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Antidiarrhoeal activity of aqueous and methanolic *Alchornea laxiflora* (Euphorbiaceae) leaves extracts in rats

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Abstract
Alchornea laxiflora (Euphorbiaceae) is a medicinal plant used in Cameroon to treat some gastrointestinal infections. The present study was undertaken to evaluate the antidiarrhoeal activity of aqueous and methanolic *Alchornea laxiflora* leaves extracts. The antidiarrhoeal activity of the extracts was evaluated *in vitro* on the motility of rat's intestine and the determination of their minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBCs) on seven bacteria; and *in vivo* at the doses of 125, 250 and 500 mg/kg on the models of infectious, secretory and osmotic diarrhoea induced respectively by *Shigella flexneri*, Castor oil and magnesium sulphate in rats. The results showed that the extracts of *A. laxiflora* significantly inhibited the three models of diarrhea. Only methanolic extract significantly ($p<0.01$) reduced the motility of rat's intestine and inhibited *Shigella flexneri* and *Salmonella typhi* with MIC of 512 and 1024 µg/ml respectively. These results justify the use of *Alchornea laxiflora* as antidiarrhoeal in traditional medicine.

Keywords: *Alchornea laxiflora,* anti-diarrhoeal, Castor oil, magnesium sulfate *Shigella flexneri,* rats

Introduction
Diarrhea can be defined as the quantity of stool send out in a large volume than normal (more than 300 g/day) in a regular interval (more than three times a day) for about three days [1]. It can be the result of infectious (bacterial and viral) or non-infectious (galactose, sorbitol, Magnesium sulphate Castor oil) origin. Amongst the enterobacteria that causes diarrhea, members of the genus *Shigella* are most prominent [2].
Diarrhea is one of the common diseases which is widely spread all over the World [3]. It causes dead to more than 5 to 8 million children of less than 5 years in the World every year especially in developing Countries [4]. According to WHO [5], the prevalence of South Sahara Africans suffer from diarrhea is 39, 1%, compared to 7, 2% in developed Counties. In fact, it is the first cause of mortality in developing countries hence, a major cause of morbidity in children before 5 years.
Modern medicine is therefore faced with anti bacterian and anti-diarrhea therapeutic urgencies to which is not only added the secondary effects of synthetic pharmaceutical drugs but also the resistance developed by the bacteria to commonly used antibiotics [6, 7]. Moreover, the usual high cost of pharmaceutical products and its inaccessibility have push the rural population to depend on traditional medicine which is readily available and have less secondary side effects.
Alchornea laxiflora is a plant of the family Euphorbiaceae, traditionally used in the treatment of diarrhea, inflammatory and some infectious diseases. Phytochemical studies carried out on *Alchornea laxiflora* leaves revealed the presence of some secondary metabolites like alcaloïds, flavonoïds, tanins saponins, phenols, sterols and reducing sugar [8, 9]. The present study was carried out to show the antidiarrhoeal properties of *Alchornea laxiflora* on Castor oil and Magnesium sulphate induced diarrhea as well as the evaluation of the antibacterial activities on six Human pathogenic species in rat model to establish the claimed biological activities of this plant.

Materials and methods

Plant material

The leaves of *A. laxiflora* were collected in South-Fotsi (West Region of Cameroon) in October 2014. The plant was identified and registered at the National Herbarium in Yaounde -Cameroun at number 34815/H14C. The leaves were then dried at room temperature under shade. The dried plant material was ground into a fine powder and used in the preparation of aqueous and methanolic extracts.

Preparation of plant extracts

Aqueous extract

A decoction was prepared by boiling 500 g of a dried powder with 5000 ml of distilled water for 30 min. The decoction once cooled at room temperature was filtered. Then the filtrate was concentrated by evaporating water at 40 °C an oven for 48 h.

Methanolic extract

The powdered plant (500 g) was macerated in 3 liters of methanol for 72 hours. The filtrate was evaporated to dryness in a rotary evaporator (Bushi R-200) at 60 °C.

