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In vitro antibacterial and antidiarraheic activity of root bark extract of *Anogeissus leiocarpa* (Combretaceae) during an experimental bacterial diarrhea induced by *Escherichia coli* extended-spectrum β-lactamases (ESBL) in albino Wistar rats

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The present study was conducted to investigate *in vitro* and *in vivo* antibacterial activity of the root bark extracts of *Anogeissus leiocarpa* (DC) Guill. & Perr, in *Escherichia coli* extended-spectrum β-lactamases (ESBL)-induced diarrhea in rat. The antibacterial activity was performed *in vitro* by determining the inhibition zone using standard agar diffusion method as well as *in vivo* on *E. coli* infected Wistar rat model. Both minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were carried through microdilution method. Results obtained in this study indicated that ethanolic and acetatic extracts were only active on bacteria presenting an inhibition zone range from 8 to 16 mm. The MIC observed in agar slant tubes ranged from 6.25 to 50 mg/ml. The ethanolic fraction of *A. leiocarpa* (ETHA) showed the highest *in vitro* antibacterial activity against strains with MICs ranging from 6.25 to 12.5 mg/ml and MBCs ranging from 12.5 to 25 mg/ml. *In vivo*, after infection, diarrhea increased faeces frequency, weight and volume faeces and bacterial faeces load to a maximum on the 2nd day after infection ($P < 0.01$). ETHA normalized the appearance, weight, volume and water content of faeces. To all doses, like ciprofloxacin it reduced significantly ($P < 0.05$) the bacterial growth compared to control Lot (infected and untreated). The death rate in diarrheic control Lot was 50% by Day 14. No death was recorded in Lot treated with ciprofloxacin and Lot treated with ethanolic fraction at dose of 2222.22 mg/kg body weight (bw). This study supports the use of *A. leiocarpa* in the traditional treatment of bacterial infections and offer many perspectives in the search for new molecules against resistant microbial strains.

Key words: *Anogeissus leiocapa*, antibacterial activity, *in vitro*, *in vivo*, therapeutic.

INTRODUCTION

Diarrheal diseases, the third leading cause of death in the world, after respiratory diseases and AIDS (OMS, 2015) continue to be one of the leading causes of morbidity and mortality. Globally, they constitute the second leading cause of infant mortality in children less than five years (Bhutta et al., 2013), with about 2.5 billion diarrhea

episodes and 2.2 million deaths all years (Bahmani et al., 2015). Of the 2.2 million annual deaths, 37% of cases occur in sub-Saharan Africa (OMS, 2016). In Côte d'Ivoire, diarrhea represents 14% of the reasons for consultation in maternal and child health centers (Kouakou, 2012) and 15% of deaths of children less than 5 years (Liu et al., 2014). The main agents involved are viruses, protozoa and bacteria among which *Escherichia coli* is one of the most important etiologic agents (Asadi et al., 2010).

Given the magnitude of this situation, World Health Organization (WHO) has adopted a treatment based on oral rehydration (OR) and oral rehydration solution (OSR). However, the annual rates of use of OR and OSR, respectively of 0.39 and 1.02% are still very low (Birger et al., 2006). In addition to this program, antibiotics, although used, are becoming increasingly ineffective because of microbial resistance and toxicity problems. They are also still beyond the reach of population grants in developing countries because of their high cost. In this situation, it is necessary and urgent to offer to disadvantaged populations more accessible new therapeutic solutions taking into account their culture and their purchasing power.

To meet this challenge, exploring the rich Ivorian medicinal flora with nearly 800 species of medicinal plants (Aké-Assi, 1991) could offer alternative therapeutic solutions. It is in this context, that the present study focused on *Anogeissus leiocarpa* (DC) Guill. & Perr, a plant of the Ivorian medicinal flora. Phytochemical studies carried out on *A. leiocarpa* root barks revealed the presence of phenolic compounds (flavonoids, tannins, leucoanthocyanins and polyphenols), saponins and sterols (Moronkola and Kunle, 2014; Gbadamosi and Ogunsuyi, 2014). These compounds are known to have antimicrobial activity (Mann et al., 2014; Adamu et al., 2017).

This study focused on the *in vitro* and *in vivo* investigation of the antibacterial potential of Combretaceae, a selected medicinal plant of Côte d'Ivoire flora by using infected rats with *E. coli* extended-spectrum β-lactamases (ESBL).

METHODOLOGY

Plant material

The root barks of *A. leiocarpa* were used. These organs were harvested in January 2013 in Kouto (Bagoué region), a town located at 725 km north of Abidjan. This plant has been authenticated by Professor Aké-Assi of the National Floristic Center of Félix Houphouët-Boigny University and compared to the voucher specimen No. CNF 14798.

Animals

Albinos white rats, male and female of Wistar strain aged 2 to 3 months and weighing between 180 to 200 g were used.

