top of the solution to immobilize the larvae which were then counted. Water was either added or removed until solution containing 13 to 15 L_3 larvae was obtained. This concentration is necessary to produce an optimal infestation in the host [15]. After 11 days, qualitative analysis was realized to detect infestation. Mice were isolated individually in a cage. After 10-20 minutes, feces were collected and crush in the mortar. For 2g of feces, 60ml of salt solution was added for floatation [3]. Suspension was then filtered using a 150 µm stitch sieve. Solution obtained was used to filled tube in top of which a slide was placed for 10 minutes. After that, slide was removed and observed under the microscope (objective 10 x). *H. bakeri* eggs were identified by the egg shape, irregular pole and blastomers occupying the whole egg's cytoplasm [6].

2.4.3 Treatment of animals

For each extract, 25 infected mice of 6 weeks and weighing 18-25 g were used. These mice were divided into 5groupes of 5 animals each [16]. Group 0 served as negative control and received distilled water or DMSO 1.24% depending on the test. Group 1served as positive control and received 15 mg / kg bwt of Albendazol. Animals of the three others groups (G2, G3, G4) received respectively 1ml, 2ml and 4ml of the extracts solutions.

2.5 Evaluation of the Fecal Egg Count Reduction (FECR) of *H. bakeri*

This was done before, during and 2 weeks after treatment using the quantitative method of McMaster. Animals whose qualitative analysis confirms the presence of *H. bakeri*'s eggs in the feces where individually isolated for 10-20minutes. Their feces were collected, weighing and crush in the mortar. Sixty milliliter of 40% saline solution was added to 2 g of feces. After homogenization, the solution was filtered and the filtrate was used to fill McMaster cells using a pipette. After 5 minutes, this preparation was observed in the microscope for eggs count [17]. Eggs count inside the two cavity of McMaster was used to obtain EPG (eggs per gram of feces) by the following formula:

$$EPG = X.200 \quad \text{where} \quad X = \frac{\text{total eggs counted}}{\text{total of cavity counted}} \quad (1)$$

The fecal eggs count reduction rate (FECR) was calculated using the formula of Coles et al. [18]:

$$FECR\ (\%) = \left(1 - \frac{T}{C}\right) x100 \quad (2)$$

Where T and c are geometric mean of EPG in treated groups and in negative control group respectively.

2.6 Determination of the Total Worm Count Reduction (TWCR)

In the 15 days after treatment, 3 animals randomly taken in each group were sacrificed to obtain worm load [15]. Mouse inside the bottle was anesthetized by introducing them inside a bottle with 40% formalin in the cotton. The bottle was then locked for 1-2 minutes. After that, asleep mouse were dorsally attached to a polystyrene support by fixing it member with needle. The abdominal cavity was open to remove intestine from pylori to 1 cm from the anus. This portion was open longitudinally and the content was put inside the Petri dish. After washing, the remaining suspension was observed to the binocular loupe for worm count. The TWCR was obtained using the Enriquez [19] formula:

$$TWCR = \frac{MIPc - MIPt}{MIPc} \times 100 \quad (3)$$

Where MIPc is the mean intensity of parasite in the negative control group and MIPt is the mean intensity of parasite in the treated group.

2.6.1 Effect of the extracts treatments in the development of *Heligmosomoïdes barteri*'s eggs

Before the sacrifice, coproculture was realized to detect the effect of the treatment in the development of *Heligmosomoïdes barteri*'s eggs taken from treated animals. For that, 0.11g of feces was collect from the animals treated with 1960 mg/kg dose (optimal anthelminthic effect) and from negative control group. After crush, water was added to form a paste. This paste was spread inside the Petri dishes having a covered base by filter paper. This activity was repeated 5 times for each group. After 7 days, L3 larva was collect using Baermann device and counted under the microscope. To obtain the larva development rate (LDR), the following formula was used:

$$LDR = \frac{number\ of\ L3\ larva\ in\ traeted\ group}{number\ of\ L3\ larva\ in\ the\ negative\ control\ group} x100 \quad (4)$$

2.7 Statistical Analysis

Results were presented as average ± standard deviation. TECR, TWCR and LDR of different groups were submitted to analyses of variance with one or two factors. Waller Duncan test was used to separate means at 5% probability. For these analyses, SPSS version 22.0 was used.