Animals

For *in vivo* antibacterial assay, Wistar albino rats of both sex weighing 130-150 g. For Castor oil and magnesium sulphate induced diarrheal assay rats used were weighed 80-120 g. The animals were raised in the animal house of Laboratory of Animal Physiology and Phytopharmacology of the University of Dschang (Cameroon) to natural luminosity conditions. They were free access to food and water. *In vivo* experiments were done according to European guidelines of the European Union on Animal Care (CEE council 86/609) that was adopted by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon.

Microorganisms

Microorganisms used were *Shigella flexneri, Salmonella typhi, Enterococcus faecalis, Staphylococcus aureus, Proteus mirabilis Pseudomonas aeruginosa* and *Escherichia coli*.

Culture media

Mûller Hilton Agar (MHA) and Mûller Hilton broth (MHB) were used for the anti-bacterial studies. *Salmonella-Shigella* Agar (SSA) (TITAN BIOTECH LTD, India) was used for the stool culture in the *Shigella* induced rats. The strains were maintained at + 4 °C on MHA slants.

Gastrointestinal motility test

The gastrointestinal motility test was done according to the method described by Rajabhau *et al*. [10] with slight modifications. Fifty four rats were divided into 9 groups of 6 animals each. They were fasted for 24 h prior to the test, but allowed free access to water. Groups 1 and 2 served as negative control received distilled water (10ml/Kg) or DMSO (5%). Group 3 received the standard drug, atropine sulphate (2,5 mg/kg bw) and served as positive control. Groups 4, 5 and 6 received aqueous extract of *A. laxiflora* (AEAL) at the doses 125, 250, 500 mg/kg respectively. Groups 7, 8 and 9 received the methanolic extract of *A laxiflora* (MEAL) at the same doses. After 60 min, charcoal meal (3% deactivated charcoal) (Tradiphar) was administered orally to all rats. They were sacrificed after 1h and distance moved by the charcoal meal from the pylorus to caecum was measured and express as a percentage of distance travelled by the charcoal meal in ratio to the intestinal length. Percentage inhibition produced by extracts was calculated.

In vitro antimicrobial activity test

The *in vitro* antibacterial activity of the extracts was performed by determining the minimum inhibitory concentrations (MICs) and Minimum Bactericidal Concentration (MBCs) using broth microdilution method described by Newton *et al.* [11]. For this purpose, stock solutions of the extracts and standard drug were prepared at a concentration of 4096 µg/ml and 64 µg/ml respectively; successive dilutions permit to obtain the tested concentrations that varied from 1024 to 8 µg/ml in a 96 micro-well plates containing 95 µl of MHB and 5 µl of inoculum (standardized at 2.0 x 106 CFU/ml by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120-01spectrophotometer). The negative control was consisted of 195 µl of MHB and 5µl of the standard inoculums. The plates were covered with a sterile plate sealer, then agitated to mix the content of the wells using a plate shaker and incubated at 37 °C for 24h. Each concentration was tested in triplicate and the experiment was repeated three times. The plates were incubated at 35 °C for 18 h. Growth was monitored using iodo-tetrazolium chloride (INT). All concentrations at which no visible pink color changes was observed were considered as inhibitory concentrations and the lowest of these concentrations was considered as the MIC. The bactericidal concentrations were determined by adding 50 µl aliquots of the preparations (without INT), which did not show any visible colour change after incubation during MIC. The determination of the MBC was done by culturing 50µl of liquid from each well that showed no change in color on MHA and incubated for 24h at 37 °C. The lowest concentration that yielded no growth after this sub-culturing was taken as the MBC.

In vivo anti-shigellosis in rat model

Ten weeks old deparasitized albino's rats were starved for 18 hours with free access to water. The inoculum was prepared at 9×10^8CFU/ml (McFarland 3 standard) [12]. This inoculum (2 ml) was administered through oral route to rats. Food was given to animals *ad libitum* as from the end of the third hour to the end of the assay that lasted 6 hours. Only infected rats showing signs of diarrhea were selected for the rest of the experiment. These rats (60) were randomly assigned into nine groups of six rats each and treated as follow: Group 1, made up of non-infected rats received distilled water (neutral control), Groups 2 and 3 made up of infected rats received distilled water or DMSO (5%) only (diarrheic control); Group 4 made up of infected rats received Ciprofloxacin (2.5 mg/kg) (positive control); Groups 5, 6 and 7 composed of infected rats received the aqueous extract of *A. laxiflora* at the doses 125 mg/kg, 250 mg/kg and 500 mg/kg respectively; Groups 8, 9 and 10 composed of infected rats received the methanolic extract of *A. laxiflora* at the same doses. In order to minimize reinfection from fecal matter, each animal was placed in a cage whose bottom was lined with a gauge that allowed the feces to pass through.