Microorganisms

Nine bacterial strains involved in gastrointestinal disorders were used: *E. coli* CIP 7624 (ATCC 25922) (reference strain), eight clinical strains isolated from biological products: *E. coli* ESBL 13Y016 (isolated from urine), *Salmonella Typhi* 1586 (isolated from stool), *S. Typhi* 43PI16 (isolated from stool), *Pseudomonas aeruginosa* 131813 (isolated from stool), *Shigella dysenteriae* 1079PI/15 (isolated from stool), *Klebsiella oxytoca* (isolated from urine) and *Staphylococcus aureus* Met-R 1532C/10 (isolated from pus) and *Streptococcus* species. These strains come from the bio-bank of the Institut Pasteur of Côte d'Ivoire.

Culture media and antimicrobial agents

The culture media used are: Müller-Hinton agar (Liofilchem®, Italia), Eosine blue agar of Methylene (Cultimed®, USA) and Mac Conkey agar (Cultimed®, USA).

Preparation of the total aqueous extract and organic fractions of *A. leiocarpa*

Total aqueous extract was prepared according to Guede-Guina (1993) and ethyl acetate, dichloromethane and ethanol fractions according to Manga et al. (2013).

Preparation of the bacterial inoculum

A volume of either 0.01, 0.1 or 1 ml of opalescent pre-culture broth was collected for *Pseudomonas*, enterobacteria and *Staphylococci*, respectively, and then diluted in a tube containing 10 ml of physiological saline to constitute dilution inoculum 10°.

Preparation of concentration ranges

A concentration range of 500 to 7.81 mg/ml similar to that of Mann et al. (2014) was prepared by the double dilution method in 7 test tubes. These tubes were then sterilized by autoclaving at 121°C for 15 min (Bolou et al., 2011) and stored in a refrigerator at +4°C.

Preparation of culture media

Culture media were prepared according to manufacturers' instructions (Liofilchem® and Cultimed®).

Antibacterial sensitivity test

The agar well diffusion method was used (Irshad et al., 2012) for assessing the *in vitro* antibacterial activity of the prepared extract. Cefotaxime (CTX, 30 µg) for enterobacteria and gentamycin (GEN10 µg) for other bacteria served as positive controls. Only concentrations of extracts or fractions with an inhibition zone

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diameter (IZD) greater than 7 mm were reported in results

Minimum inhibitory concentration (MIC)

The incorporation of the plant extracts into Muller-Hinton (MH) agar was done using the double dilution method in agar slant tubes as described by Ouattara et al. (2013). Nine experimental tubes whose concentration varies to double dilution from 50 to 0.195 mg/ml and 2 control tubes, the growth control tube (TC) and the sterility control tube (TS) are prepared. The slope of the experimental tubes and that of the TC tube was seeded. The tubes were incubated at 37°C for 24 h. The MIC was the concentration of the first tube from which no microbial visible growth (Khaleel et al., 2016).

Minimum bactericidal concentration (MBC)

MBC is the lowest concentration of substance that leaves at most 0.01% of surviving germs. Using a loop calibrated at 2 µl, the contents of the tubes in which no haze was observed were seeded on MH (Box B) in parallel streaks 5 cm in length at the surface, starting with by the MIC tube. After 24 h incubation in an oven at 37°C, the numbers of colonies on the streaks of Box B with those of Box A were compared. In practice, the CMB corresponds to the concentration of the experimental tube whose number of colonies present on the streak is less than or equal to the number of colonies present on the streak of the dilution 10^{-4}.

***In vivo* antibacterial activity**

Experimental design

Forty-two rats of both sexes previously deparasized through oral administration of 10 mg/kg body weight of tetracycline for 3 days (Ricicová et al., 2010) were used. They were divided into 7 Lots of six rats each. Rats from 6 Lots received, orally, 2 ml of the infective dose (Eman et al., 2008) evaluated at 2×10^8 CFU/ml with a sterile disposable needle-less syringe. The 7th batch is uninfected. Treatment started 48 h after the induction of diarrhea following the appearance of diarrheal faeces. For treatment, the infected rats received orally concentrations of the ethanolic fraction of *A. leocarpa* and ciprofloxacin (the reference antibiotic) according to the prescription of Venkatesan et al. (2005). Thus, daily and for 14 days, Lot 1 (uninfected and untreated) received 1 ml of distilled water; Lot 2 (infected and untreated) received 1 ml of distilled water; Lot 3 (infected and treated) received 2 ml of ETHA at dose of 69.44 mg/kg body weight corresponding to 1 × MIC; Lot 4 (infected and treated) received 2 ml at a dose of 271.87 mg/kg body weight (5 × CMI); Lot 4 (infected and treated) received 2 ml of ETHA at a dose of 631.3 mg/kg body weight (10 × CMI) ; Lot 6 (infected and treated) received 2 ml of ETHA at a dose of 2222.22 mg/kg body weight (40 × CMI) and Lot 7 (infected and treated) received 1 ml of ciprofloxacin at dose of 5 mg/kg body weight w.

