

Anthelmintic activities on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae) of *Alafia barteri*'s stem bark (Apocynaceae) administrated in the form of drinkable suspension of extracts (aqueous and ethanolic)

Abstract

Due to the resistance of helminths parasites to synthetic drugs, the International community looks for news methods of controlling gastrointestinal helminths like the traditional use of medicinal plants with anthelmintics 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*. To achieve this aim, mice were orally infected with L₃ infective larva of the parasite. 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 the unique dose of 15 mg / kg body weight of Albendazole. The three other groups (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 days. The efficacy of *A. barteri* was evaluated indirectly through the fecal egg count reduction (FECR) and directly through the total worm count reduction (TWCR). Moreover, the anthelmintic effects of the plant were assessed on the development of eggs taken from treated animals. Results showed that globally, effects of treatments were non-dose dependent. The plant showed a considerable anthelmintic effect on all parameters studied. But, ethanolic extract exhibited the highest anthelmintic activity in the reduction of the fecal eggs with 91.36 % reduction obtained 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 %). Albendazole had FECR and TWCR of 91.76 % and 86, 52 % respectively. Then we can conclude that ethanolic extract exhibited the highest anthelmintic activity in this study. From the global results obtained, we can say that *A. barteri* has compounds with vermifugal anthelmintics properties.

Key words: anthelmintic activity, *Alafia barteri*, *Heligmosomoides bakeri*

Introduction

Parasitic infections constitute a major health problem in tropical and subtropical zones (Khan Payne *et al.*, 2017). Among these infections, helminthes infections remain a serious public health problem (Nkouayep *et al.*, 2020). They are disease 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 (Matsinkou *et al.*, 2021). 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 (WHO, 2017). Apart the fact that they have chronic and insidious symptoms, helminthiasis are able to breakdown the health state 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 (Maouxuan *et al.*, 2020). Moreover, helminthiasis also represent the most important problem affecting the productivity of livestock with important economic losses like the death of highly infected animals (Onyilofe *et al.*, 2015). Anthelmintic control is done by the used of synthetic anthelmintic 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 (Selamawit *et al.*, 2017). 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 anthelmintic properties are cheaper and effective against helminthes and they have the advantage to be less toxic and largely biodegradable (Selamawit *et al.*, 2017). According to traditional practitioners of the Menoua Division, West Region of Cameroon, *Alafia barteri* used in this study has many therapeutic properties like anthelmintic and antimalarial properties. Consequently, the aim of this work was to evaluate *in vivo* the anthelmintic 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.

Materials and methods

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 (Margaret *et al.*, 2014; Kutama *et al.*, 2015). 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 Herbarium 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 obtained was conserved in the laboratory inside a plastic bag for further use (Wabo Poné *et al.*, 2013).

Preparation of extracts

Ethanol 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 plate was placed in an oven heated at 50°C for 2 days to evaporate the remaining ethanol. This drying ethanolic extract obtained was weighed and conserved (Asante-Kwatia *et al.*, 2019).

Aqueous extract

Five L 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 weighed and conserved in the labs according to usual procedure in the LABEA (Etung *et al.*, 2012).

Doses preparation

Based on the therapeutic dose, doses 980 mg/kg, 1960 mg/kg and 3920 mg/kg were retained in this work. Since the treatment had to be administered in the drinkable water, concentrations of 6,125mg/ml; 12, 25 mg/ml and 24,5mg/ml according respectively to these

doses were obtained. In fact, 3g of dry extract was weight and dissolved in the mortar with 1.5ml 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 any 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 corresponding to 0,28 mg/ml concentration.

Animal material

Animal materials were made up of laboratory white mouse (*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 (Harris *et al.*, 2013). 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*.

Obtaining of L₃ infective larva of *H. bakeri*

H. bakeri L₃ infective larva was obtained from experimentally infected mice in the RUBAE laboratory. Methods describe by Smyth (1996) 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, L₃ infective larva was collected using Baermann device.

Infection of mice

L₃ larva obtain was used to infect 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 L₃ larvas was obtained. This concentration is necessary to produce an optimal infestation in the host (Wabo Poné, 2007). 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. Suspension was then filtered using a 150 μ m 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 (Onyilofe *et al.*, 2014).

Treatment of animals

For each extract, 25 infected mice of 6 weeks and weighing 18-25 g were used. These mice were divided into 5 groups of 5 animals each (Tsala *et al.*, 2010). Group 0 served as negative control and received distilled water or DMSO 1.24% depending on the test. Group 1 served 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.

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 were individually isolated for 10-20 minutes. 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 (Thienpont *et al.*, 1979). Eggs count inside the two cavity of McMaster was used to obtain EPG (eggs per gram of feces) by the following formula:

$$\text{EPG} = X \cdot 200 \quad \text{where} \quad X = \frac{\text{total eggs counted}}{\text{total of cavity counted}}$$

The fecal eggs count reduction rate (FECR) was calculated using the formula of Coles *et al.* (1992): $\text{FECR} (\%) = (1 - \frac{T}{C}) \times 100$ where T and C are geometric mean of EPG in treated groups and in negative control group respectively.