The stools of infected animals were collected in sterile containers for the evaluation of the bacterial load. The faeces (0.5 g) were completely dissolved in 5 ml of 0.9% NaCl solution. Then, 250 µl of the suspension obtained were diluted in 9,750 ml of 0.9% NaCl. Fifty µl of the final suspension were cultured on a Salmonella-Shigella agar for each stool sample. After 18 hours of incubation at 37 °C the bacterial load was assessed and expressed in terms of number of

Colony Forming Units (CFU) per gram of faeces per animal. The relative body weight of the animals was also evaluated during the treatment period.

Castor oil -induced diarrhea

Induction of secretory diarrhea was done according to the method described by Karthik *et al*. [13] with slight modifications. The rats were all screen initially with 0,5 ml of castor oil, one week before the actual experiment. Only those showing diarrhea were selected for the final experiment. They were fasted for 24 h prior to the treatment, but had free access to water. For the experiment, 54 rats were randomly divided into nine groups of six animals each: Groups 1 and 2 served as negative control and received distilled water (10ml/Kg) and DMSO (5%) respectively; group 3 received the standard drug, Loperamide (2.5 mg/kg) and served as positive control; groups 4, 5 and 6 received the aqueous extract of *A laxiflora* at the doses 125, 250, 500 mg/kg respectively; groups 7, 8 and 9 received the methanolic extract of *A laxiflora* at the same doses. One hour after administration of these extracts, all animals received 10 ml/kg body weight of castor oil. They were then kept in separate metabolic cages, the floor of which was lined with a transparent absorbent paper to collect faces. After castor oil administration, the following parameters were measured for 6 hours: latency time, frequency of defecation and water content of stool.

Magnesium sulphate-induced diarrhea

Osmotic diarrhea was induced using the same method as that described previously with the only difference that magnesium sulfate was used at the dose of 3 mg/kg instead of Castor oil.

Statistical analysis

Data were subjected to the one way analysis of variance (ANOVA) and recorded as mean ± SD. When differences exist between treatments, means were compared using Turkey posttest for anti- diarrheal analysis and ANOVA two ways followed by Bonferroni test for anti-microbial and motility analysis of variance, where $p<0.05$ was considered statistically significant. The data were analyzed using Graph pad prism version 5.0.

Results

***In vitro* antidiarrheal activity**

Effect of AEAL and MEAL on intestinal transit in rat

Table 1 shows that the aqueous extract of *A. laxiflora* does not significantly inhibit the intestinal motility while methanolic extract significantly reduced the charcoal movement in the intestine ($p<0.01$) and the distance covered by the charcoal ($p<0.00$) at doses 250 mg/ kg and 500 mg /kg. The effect of this extract was smaller at dose 125 mg/kg where only the distance covered by the charcoal was significantly reduced ($p<0.01$).

Table 1: Effect of aqueous and methanolic extracts on rats intestine after 1 hour of treatment.

Treatment	Dose mg/kg	DMI (cm)	MDTC (cm)	MPCM (%)	PI (%)
Control	-	89.17 ± 2.87	47.67± 10.50	52.32 ±10.50	-
DMSO	-	88.50 ± 2.95	44.17 ± 9.56	48.63 ± 9.32	-
Atropine	1	91.83 ± 3.49	39.67 ± 3.94	42.99 ± 3.50	57.01
AEAL	125	98.17 ± 2.98	34.50± 12.52	34.67± 12.02	65.33
	250	93.33 ± 2.42	39.92 ± 8.55	41.89 ± 8.02	58.11
	500	97.33 ± 3.31	48.33 ± 6.97	49.41 ± 6.55	50.59
MEAL	125	98.67 ± 2.18	22.50± 8.95[b]	22.52 ± 9.07	22.21
	250	99.67 ± 1.63	6.67 ± 5.90[c]	6.63 ± 5.90[b]	93.36
	500	97.50 ± 2.88	3.33 ± 3.33[c]	3.44 ± 3.44[b]	96.56