Enumeration of faecal E. coli

Rats faeces were collected in sterile polyethylene sterile bags of 500 ml (LMR®, France) placed under the cage of the rats, twice daily for 2 weeks. One (1) g of faeces removed with a spatula was transferred to 9 ml of BMH, then vortexed for 10 s and incubated at 37°C for 3 to 4 h to obtain dilution inoculum10°. From this dilution 10°, dilutions ranging from 10^{-1} to 10^{-5} were prepared. This serial dilution were cultured (0.1 ml) in duplicate on Mac Conkey agar (two plates per dilution). After incubation at 37°C for 18 to 24 h, typical smooth pink color colonies were counted on two successive dilutions. Plates inoculated with a sample dilution that yields between 10 and 300 colonies per plate were read (ISO 7218, 2007). The *E. coli* faeces load (UFC per g of faeces) was calculated according to the formula proposed by ISO 7218 (2007):

$$\textit{E. coli} \text{ faeces load (UFC/g of faeces)} = \frac{\sum C}{V \times 1.1d}$$

where ΣC: sum of colonies counted on the two Petri dishes retained, V: volume of seeded inoculum on each Petri dish, and d: dilution corresponding to the first retained Petri dish, with the least diluted inoculum.

Evaluation of the water content of faeces

Fresh faeces was collected after 6 h and weighed individually to determine the wet weight (WW). Faecal samples were dried in a conventional oven at a temperature of 70°C. After 24 h, dried faeces were weighed again for determining their dry weight (DW) (Navarro et al., 2006). The difference between the WW and the DW helped to determine the water content of faeces (Navarro et al., 2006) expressed as a percentage, by the following relation:

$$\text{Water content (\%)} = \frac{WW - DW}{WW} \times 100$$

Mortality rate and pathological manifestations

The mortality rate of the rats in the different groups is calculated as the number of dead rats during the experiment compared to the total number of rats used in each Lot (Eman and Hoda, 2008). Faeces were checked continuously for color and consistency, the development of diarrhea in infected Lots, as well as any change in activity and behavior were recorded weekly throughout the experiment.

Statistical analysis

All data during this experience were expressed in three replicates. The results are expressed as mean ± standard error of means. They were determined by Dunnett's test using GraphPad Prism 7.0 Statistic Software and were considered significant at *p*-value less than 0.05.

Ethical approval

The experiments were conducted according to the ethical guidelines of Ethics Committee of Pasteur Institute of Côte d'Ivoire (Charter of Ethics of the Pasteur Institute, Text of September, 2012).

RESULTS

Antimicrobial activity of aqueous extract and organic fractions of *A. leiocarpa*

The effects of total aqueous extract and organic A. *leiocarpa* fractions obtained by agar well diffusion method on bacterial growth are shown in Table 1. The total aqueous extract and the dichloromethane fraction showed no activity on all the bacteria tested with inhibition zones

Table 1. Sensitivity of bacterial strains tested to aqueous extract and organic fractions of *Anogeissus leiocarpa*.

Bacterial strains	Concentration of aqueous extract and organic fractions of *Anogeissus leiocarpa* (mg/ml)							Antibiotics (µg)	
	ETHA			EAA			EDMA ETAA		
	C_1= 500	C_2= 250	C_3= 125	C_1= 500	C_2= 250	C_3= 125	C_1=500, C_2=250, C_3=125	CTX (30)	GEN (10)
	Diameters of inhibition zone (mm)								
E. coli ATCC 25922	13.42±1.22	10.19±0.50	8.46±0.32	11.40±0.40	9.61±0.88	<7		30	ND
*E. coli*ESBL	12.02±0.19	9.62±0.52	ND	10.71±0.56	8.14±0.11	<7		10	ND
P. aeruginosa 131813	11.00±0.86	9.24±0.49	8.12±0.60	10.00±0.50	8.00±0.23	<7		ND	21
S. Typhi 43PI16	15.33±0.88	14.00±0.58	10.67±0.68	14.01±0.84	11.30±0.25	9.46±0.77		25	23
S. Typhi 1586	14.67±1.33	12.33±0.33	9.08±0.86	15.07±0.61	12.37±0.33	10.05±0.14	<7	28	30
S. dysenteriae 1079PI15	12.71±0.28	10.22±0.33	8.03±0.50	11.33±0.58	9.09±0.47	8.43±0.46		ND	ND
K. oxytoca	13.01±0.84	10.22±0.21	8.06±0.77	10.21±0.79	8.33±0.22	<7		ND	ND
S. aureus Meti-R	16.00±0.12	14.10±0.88	11.45±0.18	15.00±0.44	13.58±1.08	11.58±0.86		ND	ND
Streptococcussp	16.64±0.56	13.05±0.83	9.14±0.23	14.17±0.20	12.48±0.31	9.69±0.27		ND	15