3. RESULTS

Anthelminthic activities of *A. barteri* stem bark was evaluated on three parameters namely fecal egg count reduction, worm load and the development of eggs taken from treated animal.

3.1 Effect of Treatments on the Fecal Egg Count Reduction (FECR)

3.1.1 Effect of aqueous extract of *Alafia barteri* on the FECR

Table 1 shows how the fecal eggs concentrations varies depending on the products (aqueous extract and Albendazole), doses, and treatment period.

Table 1. Variation of the fecal egg concentration (of *Heligmosomoides bakeri* ± standard deviation) and the fecal egg count reduction rate (FECR) during and after administration of Albendazole and aqueous extract of *Alafia barteri*

Products	Doses (mg /kg)	Treatment period	Post-treatment period	
		Egg concentration in the feces ± SD (FECR rate ± SD)		
		week 1	week 2	week 3
Distilled water (negative control)	-	38695.39 ± 26943.36[a] (0 ± 0)[*]	45577.22 ± 25187.21[a] (0 ± 0)[*]	53038.31 ± 26901.86[a] (0 ± 0)[*]
Albendazole (positive control)	15	5202.77 ± 5494.36[b] (86.55 ± 14.2)[#]	3757.49 ± 2834.18[b] (91.76 ± 6.22)[#]	5919.18 ± 6579.19[b] (88.84 ± 12.40)[#]
Aqueous extract	980	6520.88 ± 4393.81[b] (83.15 ± 11.35)[#]	34567.82 ± 30544.4[a] (24.16 ± 67.02)[*]	23279 ± 26899.59[b] (56.11 ± 50.72)[#]
	1960	4871.51 ± 3819.35[b] (87.41 ± 9.87)[#]	26197.44 ± 14621.73[ab] (42.52 ± 32.08)[*]	27173.12 ± 16056.17[ab] (48.77 ± 30.27)[#]
	3920	10031.98 ± 7339.25[b] (74.07 ± 18.97)[#]	34672.31 ± 13834.61[a] (23.93 ± 30.35)[*]	29646.97 ± 19378.82[ab] (44.10 ± 36.54)[#]

"a, b" compare values inside the same column. Values having the same letter in their top inside the column are not significantly different (P > 0. 05). ", #" compare values inside the same column. Values having the same sign in their top inside the column are not significantly different (P > 0. 05)*

From this table, it can be observed that the fecal egg count in the negative control group was gradually increasing during all the experimental period. During the first week after treatment, a significant difference (P < 0.05) was observed between fecal egg count reduction of treated animals and fecal egg count reduction of the negative control animals. Very significant FECR rate (87%) appeared only during the treatment period inside the group treated with 1960 mg/kg plant infusion. During this treatment period, Albendazole showed a FECR rate of 86.55% lesser than 87.41% obtained with plant extract at 1960 mg/kg, even that the difference was not significant (P > 0. 05) So that, during the treatment period, the plant effectiveness was not dose dependent. After the treatment, a fluctuating drop of the FECR rate was observed with the entire treated group.

During the first week after treatment, a significant drop of the fecal egg concentration was observed inside the entire animals groups treated with plant extract. The lesser FECR rate (23.93 %) was obtained with the highest dose (3920 mg/kg) while the most important FECR rate (91.76 %) obtained one week after treatment was with albendazole.

At the second week after treatment, there was a light drop observed with albendazole (88.84 %), light growth of FECR rate was also noticed with the aqueous extract, particularly at the weak dose of 980mg/kg (56.11 %).

3.1.2 Effect of *Alafia barteri* ethanolic extract on the FECR

Table 2 shows how the fecal eggs concentrations varies depending on the products (ethanolic extract and Albendazole), doses, and treatment period.