Each value represents the mean ± SEM. [b]$p<0.01$; [c]$p<0.001$: significant differences compared to the negative control

DMI = Distance Mean of intestine; MDTC = Mean distance traveled by charcoal; MPCM = Mean Percentage of charcoal movement; PI = Percentage of inhibition

***In vitro* anti-microbial activity**

Only the methanolic extract of *A. laxiflora* presented a remarkable inhibitory activity on *S. flexneri* and *S. typhi* with MIC of 512 and 1024 µg/ml respectively. The ciprofloxacin used as reference substance was active on all bacterial strains and isolates (table 2).

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of AEAL and MEAL and Ciprofloxacin on bacterial strains and isolates

Microorganisms	Parameters	Extracts (µg/ml)		ciprofloxacin (µg/ml)
		Aeal	Meal	
E. coli ATCC 10536	MIC	-	-	2
E. faecalis ATCC 1054	MIC	-	-	8
	MBC	-	-	2
E. aerogenus ATCC13048	MIC	-	-	2
	MBC	-	-	1
S. flexneri	MIC	-	512	8
	MBC	-	-	2
S. typhi ATCC 6539	MIC	-	1024	1
	MBC	-	-	2
	MIC	-	-	2
S. aureus	MBC			1

(-): MIC >1024 µg/ml. AEAL: aqueous extract of *A. laxiflora*; MEAL: methanolic extract of *A. laxiflora.*

***In vivo* anti-diarrhoeal activity**

***In vivo* antibacterial activity of the AEAL and MEAL on *S. flexneri* induced diarrhea**

Figure 1 shows that aqueous extract caused a significant decrease (p<0.001) in the number of bacteria colonies in the faeces of animals compared to the negative control. This decrease was 50.35, 72.84 and 60.02% in animals treated at the doses 125, 250 and 500 mg/kg respectively. This decrease was maximum in animals that received the extract at the dose 250 mg/kg of AEAL where the number of colonies passed from 58.33 ± 6.12 x 10^2 UFC/g faeces at the second day to 0.00 ± 0.00 x 10^2 UFC/g faeces at the fourth day that is 100%, compared with animals that received distilled water in which the number of colonies passed from 214.83 ± 51.37 x 10^2 UFC/g faeces in day 2 to 50.33 ± 6.20 x 10^2 UFC/g faeces in day 16. The ciprofloxacin also caused a significant decrease (p<0.001) in the animals faeces passing from 63.07% at the second day to 100% at the 8th day. During the whole treatment, bacteria colonies are totally absents in non-infected animals.

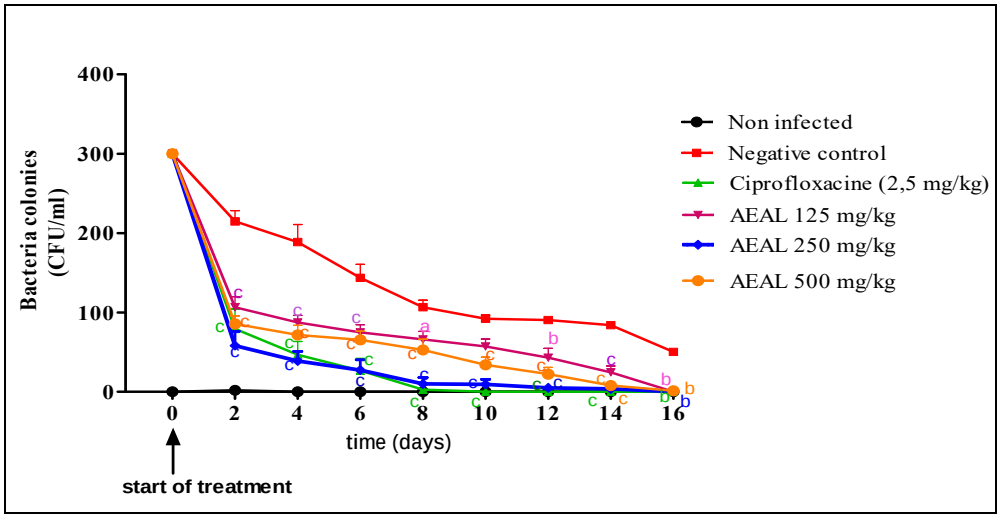

Fig 1: Number of *Shigella flexneri* in infected rat stool over 16 days of treatment with AEAL and ciprofloxacin. Each value represents the mean ± SEM. $^b p$<0.01; $^c p$<0.001: significant differences compared to the negative control. AEAL: aqueous extract of *A. laxiflora*