The values were expressed as Mean±SEM. CTX: Cefotaxim; GEN: gentamycin; ESBL: extended spectrum beta-lactamase; Meti-R: meticillin resistant; ND: not determined. ETHA: ethanol fraction of *A. leiocarpa*, ETAA: total aqueous extract of *A. leiocarpa*; EDMA: dichloromethane fraction of *A. leiocarpa*; EAA: ethyl acetate fraction of *A. leiocarpa*.

diameters less than 8 mm.

Both ethanol (ETHA) and ethylacetate (EAA) fractions were active against all infectious bacteria tested with an IZD values ranging from 8 to 16 mm and 8 to 15 mm, respectively. Diameter of zone of inhibition decreased when reducing gradually the extract or fraction concentration excepted for EDMA and ETAA. On a general note ETHA exhibited higher sensitivity than EAA. Only *S. aureus* Meti-R and *Streptococcus* spp. displayed the highest sensitivity with an IZD up to 16.64 at 250 and 500 mg/ml, highest zone of inhibition observed with ETHA compared to EAA. With the exception of *E. coli* ESBL which is the main resistant strain at 125 mg/ml, the IZD of most bacteria tested with ETHA are larger than those of EAA. In addition, EAA at the concentration of 125 mg/ml revealed 3 resistant strains which are *E. coli* ATCC, *E. coli* ESBL and *P. aeruginosa* with an IZD below 8 mm. The inhibition zone of total aqueous extract and dichloromethane fraction at any concentration is below 8 mm. For standard drugs, extreme sensitivity was observed with an IZD that ranged from 10 to 30 mm.

Antibacterial parameters of the total aqueous extract and organic fractions of *A. leiocarpa*

MIC, MBC and MBC/MIC ration values are shown in Table 2. The MICs values of all strains studied with both ETAA and EDMA extracts were above 50 mg/ml, thus their MBC and MBC/MIC ratio were not determined. The MICs of *A. leiocarpa* ranged from 6.25 to 12.5 mg/ml in ETHA fraction. For EAA fraction, it was 6.25 mg/ml for *S. Typhi* 43PI16 and *S. Typhi* 1586, but was up to 25 mg/ml for *E. Coli* ATCC, ESBL, and *P. aeruginosa* but it was up to 50 mg/ml for *Shigella dysenteriae* and *K. oxytoca*. The MBC/MIC ratio of EAA and ETHA fractions observed were strictly inferior to 4 corroborating the bactericidal effect against all bacteria tested.

In vivo antibacterial activity

Infection rate, mortality in rats and faeces appearance

The antibacterial activity *in vivo* was evaluated by increasing the concentration of ETHA shown in Table 3.

These results indicated antibacterial activity *in vivo* after 7 days of treatment especially from Lot 3 (ETHA 69.44 mg/kg body weight) to Lot 7 (ETHA 2222.22 mg/kg body weight) was correlated to a gradual decrease of mortality rate from 30 to 0%. However, apart from Lot 1 as control and untreated rats in Lot 2 which scored 40 and 10% mortality rate at 7 and 14 days, respectively, there was no mortality rate

Table 2. Antibacterial parameters of the total aqueous extract and organic fractions of *A. leiocarpa*.

Extract	Antibacterial parameters (mg/L)	Gram negative bacteria							Gram positive bacteria	
		***E. coli* ATCC**	***E. coli* ESBL**	***S. Typhi* 43PI16**	***S. Typhi* 1586**	***P. aeruginosa* 131813**	***S. dysenteriae* 1079PI15**	***K. oxytoca***	***S. aureus* Meti-R**	***Streptococcus* spp.**
EDMA	MIC	>50	>50	>50	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50	>50	>50	>50
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Effect	ND	ND	ND	ND	ND	ND	ND	ND	ND
EAA	MIC	25	25	6.25	6.25	25	50	50	6.25	6.25
	MBC	25	50	6.25	6.25	50	50	50	6.25	12.5
	MBC/MIC	1	2	1	1	2	1	1	1	2
	Effect	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid
ETHA	MIC	12.5	12.5	6.25	6.25	12.5	12.5	12.5	3.12	3.12
	MCB	12.5	12.5	12.5	12.5	25	25	25	6.25	6.25
	MBC/MIC	1	1	2	2	2	2	2	2	2
	Effect	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid	Bcid
ETAA	MIC	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
	MBC	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
	MBC/MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Effect	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: Not determined; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; ETHA: ethanol fraction of *A. leiocarpa*; ETAA: total aqueous extract of *A. leiocarpa*; EDMA: dichloromethane fraction of *A. leiocarpa*; EAA: ethyl acetate fraction of *A. leiocarpa*.Bcid: bactericidal.

observable from Lots 3 to 7 (ciprofloxacin) recorded after 14 days (2 weeks) of treatment. Notably, Lot 6 with 2222.22 mg/kg body weight corresponding to 40 × MIC and the reference drug ciprofloxacin cleared the infection after 7 days of treatment.