According to ethanolic extract, FECR rate increases with time at the entire doses. The most prominent FECR rate (91.36%) was observed in the second week after treatment with the administration of 1960mg/kg of ethanolic extract. A difference (P < 0. 05) was observed at this last week of the experimentation between the fecal egg concentration in animals treated with plant extract and those of animals of the negative control groups. Albendazole showed a maximal reduction rate of the fecal egg concentration of 91.76% at the first week after treatment. The treatment showed a non-dependent dose effect.

3.2 Effects of Treatments on the Parasitic Load (TWCR rate)

After evaluation of the reduction rate of the fecal egg concentration, three animals were taken per group and sacrificed for the determination of the parasitic load.

Table 2. Variation of the fecal egg concentration (of *Heligmosomoides bakeri* ± standard deviation) and the fecal egg count reduction rate (FECR) during and after administration of Albendazole and ethanolic extract of *Alafia barteri*

Products	Doses (mg / kg)	Treatment period	Post-treatment period	
		Fecal egg concentration ± SD (FECR rate ± SD)		
		week 1	Week 2	Week 3
DMSO 1.24% (negative control)	-	37089.54 ± 9813.90^{a} (0 ± 0)*	35918.69 ± 19948.87^{a} (0 ± 0)*	32120.02 ± 8651.68^{a} (0 ± 0)*
Albendazole (positive control)	15	5202.77 ± 5494.36^{b} (86.55 ± 14.2)$^{\#}$	4164.81 ± 3255.02^{b} (91.76 ± 6.22)$^{\mu}$	5919.18 ± 6579.19^{b} (88.84 ± 12.40) $^{\mu}$
Ethanolic extract	980	35366.82 ± 29956.04^{a} (4.64 ± 80.77)*	30234.96 ± 29706.28^{a} (15.82 ± 82.70)$^{*\#}$	11796.5 ± 9090.72^{b} (63.27 ± 28.30)$^{\#}$
	1960	14958.51 ± 6718.81^{ab} (59.67 ± 18.11)$^{*\#}$	11674.49 ± 8278.74^{ab} (67.5 ± 23.05)$^{\# \mu}$	2775.44 ± 2785.22^{b} (91.36 ± 8.67) $^{\mu}$
	3920	25611.37 ± 15657.02^{ab} (30.95 ± 42.21)$^{*\#}$	18309.57 ± 15860.96^{ab} (49.02 ± 44.16)$^{*\# \mu}$	10075.71 ± 7305.23^{b} (68.63 ± 22.74)$^{\# \mu}$

*"a, b, *, #" compare values inside the same column. Values having the same letter or the same sign in their top, inside the column are not significantly different (P > 0, 05)*

3.2.1 Effects of the aqueous extract on the TWCR rate

The variation of the average parasitic load as well as the variation of the TWCR rate is presented in Table 3, depending on doses and treatments (albendazole and aqueous extract).

From this Table III, we noticed that the parasitic load of animals treated with 980 mg/kg of the infused extract is comparable (P > 0.05) to that of animals treated with albendazole. Moreover, the prominent TWCR rate was 89.89% obtained at the dose 980 mg/kg. That rate was relatively higher than the rate obtained with the reference drug (86.52%) (P = 0.05). So, in groups treated with aqueous plant extract, the effect was inversely proportional to the dose.

3.2.2 Effects of the ethanolic extract on the TWCR

The variations of the average parasitic load as well as the variation of the TWCR rate are presented in Table 4, depending on doses and treatments (albendazole and ethanolic extract).

Table 3. parasitic load variation of *Heligmosomoides bakeri* ± SD and the TWCR rate after treatment with Albendazole and aqueous extract of *Alafia barteri*

Products	Doses (mg/kg)	Average parasitic load ± SD (TWCR ± SD)
Distilled water (negative control)	-	29.67 ± 32.35^{a} (0 ± 0)*
Albendazole (positive control)	15	4 ± 2^{b} (86.52 ± 6.74)$^{\#}$
Aqueous extract	980	3 ± 1.73^{b} (89.89 ± 5.84)$^{\#}$
	1960	20.67 ± 15.30^{a} (30.34 ± 5.6)$^{*\#}$
	3920	28 ± 14.73^{a} (5.62 ± 4.65)$^{*\#}$