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 (Wabo Poné, 2007). 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 (1993) formula: $TWCR = \frac{MIPc - MIPt}{MIPc} \times 100$ 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.

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

Before the sacrifice, coproculture was realized to detect the effect of the treatment in the development of *Heligmosomoides 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 anthelmintic 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:

$$LDR = \frac{\text{number of L3 larva in traeted group}}{\text{number of L3 larva in the negative control group}} \times 100$$

Statistical analysis

Results were presented as average \pm 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.

Results

Anthelmintic 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.

1. Effect of treatments on the fecal egg count reduction (FECR)

1.1. Effect of aqueous extract of *Alafia barteri* on the FECR

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

Table I: Variation of the fecal egg concentration (of *Heligmosomoides bakeri* \pm 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 \pm SD (FECR rate \pm SD)			
		week 1	week 2	week 3	
Distilled water (negative control)	-	38695,39 \pm 26943,36 ^a (0 \pm 0) [*]	45577,22 \pm 25187,21 ^a (0 \pm 0) [*]	53038,31 \pm 26901,86 ^a (0 \pm 0) [*]	
	Albendazole (positive control)	15	5202,77 \pm 5494,36 ^b (86,55 \pm 14,2) [#]	3757,49 \pm 2834,18 ^b (91,76 \pm 6,22) [#]	5919,18 \pm 6579,19 ^b (88,84 \pm 12,40) [#]
980		6520,88 \pm 4393,81 ^b (83,15 \pm 11,35) [#]	34567,82 \pm 30544,4 ^a (24,16 \pm 67,02) [*]	23279 \pm 26899,59 ^b (56,11 \pm 50,72) [#]	
Aqueous extract		1960	4871,51 \pm 3819,35 ^b (87,41 \pm 9,87) [#]	26197,44 \pm 14621,73 ^{ab} (42,52 \pm 32,08) [*]	27173,12 \pm 16056,17 ^{ab} (48,77 \pm 30,27) [#]
	3920	10031,98 \pm 7339,25 ^b (74,07 \pm 18,97) [#]	34672,31 \pm 13834,61 ^a (23,93 \pm 30,35) [*]	29646,97 \pm 19378,82 ^{ab} (44,10 \pm 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 (3920mg/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%).

1.2. Effect of *Alafia barteri* ethanolic extract on the FECR

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

Table II: Variation of the fecal egg concentration (of *Heligmosomoides bakeri* \pm 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 \pm SD (FECR rate \pm SD)					
		week 1	Week 2	Week 3			
DMSO 1,24 % (negative control)	-	37089,54 \pm 9813,90 ^a	35918,69 \pm 19948,87 ^a	32120,02 \pm 8651,68 ^a			
		(0 \pm 0) [*]	(0 \pm 0) [*]	(0 \pm 0) [*]			
Albendazole (positive control)	15	5202,77 \pm 5494,36 ^b	4164,81 \pm 3255,02 ^b	5919,18 \pm 6579,19 ^b			
		(86,55 \pm 14,2) [#]	(91,76 \pm 6,22) ^μ	(88,84 \pm 12,40) ^μ			
Ethanollic extract	980	35366,82 \pm 29956,04 ^a	30234,96 \pm 29706,28 ^a	11796,5 \pm 9090,72 ^b			
		(4,64 \pm 80,77) [*]	(15,82 \pm 82,70) ^{*#}	(63,27 \pm 28,30) [#]			
	1960	14958,51 \pm 6718,81 ^{ab}	11674,49 \pm 8278,74 ^{ab}	2775,44 \pm 2785,22 ^b			
		(59,67 \pm 18,11) ^{*#}	(67,5 \pm 23,05) ^{#μ}	(91,36 \pm 8,67) ^μ			
	3920	25611,37 \pm 15657,02 ^{ab}	18309,57 \pm 15860,96 ^{ab}	10075,71 \pm 7305,23 ^b			
		(30,95 \pm 42,21) ^{*#}	(49,02 \pm 44,16) ^{*#μ}	(68,63 \pm 22,74) ^{#μ}			

“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).

According to ethanollic 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 ethanollic 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.

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.

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 III, depending on doses and treatments (albendazole and aqueous extract).

Table III: parasitic load variation of *Heligmosomoides bakeri* \pm SD and the TWCR rate after treatment with Albendazole and aqueous extract of *Alafia barteri*

Products	Doses (mg/kg)	Average parasitic load \pm SD (TWCR \pm SD)
Distilled water (negative control)	-	29,67 \pm 32,35 ^a (0 \pm 0) [*]
Albendazole (positive control)	15	4 \pm 2 ^b (86,52 \pm 6,74) [#]
	980	3 \pm 1,73 ^b (89,89 \pm 5,84) [#]
Aqueous extract	1960	20,67 \pm 15,30 ^a (30,34 \pm 51,6) ^{*#}
	3920	28 \pm 14,73 ^a (5,62 \pm 49,65) ^{*#}

“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 ($P > 0,05$).