Effect of ETHA on E. coli enumeration in rats diarrheal faeces

The effect of ethanolic fraction on bacterial load in diarrheic faeces is as shown in Figure 1. It can be seen that after the 2 days of rats infection, the *E. coli* colony forming units per g of faeces (UFC) counted decrease from Lots 3 to 7 when the concentration of ETHA was increased progressively. Outstandingly, the UFC of Lot 6 is close to that of the Lot 7, the standard drug that drops rapidly to zero after 6 days of treatment but the UFC of Lot 2 remained higher than those with treatment during the 13 days of study.

Effect of ETHA on water content

From the Figure 2 that shows the percent of facael water content, a significant decrease of water lost during the ETHA treatment was observed and it is strongly correlated to the diminishing feacal *E. coli* load in Figure 1. Only the rats treated with ciprofloxacin or cefotaxime showed no statistically significant difference ($p > 0.05$) in water content when compared with Lot 1 which represent not uninfected and untreated rats.

Effect of ETHA on the volume of diarrheal faeces

To gain additional information on the antibacterial activity of ETHA, the effect of ETHA on the

Table 3. Infection and mortality rate during experiment.

Lot	Before infection		24 h after infection		7 days after treatment	14 days after treatment	Death rate (%)
	IR (%)	MR (%)	IR (%)	MR (%)	MR (%)	MR (%)	
Lot 1	0	0	0	0	0	0	0
Lot 2	0	0	100	0	40	10	50
Lot 3	0	0	100	0	30	0	30
Lot 4	0	0	100	0	20	0	20
Lot 5	0	0	100	0	10	0	10
Lot 6	0	0	100	0	0	0	0
Lot 7	0	0	100	0	0	0	0

MR: Mortality rate, IR: infection rate; Lot 1: no infected and no treated; Lot 2: infected and untreated; Lot 3: infected and treated with ETHA at dose of 69. 44 mg/kg bw corresponding to 1 × MIC; Lot 4: infected and treated with ETHA at dose of 271. 87 mg/kg bw corresponding to 5 × MIC; Lot 5: infected and treated with ETHA at dose of 631. 3 mg/kg bw corresponding to 10 × MIC; Lot 6: infected and treated with ETHA at dose of 2222.22 mg/kg bw corresponding to 40 × MIC; Lot 7: infected and treated with ciprofloxacin at dose of 5 mg/kg bw.

volume of diarrheic faeces investigation was perfomed on both volume and weight of the rats' faeces (Figures 3 and 4). There was a significant difference ($p < 0.01$) on both volume and weight of diarrheic faeces. Also, there was no significant ($p > 0.05$) decrease in infected and treated rats from 69.99 to 2222.22 mg/kg body weight when compared with uninfected and untreated rats. Notably, it is observed that the weight and volume estimated for the Lot 6 is almost similar to that of the drug control (Lot 7).

DISCUSSION

The purpose of this study was to summarize the investigation of the antimicrobial properties of both the aqueous extract and organic fractions (ethyl acetate, dichloromethane and ethanol) of *A. leiocarpa* using antibacterial activity *in vitro* and *in vivo,* respectively.

This work made clear the advantages of the combination of these two experiments to obtain interesting information not only to gain insights into sensitivity or inhibitory measurement but also to obtain real time data from the direct effect of the fraction test on selected laboratory animal (the Wistar albino rats).

The results showed that microorganisms tested were susceptible to plant extracts and the highest inhibitory activity was observed for ethanol and ethyl acetate fractions which presented antibacterial activity against all bacteria examined.

These observations were consistent with previous study realized by Biyiti et al. (2004) who indicated that by a diffusion method, bacteria were sensible for IZD of 9 to 14 mm, very sensitive for IZD of 15 to 19 mm, highly sensitive for IZD larger than 20 mm whereas not sensible for IZD less than 8 mm, which is in accordance with the present results. Strains of *E. coli* ATCC 25922, *E. coli* ESBL, *P. aeruginosa*

131813, *S. Typhi* 43PI16, *S. Typhi* 1586, *S. dysenteriae* 1079PI15 and *K. oxytoca* were therefore sensitive to ethanolic fraction of *A. leiocarpa*. *S. aureus* Meti-R 1532C/10 and *Streptococcus* spp. were very sensitive to the ethanolic and ethyl acetate fractions of *A. leiocarpa* according to their inhibition diameters (14.57 ± 0.20 to 16.64 ± 0.56 mm). Similar results were obtained by Ichor et al. (2011) and Mann (2012), who obtained diameters of inhibition with strains of *S. Typhi*, *E. coli*, *Shigella* spp. and *P. aeruginosa*, ranging from 9 to 17 mm in the presence of the methanolic leaves extract of *A. leiocarpa*. In addition, Mann et al. (2008) showed that the ethanolic leaves extract of *A. leiocarpa* inhibit the *in vitro* growth of strains of *Pseudomonas* MDR, *S. aureus* Meti-R and *E. coli*.