Figure 2 shows the effects of methanolic extract on the evolution of bacteria charge on the faeces of infected rats. A significant dose dependent decrease of bacteria charge was observed 6 days after the treatment as compared to the negative control. In fact, bacteria charge passed to 24,33; 27,66 and 37.10% on the 2nd day at respective doses of 125, 250 and 500 mg/kg at 100% on day 14 for all the doses. On the other hand animals treated at the dose 2.5 mg/kg of ciprofloxacin were cured at day 8 when the bacteria charge decreased to 63.07% at day 2 passed to 100%.

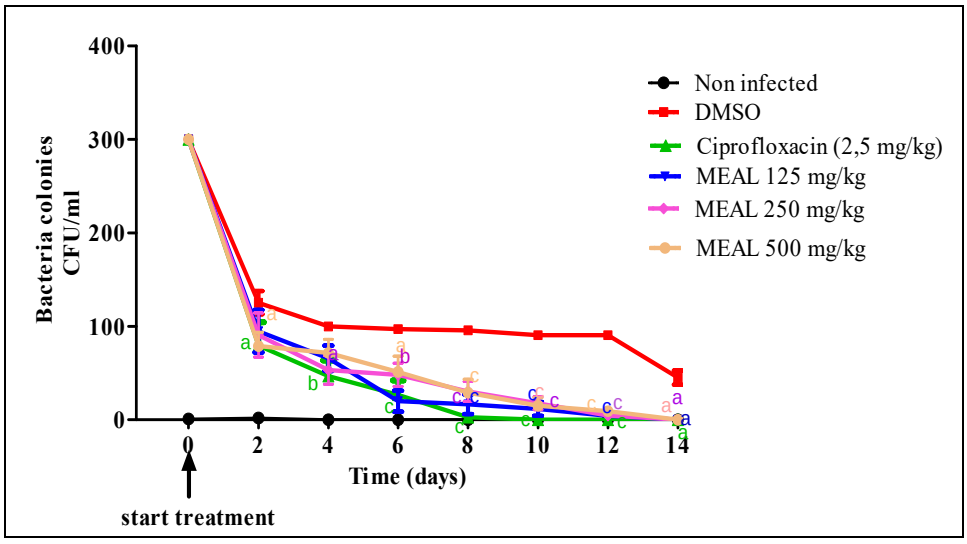

Fig 2: Number of *Shigella flexneri* in infected rat stool over 14 days of treatment with MEAL and ciprofloxacin. Each value represents the mean ± SEM. $^a p$<0.05 ; ^{b}P < 0.01; $^c p$<0.001 : significant differences compared to the negative control. MEAL: methanolic extract of *A. laxiflora*

Effect of the AEAL and MEAL on body weight during the treatment period

The aqueous extract at the dose 250 mg/kg significantly increased the body weight of animals compared to negative control (figure 3). In animals treated with ciprofloxacin the increase was also significant as from the 11th day.

The effect of methanolic extract on body weights of infected rats is illustrated in figure 4. An increase in body weight of animals was observed from day 8 in all groups compared to the negative control.