"a, b,,#" are used to compare values inside the column. Values having the same letter or the same sign in their top, inside the column are not significantly different (P > 0, 05)*

Table 4. parasitic load variation of *Heligmosomoides bakeri* ± SD and the TWCR rate after administration of Albendazole and ethanolic extract of *Alafia barteri*

Products	Doses (mg/kg)	Average parasitic load ± SD (TWCR ± SD)
DMSO 1.24 % (Negative control)	-	20 ± 17.09^{a} $(0 \pm 0)^{*\#}$
Albendazole (positive control)	15	4 ± 2^{b} $(86.52 \pm 6.74)^{\#}$
Ethanolic extract	980	14 ± 10.58^{a} $(30 \pm 52.92)^{*\#}$
	1960	18.67 ± 9.71^{a} $(6.67 \pm 48.56)^{*\#}$
	3920	22 ± 15.72^{a} $(-10 \pm 78.58)^{*}$

"a,b,,#" are used to compare values inside the column. Values having the same letter or the same sign in their top, inside the column are not significantly different (P > 0, 05)*

Table 5. L_3 larval development rate ± SD after the exposition of eggs to 1960 mg/kg dose of plant extracts (aqueous and ethanol)

Products	Dose (mg/kg)	Average number of L_3 larvae obtained	Larval development rate (in %) ± SD
Negative control	-	147.67 ± 82.45^{a}	$100 \pm 0.0^{*}$
Aqueous extract	1960	75.2 ± 52.75^{b}	$50.93 \pm 35.72^{\#}$
Ethanolic extract		125 ± 75.84^{a}	$84.65 \pm 51.36^{*\#}$

"a, b,,#" are used to compare values inside the column. Values having the same letter or the same sign in their top, inside the column are not significantly different (P > 0, 05)*

From this Table 4, we notice that albendazole reduced efficiently the parasitic load in mice with 86.52% of TWCR rate, far from the best result of 30 % reduction rate obtained with plants extracts at 980 mg/kg bwt. As in the case of the treatment with the aqueous extract, the reduction of the parasitic low was not dose dependent. It even seem like the highest dose (3920 mg/kg) did not killed parasites or may had contributed to their multiplication.

3.3 Effect of the Treatments on the Development of Eggs taken from Treated Animals

The average number of L_3 lava as well as the larval development rate noticed in negative control group and in the group treated with 1960 mg/kg dose of plant product is presented in Table 5.

From this table, it stands out that the egg development rate until the L_3 larval stage inside the negative control group was 100% which is significantly different ($P < 0.05$) from the development rate of 50.93% obtain with aqueous extract. So, we can conclude that eggs laid by parasite of treated mice do not developed optimally.

4. DISCUSSION

The objective of this work was to evaluate the Anthelminthic effect of *Alafia barteri* plant products (aqueous and ethanolic extracts) in certain parameters which are the fecal egg concentration of *H. bakeri* eggs, the parasitic load and the inhibitory effect of the plant on eggs taken from treated animals.

The infused aqueous extract of *A. barteri* presented a fleeting anthelminthic activity when animals take the treatment. During the treatment period, the maximum fecal egg reduction rate was 87.41% obtained with the dose 1960 mg / kg bwt. After the treatment period, the efficacy was compromised for all doses even that a slight increase was observed during the last week of the experimentation. Similar result was obtained by Hounzangbe et al. [20] who found a fleeting activity of 92 % in the tenth day after treatment of sheep with 400 mg / kg bwt of papaya seed powder. Alafia barteri is a creeper plant rich in tannins, phenolic acid, flavonoids, reduction sugar, steroids, anthraquinones, alkaloids, triterpenoïds and saponines components [21]. According to Andrew et al. [22], some of these metabolites like tannins have anthelminthic properties. The anthelminthic activities of a plant depend not only on the type of extraction solvent but also on the quality and the quantity of product obtained after extraction [23]. This fleeting anthelminthic activity obtained with the aqueous extract can be explained by the fact that water has extracted mainly hydro-soluble components like flavonoïds (heterosides), monoterpens

(iridoïdes) and tannins [24] in very low proportion. These components (tannins particularly) may have interfere with coupled oxidative phosphorylation, thus blocking ATP synthesis in the parasites [7]. Moreover, the delusive activity observed with that aqueous extract can be an accommodation strategy of the parasite. In fact, as many other parasites, gastrointestinal nematodes are species of the "r" adaptative strategy (small size, high prolificity, short life span, vulnerable, rapid sexual intercourses).