MICs determined using the broth dilution method were correlated to those obtained by the diffusion method. The MIC value gave a measure of the antibacterial performance of antibiotics but it appeared that the MICs values of plant extracts and essential oils were not standardized. Also, it was important to note that there were no consensus on the inhibition concentration for natural products, hence the consistency of the antimicrobial activity results are based on the growth inhibition zones observed and the ratio of MBC/MIC estimated. The results of this study showed that, ethanolic fraction (ETHA) was the most active fraction which displayed the lowest MICs values, ranging from 3.12 to 12.5 mg/ml, while the ethyl acetate fractions (EAA) gave the

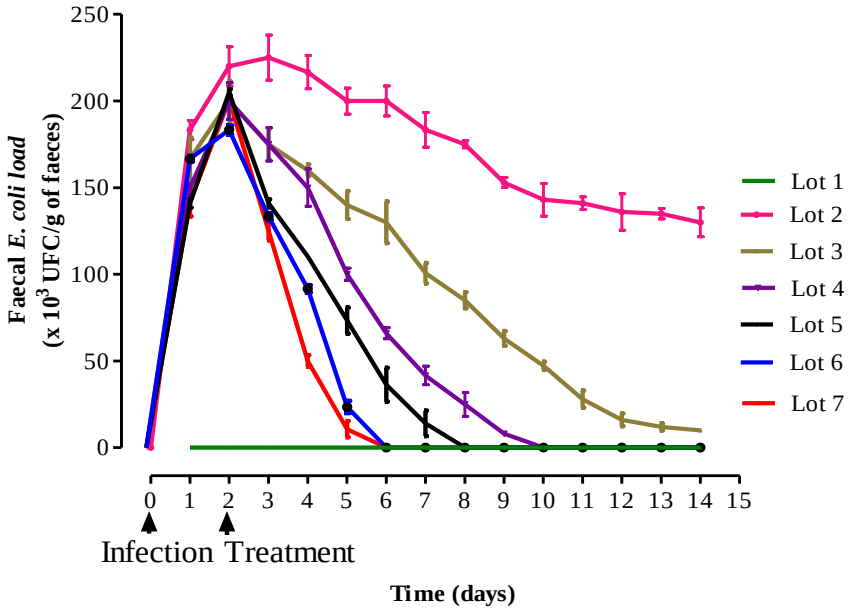

Figure 1. Evolution of faecal *Escherichia coli* ESBL load during experiment.

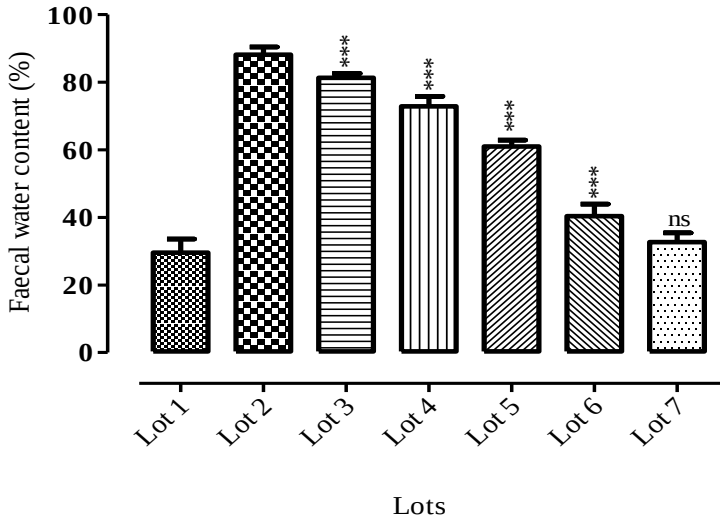

Figure 2. Variation of faecal water content during experiment. The values were expressed as Mean±SEM (n = 6 rats/Lot). ***p<0.001: significant difference compared to the infected and untreated Lot, ns: no significant when compared with the uninfected and untreated Lot at p<0.05. Lot 1: No infected and no treated, Lot 2: infected and untreated, Lot 3: infected and treated with ETHA at dose of 69,44 mg/kg bw corresponding to 1 × MIC, Lot 4: infected and treated with ETHA at dose of 271,87 mg/kg bw corresponding to 5 × MIC, Lot 5: infected and treated with ETHA at dose of 631,3 mg/kg bw corresponding to 10 × MIC, Lot 6: infected and treated with ETHA at dose of 2222.22 mg/kg bw corresponding to 40 × MIC, Lot 7: infected and treated with ciprofloxacin at dose of 5 mg/kg bw.