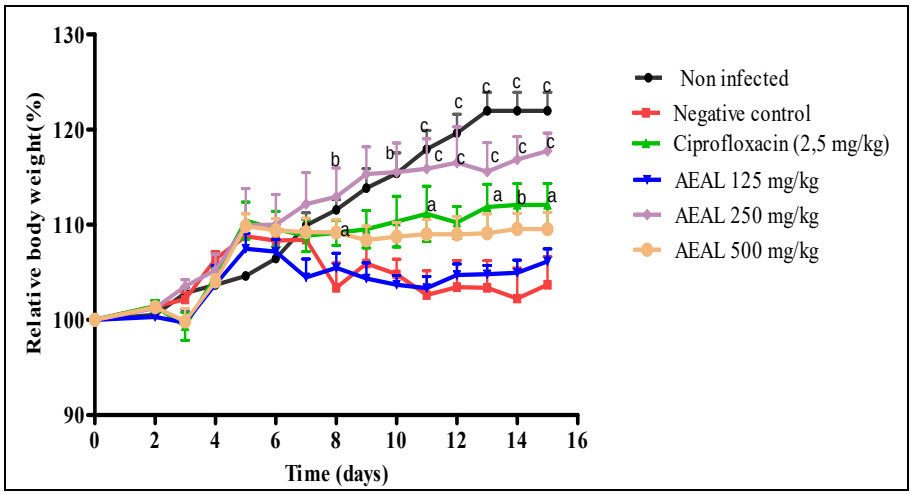

Fig 3: Variation of relative body weight of *Shigella flexneri* in diarrheic rats treated with AEAL and Ciprofloxacin. Each value represents the mean ± SEM. [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$: significant differences compared to the negative control. AEAI: aqueous extract of *A. laxiflora.*

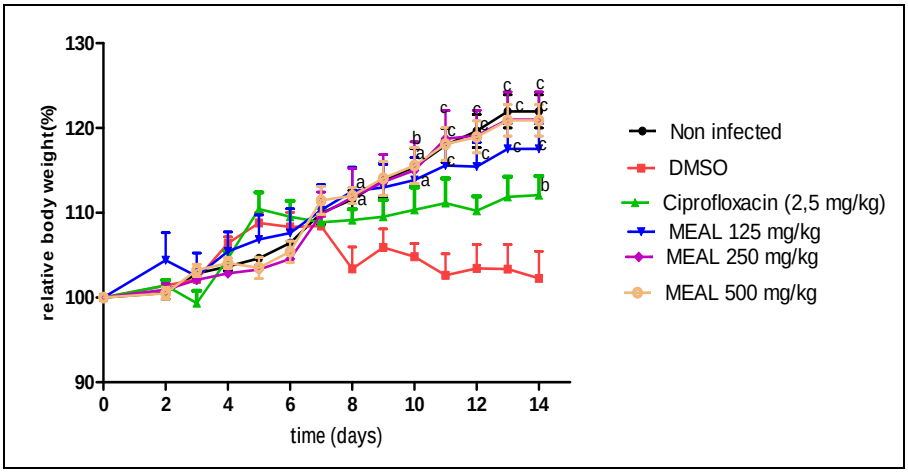

Fig 4: Variation of the relative body weight of *Shigella flexneri* in diarrheic rats treated with MEAL and Ciprofloxacin. Each value represents the mean ± SEM. [a]$p<0.05$; [c]$p<0.001$: significant differences compared to the negative control. MEAL: methanolic extract of *A. laxiflora*.

***In vivo* antidiarrhoeal activities of the AEAL and MEAL on Castor oil induced diarrhea**

Table 3 shows the evolution of the different parameters after 6 hours of observation of rats when studying effects of *A. laxiflora* on Castor oil induced diarrhea. AEAL significantly inhibited at 70.98% the frequency of defecation ($p<0.001$) and number of wet stools ($p<0.01$) at dose 125 mg/kg compared to negative control; whereas MEAI significantly increased the latent period at doses 250 mg/kg ($p<0.05$) and 500 mg/kg ($p<0.001$). MEAI also significantly reduced ($p<0.05$) the number of wet stools. Loperamide administrated at dose 2.5 mg/kg significantly increased latent period ($p<0.05$), and reduced frequency of defecation ($p<0.001$) and number of wet stools ($p<0.05$).

Table 3: Effect of aqueous and methanolic extracts of A. laxiflora in Castor oil induced diarrhea in rats after 6 hours of treatment.