At the third week representing the last week of the test, the fecal egg count reduction rate has slightly increase, evidence that some parasites died hence the reduction of the number of eggs in the mice's feces. This could also be as a result of the fact that during treatment, female parasites may have reduced their laying frequency so as to use their energy to fight against the effect of the drugs. So after the treatment, they just assume their normal rate of egg-laying.

After the aqueous extract, the FECR rate was evaluated with ethanolic extract. Results showed a time dependent but not dose dependent effect. The maximal rate of the fecal egg count reduction of 91.36% was observed with the intermediate dose (1960 mg / kg bwt) during the last week of experimentation. This result is similar to the one obtained by Azando et al. [25] who founded that the treatment at 3,2 g/kg had the similar result with the treatment at 4,8 g/kg, in their study on the anthelminthic activity of *Zanthoxylum zanthoxyloïdes* in gastro-intestinals nematodes. The anthelminthic activity of the ethanolic extract can be attributed to the ethanol's capacity to extract at the same time hydrophilic and hydrophobic compounds which among others are tannins, phenolic acids, flavonoïds, steroids, alkaloids, triterpenoïds and saponines [21]. These components may have acted conjointly to produce the observed effect. The main question at this level is to know how each compound has acted. From all evidence, the action of ethanolic extract can be related to it phenolic compounds because, according to Cosme et al. [26], these phenolic compounds are the most widespread secondary metabolites of the plant kingdom and are known for their important role in parasitic control. Tannins may fix their self on proteins and may act by many mechanisms. In vivo, they may act by creating a hostile gastro-intestinal environment reducing the parasite fecundity [27,28].

Another group of phenolic compound, flavonoids may produce oxygenated substances creating an oxidant stress in parasite [29]. So the anthelminthic activity of a plant is a function of it richness in phenolic compound because they may mimic the action of their synthetic homologue by disturbing the reproductive system of the parasite [29]. Beside these phenolic compounds, Al-Shaibani et al. [27] showed in their works that alkaloids present in plant may have the capacity to diffuse through the cuticle and to slip in the process of parasites' DNA synthesis. Moreover, terpenoïds contained in *Alafia barteri* may play an important anthelminthic activity. In fact, according to Yondo [29], these bioactive molecules may act in the *H bakeri* cellular membrane by inducing structural changes of the internal compartment, resulting in the destruction of organic cells such as mitochondria which is the main site of energy production of the parasite.

Let's note elsewhere that the efficacy was not dose-dependent because in the second week after treatment, the fecal egg count reduction rate (68.63%) at the dose 3920 kg/kg bwt was less than the rate obtained with the dose 1960 mg/kg bwt (91.36%). This is similar to the study conducted by Mpoame and Essomba [30] who, evaluating the effect of papaya seeds on gastro-intestinal parasite of chicken revealed that there was no significant difference between the efficacy in the dose 5 g/l and 10 g/l of aqueous decoction.

In fact, the product administrated in a very few quantity may have diffused through the receptors of the cuticle such that it has attack the parasite. Nevertheless, when the product arrived in a very high quantity than the number of available receptors, these receptors may become saturated making the diffusion difficult and by that fact reducing the plant effect. Finally, we can also say that the increase reduction of the fecal egg concentration observe during this experimentation can be caused by the mortality of adults parasites or the inhibition of egg-laying in female worms [25].

After the sacrifice, the effect of *A. barteri* extract was evaluated on the viability of adult worms. The parasitic low reduction rate was not dose dependent. That rate was highest with the lowest dose (980 mg/kg). Similar observation was done by Azando et al. [25]. This could be due to the fact that bioactive compounds seem to diffuse easily through the cuticle of worms; that passage

may have disturbed the metabolism of parasite producing a stressing effect on parasites [31]. Under the effect of stress, the survivorship may have made worms to relieve their propagule to ensure the perennity of their species [32].