highest MICs that ranged from 6.25 to 25 mg/ml apart from *S. dysenteria* and *K. oxytoca* but no antibacterial activity were recorded with ETAA and EDMA. These results were similar to those of Timothy et al. (2015) and

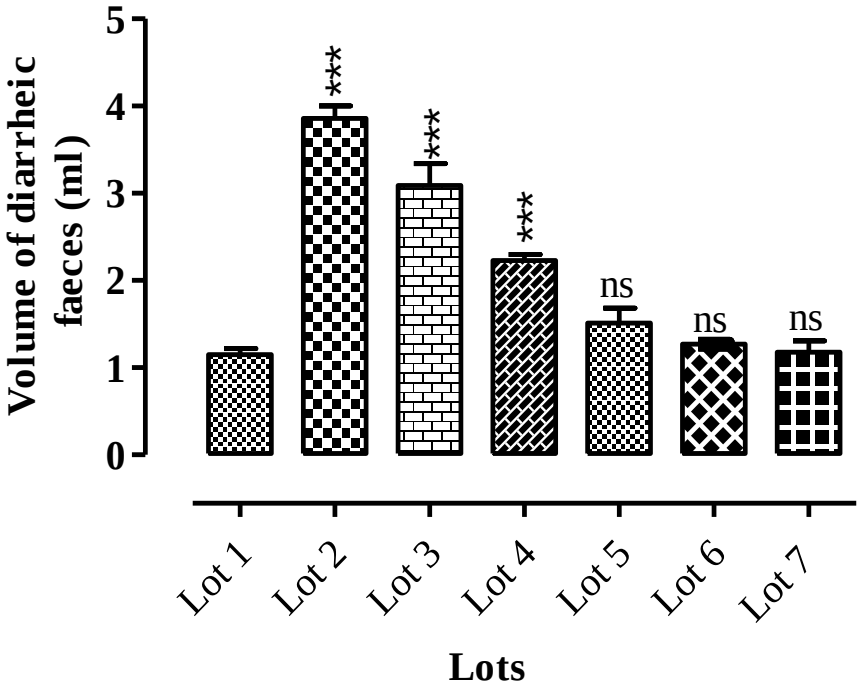

Figure 3. Volume of faeces of rats treated and untreated during experiment. Values are means±SEM. Each Lot includes 6 animals (n=6/Lot). ***p<0.001: significant difference compared to the uninfected and untreated Lot, ns: no significant at p<0.05 when compared with the uninfected and untreated Lot (Lot1). Lot 1: no infected and no treated, Lot 2: infected and untreated, Lot 3: infected and treated with ETHA at dose of 69.44 mg/kg bw corresponding to 1 × MIC, Lot 4: infected and treated with ETHA at dose of 271.87 mg/kg bw corresponding to 5 × MIC, Lot 5: infected and treated with ETHA at dose of 631.3 mg/kg bw corresponding to 10 × MIC, Lot 6: infected and treated with ETHA at dose of 2222.22 mg/kg bw corresponding to 40 × MIC, Lot 7: infected and treated with ciprofloxacin at dose of 5 mg/kg bw.

Ali et al. (2017) with MICs ranging from 6.3 to 44.6 mg/ml and 5 to 20 mg/ml, respectively with ethanolic and ethyl acetatefraction extract of *A. leiocarpa* barks. Although antibacterial activity was detected in both EAA and ETHA fractions against almost tested bacteria strains, the MBC values (minimum bactericidal concentration) also showed a similar pattern of activity from that of MIC. The extract action was bactericidal when the ratio of the MBC/MIC is <4 and bacterioatatic for MBC/MIC >4 (Berché et al., 1991).

Based on results scored, the EAA and ETHA extracts on sensitive bacteria were especially bactericidal against both Gram positive and Gram negative bacteria. This result could be explained by the fact that most antimicrobial active components were less polar compounds that were not water soluble and so the organic solvent extracts showed a more potent activity. Contrary to the use of dichloromethane which was an organic solvent, no bacterial activity has also been observed. That was in accordance with previous work of Mabiki et al. (2013) that showed for the most of part of terpenes/terpenoids compounds in dichloromethane extract. It was known that because of this chemical diversity, terpenes/terpenoids have great industrial uses as flavors, fragrances (Schwab et al., 2008), high grade lubricants, biofuels, agricultural chemicals and in the near future will play a more significant role in medicines (Niehaus et al., 2011).