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.

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 IV, depending on doses and treatments (albendazole and ethanolic extract).

Table IV: parasitic load variation of *Heligmosomoides bakeri* \pm SD and the TWCR rate after administration of Albendazole and ethanolic extract of *Alafia barteri*

Products	Doses (mg/kg)	Average parasitic load \pm SD (TWCR \pm SD)
DMSO 1,24 % (Negative control)	-	20 \pm 17,09 ^a (0 \pm 0) ^{*#}
Albendazole (positive control)	15	4 \pm 2 ^b (86,52 \pm 6,74) [#]
	980	14 \pm 10,58 ^a (30 \pm 52,92) ^{*#}
Ethanolic extract	1960	18,67 \pm 9,71 ^a (6,67 \pm 48,56) ^{*#}

3920

 $22 \pm 15,72^a (-10 \pm 78,58)^*$

“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 ($P>0, 05$).

From this table IV, 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. Effect of the treatments on the development of eggs taken from treated animals

The average number of L₃ 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 V.

Table V: L₃ larval development rate \pm 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 %) \pm SD
Negative control	-	147.67 ± 82.45^a	$100 \pm 0.0^*$
Aqueous extract	1960	75.2 ± 52.75^b	$50.93 \pm 35.72^\#$
Ethanollic 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, it stands out that the egg development rate until the L₃ 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.

Discussion

The objective of this work was to evaluate the Anthelmintic 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 anthelmintic activity when animals were taken 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. (2001)** 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, triterpenoids and saponines components (**Lasisi et al., 2016**). According to **Andrew et al. (2014)**, some of these metabolites like tannins have anthelmintic properties. The anthelmintic 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 (**Krishnananda et al., 2017**). This fleeting anthelmintic 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 (**Yeza and Bouchama, 2014**) in very low proportion. These components (tannins particularly) may have interfere with coupled oxidative phosphorylation, thus blocking ATP synthesis in the parasites (**Selamawit, et al., 2017**). 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. (2011)** 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 anthelmintic activity of *Zanthoxylum zanthoxyloides* in gastro-intestinal nematodes. The anthelmintic 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, flavonoids, steroids, alkaloids, triterpenoids and saponines (**Lasisi et al., 2016**). 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 its phenolic compounds because, according to **Cosme et al. (2020)**, 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 themselves on proteins and may act by many mechanisms. In vivo, they may act by creating a hostile gastro-intestinal environment reducing the parasite fecundity (**Al-Shaibani et al., 2009; Perla et al., 2020**).

Another group of phenolic compound, flavonoids may produce oxygenated substances creating an oxidant stress in parasite (**Yondo, 2014**). So the anthelmintic activity of a plant is a function of its richness in phenolic compound because they may mimic the action of their synthetic homologue by disturbing the reproductive system of the parasite (**Yondo, 2014**). Beside these phenolic compounds, **Al-Shaibani et al. (2009)** 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, terpenoids contained in *Alafia barteri* may play an important anthelmintic activity. In fact, according to **Yondo (2014)**, these bioactive molecules may act in the *H bakeri* cellular membrane by inducing structural changes of the internal compartment, resulting in the destruction of organelles 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 mg/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 (2000)** 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 administered in a very few quantity may have diffused through the receptors of the cuticle such that it has attacked 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 (**Azando et al., 2011**).

After the sacrificed, 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. (2011)**. 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 (**Yondo et al., 2013**). Under the effect of stress, the survivorship may have made worms to relieve their propagule to ensure the perennity of their species (**West et al., 2001**).

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 (1989)**. 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 (**Gofarana et al., 2014**).

Conclusion

The aim of this study was to evaluate *in situ* the anthelmintic activity of drinkable suspension of *Alafia barteri* on *Heligmosomoides bakeri*. We can conclude that:

- Aqueous extract had a fleeting activity when animals where receiving treatment. The maximal fecal egg count reduction rate and parasitic load reduction rate where respectively of 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%;
- Ethanolic extract presented encouraging activities during the test with the maximal fecal egg concentration reduction rate of 91.36% in the end of the experiment with the dose 1960 mg/kg. The maximum parasitic load reduction rate was 30 % in the dose 980 mg/kg and the larval development rate was 51.48%;
- Albendazole shows a fecal egg count reduction rate of 91.76% and a parasitic load reduction rate of 86.32%.

These results confirm the importance of *A. barteri* in the treatment of gastrointestinal helminths. *Alafia barteri* can be retained among alternative solution for the control of gastrointestinal nematodes. Thus, deep study is necessary to determine the toxicological effect of the plants.

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UNDER PEER REVIEW