In highest dose, the inverse phenomenon take place; that means there is an increase of the fecal egg count reduction rate and a decrease of the parasitic low reduction rate. This result reflects the effect of the worm population density on the egg production. That can be explained by the theory of mass effect put into evidence by Smyth and McManus [33]. According to that theory, as the number of worm present in a host is high, in the same way the number of egg lays by each worm if low, that can explain the low fecal egg count reduction rate and the high parasitic burden reduction rate observed [34].

5. CONCLUSION

At the end of this study, we can conclude that aqueous extract had a fleeting activity when animals where receiving treatment. The maximums FECR and FWCR were respectively 87.41% in the dose 1960 mg/kg and 89.89% in the dose 980 mg/kg. The larval development rate obtained with this extract was 77.53%. With ethanolic extract, encouraging activities was observed during the test with the maximal FECR rate of 91.36% in the end of the experiment with the dose 1960 mg/kg. The maximum FWCR rate was 30 % in the dose 980 mg/kg and the larval development rate was 51.48%. Albendazole shows a FECR rate of 91.76% and a FWCR rate of 86.32%. These results confirmed that *Alafia barteri* can be retained among alternatives solutions for the control of gastrointestinals nematodes. Thus, deep study is necessary to determine the toxicological effect of the plant.

ACKNOWLEDGEMENT

We wish to thank all the members of the Research Unit of biology and applied ecology for their multifaceted supports during tests realization.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Khan Payne V, Lontuo FR, Ngangnang GR, Megwi L, Mbong E, Yamssi C, Bamou R, Mpoame M. Prevalence and Intensity of Infection of Gastro-Intestinal Parasites in Babadjou, West Region of Cameroon . International Journal of Clinical and Experimental Medical Sciences. 2017;3(2):14-22.
   DOI: 10.11648/j.ijcems.20170302.11
2. Nkouayep VR, Nejsum P, Dzune FDC, Noumedem ACN, Atiokeng TRJ, Mpoame, M. Prevalence and Risk Factors of Infection with Soil Transmitted Helminths in Children from Bandjoun, the West Region of Cameroon. International Journal of Tropical Disease & Health. 2020; 41(17): 34-43.
   DOI: 10.9734/ijtdh/2020/v41i17303773
3. Matsinkou MRR, Yamssi C, Mbong EM, Noumedem ACN, Tateng NA, Megwi L, and Vincent KP. Intestinal Helminth Infections and Associated Risk Factors among School-Aged Children of Bamendjou Community, WestRegion of Cameroon. Journal of Parasitology Research. 2021; 8.
4. World Health Organization. Guideline: preventive chemotherapy to control soil-transmitted helminth infections in at-risk population groups. 2017; 87 P.
5. Maoxuan L, Sujogya KP, Walter L. Plant-Based Natural Products for the Discovery and Development of Novel Anthelmintics against Nematodes. Biomolecules. 2020; 10:426.
6. Onyilofe S, Enejoh Khadijah Shaibu, Mohammed M, Suleiman, Oseph O, Ajanusi. Evaluation of anthelmintic efficacy of Citrus aurantifolia fruit juice against *Heligmosomoides bakeri*. International Journal of Advanced Biological Research. 2015;5(1):5-10.
7. Selamawit Z, Teka F, Solomon A. In Vitro Anthelmintic Activity of Crude Extracts of Aerial Parts of Cissus quadrangularis L. and Leaves of Schinusmolle L. against *Haemonchus contortus*. BioMed Research International. 2017; 6 P.
8. Margaret OS, Essien I, Chidebelu E, Flora R, Aigbe Abidemi JA. Antinociceptive and anti-inflammatory activities of ethanolic extract of *Alafia barteri*. Brazilian Journal of Pharmacognosy. 2014;24:348-354.
   Available:https://doi.org/10.1016/j.bjp.2014.07.013