Treatements	Doses (mg/kg)	Latency time (min)	Frequency of defecation in 6H	Water content (%)	Number of wet stools
Negative control	1 ml/100g/pc	44.00 ± 5.89	03.17 ± 0.17	82.94 ± 2.23	3,00 ± 0,00
DMSO 5%	0	49.00 ± 7.53	02.50 ± 0.62	67.94 ± 13.76	2,50 ± 0,62
Loperamide	2.5	262.20 ±59.91[a]	01.33 ± 0.80[c]	38.07 ± 17.31	1,00 ± 0,68[a]
AEAL	125	188.20 ±62.38	0.83 ± 0.31[c]	42.74 ± 19.14	0,50 ± 0,22[b]
	250	71.60 ± 7.67	02.17 ± 0.31	69.65 ± 14.04	1,50 ± 0,34
	500	147.80± 48.20	02.50 ± 0.85	59.37 ± 18.86	1,50 ± 0,62
Meal	125	92.80 ±8.77	02.67 ± 0.21	79.06 ± 2.11	1,17 ± 0,31
	250	232.80± 44.34[a]	01.33 ± 0.42	62.87 ± 12.99	1,00 ± 0,52
	500	321.00± 23.92[c]	05.00 ± 0.22	37.39 ± 17.46	0,33 ± 0,21[a]

Each value represents the mean ± SEM. [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$: significant differences compared to the negative control.
AEAI: aqueous extract of *A. laxiflora*; MEAI: methanolic extract of *A. laxiflora*

***In vivo* antidiarrhoeal activity of the AEAL and MEAL on osmotic diarrhea induced by Magnesium sulphate**

AEAL and MEAL significantly prolonged latency period ($p<0.05$; $p<0.01$) at all the doses in the model of osmotic diarrhea induced by magnesium sulfate. The maximum of prolongation was observed with MEAL at doses of 125 and 250 mg/kg with a percentage of prolongation of 62.46% and 71.46% respectively. Except the dose 125 mg/kg of AEAL, all the doses of different extracts and loperamide significantly reduced number of wet stools (table 4).

Table 4: Effect of aqueous and methanolic extracts of *A. laxiflora* on magnesium sulphate induced diarrhea in rats after 6 hours of treatment.

Treatments	Doses (mg/kg)	Latency time (min)	Frequency of defecation in 6H	Water content (%)	Number of wet stool
Negative control	0	176.80± 45.00	01.00 ± 0.36	45.21 ± 14.62	0,67 ± 0,21
DMSO 5%	1 ml/100g pc	173.6 ± 50.33	01.00 ± 0.32	46.88 ± 13.6	0,62 ± 0,20
Loperamide	2.5	340.40±15.30[a]	0.33 ± 0.21	20.13 ± 12.78	0,00 ± 0,00[a]
Aeal	125	295.80±44.44[a]	0.5± 0.37	22.00 ± 15.38	0,17 ± 0,18
	250	329.20±30.80[a]	0.17 ± 0.07	8.84 ± 4.42	0,00 ± 0,00[a]
	500	354.20± 5.80[a]	0.17 ± 0.1	9.73 ± 3.24	0,00 ± 0,00[a]
Meal	125	332.20 ± 27.80[b]	0.21 ± 0.10	11.88 ± 11.84	0,00 ± 0,00[b]
	250	360.00 ± 0.00[b]	0.00 ± 0.00	0.00 ± 0.00[a]	0,00 ± 0,00[b]
	500	339.6± 20.40[b]	0.20 ± 0.20	14.85 ± 14.85	00,00 ± 0,00[a]

Each value represents the mean ± SEM. [a]$p<0.05$; [b]$p<0.01$: significant differences compared to the negative control.
AEAL: aqueous extract of *A. laxiflora*; MEAL: methanolic extract of *A. laxiflora*.

Discussion

In the evaluation of intestinal transit, Atropine Sulphate was use as positive control. Atropine is well known as a transit intestinal inhibitor probably due to its anticholinergic effect. It acts as muscarinic receptors of type M_1 of acetycholine in the central nervous system and peripheral nervous system [14]. Temitope and Omotayo [9] have described the presence of tannin, flavonoids and terpenoids in this plant. The inhibition of intestinal movement could be due to the presence of flavonoids and tanins known for their antidiarrheic activities through the inhibition of intestinal mobility [15, 10]. Tanins reduces peristaltic movements and intestinal secretion by the formation of a complex with luminal protein [7]. On the other hand flavonoids inhibit intestinal motility and hydro-electrolytic secretions.