9. Kutama AS, Dangora II, Aisha W, Auyo MI, Sharif U, Umma M, Hassan K Y. An overview of plant resources and their economic uses in Nigeria. Global Advanced Research Journal of Agricultural Science. 2015;4:042-067.
Available:https://doi.org/10.1155/2020/7059323
10. Wabo Poné J, Payne VK., Mbogning Tayo G, Komtangi MC, Yondo J, Ngangout AM, Mpoame M, Bilong Bilong CF. In vitro anthelminthic efficacy of *Dichrocephala integrifolia* (Asteraceae) extracts on the gastro-intestinal nematode parasite of mice: *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). Asian Pacific Journal of Tropical Biomedicine. 2013; 3(2): 100-104.
11. AsanteKwatia E, Jibira Y, Mensah AY, Osei-Sarfoh D. *Macaranga barteri* stem bark extract exerts anti-inflmmatory and anti-hyperalgesia activity in murine models. Discovery Phytomedicine. 2019; 6(3):130-137.
DOI: 10.15562/phytomedicine.2019.104
12. Etung kN, Wabo PJ, Payne VK, Yondo J, Komtangi MC, Mpoame M. Bilong BCF. In vitro Comparative Effect of Aqueous (Cold and Hot Water) and Ethanolic Extracts of the Seeds of *Aframomum Danielli* (Zingiberaceae) on Three Life Cycle Stages of the Parasitic Nematode *Heligmosomoides Bakeri* (Nematoda; Heligmosomatidae), Parasite of the Laboratory Mice (*Mus Musculus*). Medicinal & Aromatic Plants. 2012;1(7):5.
13. Harris NL, Pleass JR, Behnke JM. Understanding the role of antibodies in murine infections with *Heligmosomoïdes* (polygyrus) *bakeri*: 35 years ago, now and 35 years after. Parasite Immunology. 2013; 36(3).
DOI: 10.1111/pim.12057
14. Smyth JD. Animal parasitology. Cambridge university press, Great Britain. 1996;549.
15. Wabo Poné J. Effets des extraits de Canthium mannii Hein, 1877 (Rubiacées) sur deux nématodes parasites strongylidés: *Ancylostoma caninum* Ercolani, 1859 et *Heligmosomoides Polygyrus* Dujardin, 1845. Thèse de doctorat en Biologie Animale, Université de Yaoundé 1. 2007;145.
16. Tsala DE, Penlab BV, Nnanga N, Mendimi NJ, Kouamouo J, Dimo T. Protective activities of the stem bark methanol extract of *Alafia multiflora* against carbon tetrachloride-induced hepatotoxicity in rats. International Journal of Pharmaceutical Sciences Review and Research. 2010; 3(2):157-163.
Available: www.globalresearchonline.net
17. Thienpont D, Rochette F, Vamparijs OFJ. Diagnostic de verminose par examen coprologique. Jansen Research Foundation, Beerse. 1979;187.
18. Coles GC, Bauer C, Borgsteede FH, Greerts S, Klei TR, Taylor MA, Waller PJ. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary parasitology. 1992;44:35-44.
doi: 10.1016/0304-4017(92)90141-U
19. Enriquez JB. Les médicaments anthelminthiques utilisés en médecine des carnivores domestiques. Activité et toxicité. Recueil de Médecine Vétérinaire. 1993; 189(5/6):499-512.
20. Hounzangbe-Adote MS, Zinsou FE, Affognon KJ, Koutinhouin B, Adamou N'Diaye M, Moutairou K. Efficacité antiparasitaire de la poudre de graines de papaye (*Carica papaya*) sur les strongles gastro-intestinaux des moutons Djallonké au sud du Bénin Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux. 2001;54(3-4):225-229.
Available :https://doi.org/10.19182/remvt.9778
21. Lasisi AA, Olayiwola MA, Balogun SA, Akinloye OA, Ojo DA. Phytochemical composition, cytotoxicity and in vitro antiplasmodial activity of fractions from Alafia barteri olive (Hook F. Icon)-Apocynaceae. Journal of Saudi Chemical Society. 2016;20:2-6.
DOI: 10.1016/j.jscs.2012.05.003
22. Andrew RW, Honorata MR, Christos F, Olivier D, Irene M-H, Stig MT. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of *Oesophagostomum dentatum*. Parasites & Vectors. 2014;7:518.
Available: https://doi.org/10.1186/s13071-014-0518-2
23. Krishnananda PI, Amit GD, Dipika AP, Mahendra SD, Mangesh PM, Vaibhav CK. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. Journal of