The results show that A. *laxiflora* showed relatively good antibacterial properties. Secondary metabolites groups that have previously been described in this plant species including flavonoids, tannins, triterpens, steroids and phénols are known to possess antimicrobial properties [16, 17]. The census of pathogens revealed that *E. coli*, entero- invasive (EIEC) and entero-hemorragic (EHEC), Salmonella, Shigella, Vibrio cholerae are the major bacterial pathogens most often responsible for pandemic and epidemic diarrheal infectious diseases in developing countries [18]. *Shigella flexneri* is known to secrete verotoxins which helps it to colonize the colon mucosa and get inside the epithelial cells, inducing inflammation and degeneration of the lamina propria [19] and increase the cyclic AMP rate in the intestine epithelium [20]. Consequently, the desquamation and ulceration of the mucosa leads to the loss of blood and mucus in the intestinal lumen, which in turns hinders reabsorption. This result interesting since in the in vitro test the MIC of ciprofloxacin (0.25µg/ml) was 1024 time lower than that of the methanolic extracts (512 µg/ml). So, it seems that the immune system may have play a role in the final antimicrobial effect of the plant extracts in infected rats. Arokiyaraj *et al.* [21] and Kamtchueng *et al*., [22] showed that polyphenolic compound and alkaloids possess the immune-stimulating properties. Beside, some substances administered orally can undergo transformations during absorption, transport and distribution processes (pharmacokinetics), thereby, producing metabolites which could act by binding on toll-like receptors to potentiate the immune system especially by stimulating macrophages and dendritic cells which may have phagocyte and digest the bacteria. In the case of our extracts, the metabolite would be more active, thus, could explain this result. The infected rats show variation in body weight after treatment, compared to the control. This could be due to the increase in appetite, possibly induced by the hyperphagial properties of the plant extract. Indeed, the extracts could trigger the secretion of Leptine which is capable of stimulating Y-neuropeptid, the most powerful appetite stimulator to the best of our knowledge.

Diarrhea originates from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry, resulting in an excess loss of fluid in faeces [23]. Magnesium sulphate is known to increase the permeability of electrolytes at the level of the intestinal mucosa, alongside, the secretion of cholecystokinin in the duodenum, leading to hyper secretion which inhibits fluids' reabsorption [24]. The tested extract may content active substances that have increased the absorption of water and electrolytes from the gastrointestinal tract of rats.

In contrast, the active principle found in Castor oil, ricinoleic acid, irritates the intestinal mucosa and this results in the biosynthesis of inflammatory mediators such as Prostaglandins (PgE2) and Histamine and consequently an increase in intestinal motility and hyper secretion [25-27, 14, 28]. It is obvious that the extracts may have the capacity of inhibiting the effect of ricinoleic acid on the muscosa of the intestine. In castor oil induced diarrhea, the anti-diarrheic effect of the *A. laxiflora* could result from inhibition of prostaglandin/histamine synthesis or by installing an anti-secretory mechanism.

Loperamide used in this study as reference drug act by inhibiting the peristaltic activity, through indirect effect on circular and longitudinal muscle of the intestinal wall, also by stimulating the absorption of water and electrolytes by enterocytes by increasing the intestinal transit time (anti-spasmodic) of the bowel content [29]. It is then possible that the *A. laxiflora* aqueous and methanolic extracts act in the same manner. Moreover, the anti-diarrhea activity of the extracts could be due to the presence of flavonoids and tannins which have the capacities to inhibit intestinal motility and hydroelectrolytes' secretion. Otherwise, tannins could act by reducing intracellular calcium ion concentration or by activating the calcium pomp which would lead to muscle relaxation.

Conclusion

From the results, it appears that aqueous extract and methanolic extract of *A. laxiflora* possess antidiarrheal activity. This activity could result from their ability to increase the absorption of water and electrolytes from the gastrointestinal tract and to inhibit prostaglandin/histamine synthesis, intestinal motility and hydro-electrolytic secretions. The antidiarrheal activity of *A. laxiflora* is also associated to its antibacterial properties. Methanolic extract seems to have the most potent effect. It brought from these findings, that *A. laxiflora* is a good candidate for the development of an antidiarrheic phytomedicine.

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Competing Interests
The authors declare that they have no competing interests

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