Pharmacognosy and Phytochemistry. 2017;6(1):32-36.
Available : www.phytojournal.com

24. Yezza S, Bouchama S. Index des métabolites secondaires végétaux. Projet de fin d'Etudes en vue de l'obtention du diplôme de Licence en Sciences de la nature et de la vie. Université Kasdi Merbah, Ouargla. 2014 ; 73P.
25. Azando EVB, Olounlade AP, Hounzangbe-Adote, Hoste H. Effet anthelminthique in vivo de la poudre de feuilles de *Zanthoxylum zanthoxyloïdes* et de *Newbouldia laevis* sur les nématodes parasites gastro-intestinaux des chevreaux Djallonké. International Journal of Biological and Chemical Sciences. 2011; 5 (3):1054-1062.
DOI: 10.4314/ijbcs.v5i3.72208
26. Cosme P, Rodríguez AB, Espino J, Garrido M. Plant Phenolics: Bioavailability as a Key Determinant of Their Potential Health-Promoting Applications. Antioxidants 2020; 20.
27. Al-Shaibani IRM, Phulan MS, Shiekh M. Anthelminthic activity of *Fumaria parviflora* (Fumariaceae) against gastrointestinal nematodes of sheep. International Journal of Agriculture and Biology. 2009;11:431-436.
28. Perla MC A-R, Claudia H-C, Iván F-P, Fernando A-H, María Berenit M-G, Rubén B. Nematicidal Effect and Histological Modifications Induced by Hydrolysable Tannin Extract on the Third-Stage. Infective Larvae of *Haemonchus contortus*. Biology. 2020;9 (442):1-12.
29. Yondo J. Propriétés anthelminthiques et antioxydantes des extraits aqueux et au mélange méthanol/chlorure de methylène des écorces de *Pseudospondias microcarpa* Engl. 1877 (Anacardiaceae), *Schumanniophyton magnificum* Harms 1897 (Rubiaceae) et *Rauvolfia Vomitoria* Afzel. 1817 (Apocynaceae). Thèse de Doctorat, Département de Biologie Animale, Université de Dschang, Cameroun. 2014 ;212.
30. Mpoame M, Essomba LI. Essai de traitement contre les parasitoses gastro-intestinales du poulet avec des décoctions aqueuses de graines de papaye (*Carica papaya*). Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux. 2000; 53:23-25.
DOI : 10.19182/REMVT.9761
31. Yondo J, Komtangi M-C, Wabo Poné J, Bilong Bilong CF, Kuiate JR, Mpoame Mbida. Nematicidal efficacy of methanol/methylene chloride extract of *Rauwolfia vomitoria* (Apocynacae) on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae) parasite of the white mouse (*Mus musculus*). Journal of Medicinal Plant Research. 2013;7(34): 3220-3225.
Available:http://www.academicjournals.org/artic...
32. West SA, Gemmill AW, Graham A, Viney ME, Read AF. Immune stress and facultative sex in a parasitic nematode. Journal of Evolutionary Biology. 2001;14:333-337.
Available: https://doi.org/10.1046/j.1420-9101.2001.00266.x
33. Smyth JD, McManus DP. The Physiology and Biochemistry of Cestodes. Cambridge: Cambridge University Press. 1989;398.
34. Gofarana W, Sri AFK, Yusuf R, Fairuz A. In Vivo anthelminthics activities of ethanol Extract of Croton (*Codiaeum variegatum* L. Blume) against Tapeworm *Hymenolepis microstoma*. Scholars Academic Journal of Pharmacy. 2014; 3(2): 108-115.
Available: www.saspublisher.com

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/91981