On the whole, Gram-positive bacteria (*S. aureus* Meti-R 1532C/10 and *Streptococcus* spp.) were more sensitive than Gram-negative bacteria. Bari et al. (2010) corroborated the high resistance of some Gram-negative bacteria compared to Gram-positive bacteria. In fact, Gram-negative staining bacteria had efflux pumps that prevented the intracellular accumulation of antibacterial agents (Demetrio et al., 2015). It was noted that ethanolic fraction of *A. leiocarpa* demonstrated *in vitro* the most active antibacterial activity during this study. In order to confirm this interesting observation, this *in vivo* therapeutic activity was evaluated using an experimental bacterial diarrhea induced by the *E. coli* ESBL, a strain resistant to β-lactams.

Diarrhea could be measured by several parameters such as the water content, weight, volume, bacterial load

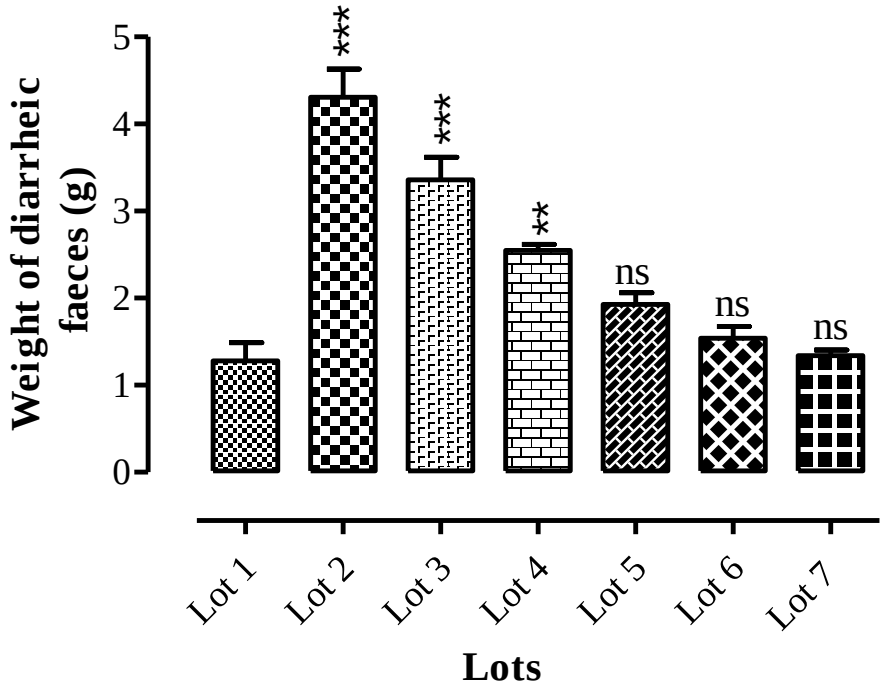

Figure 4. Weight of faeces of rats treated and untreated during experiment. Values are means±SEM. Each Lot includes 6 animals (n=6/Lot). ***p<0.001 and **p<0.01: significant difference compared to the uninfected and untreated Lot, ns: no significant at p<0.05 when compared with the uninfected and untreated Lot (Lot1). Lot 1: no infected and no treated, Lot 2: infected and untreated, Lot 3: infected and treated with ETHA at dose of 69.44 mg/kg bw corresponding to 1 × MIC, Lot 4: infected and treated with ETHA at dose of 271.87 mg/kg bw corresponding to 5 × MIC, Lot 5: infected and treated with ETHA at dose of 631.3 mg/kg bw corresponding to 10 × MIC, Lot 6: infected and treated with ETHA at dose of 2222.22 mg/kg bw corresponding to 40 × MIC, Lot 7: infected and treated with ciprofloxacin at dose of 5 mg/kg bw.

in the diarrhea feces and death of the specimen. For this instance, an infective dose of *E. coli* of 2×10^8 CFU/ml similar to that used by Mushtaq et al. (2005) was used to induce experimental *E. coli* diarrhea in rats. This dose brings about a colonization of the gastrointestinal tract in 24 h, and was responsible for dehydration, loss of appetite, appearance of demolded, and soft, liquid or semi-liquid light brown faeces (Forrester, 2002).

Mortality rate of 50% was observed in the untreated and infected Lot but no death was noted for both Lots treated with Ciprofloxacin and the ETHA fraction of 631.3 and 2222.22 mg/kg body weight, respectively. In fact, a significant reduction ($p < 0.001$ and $p < 0.01$) of rats' death was observed from 30 to 0% when increasing the dose of ETHA and a decrease of the rats weight and volume of diarrheal faeces indicating a progressive eradication of the *E. coli* inducted infection compared to infected and untreated Lots. These results illustrated the therapeutic action of the ETHA fraction.

These observation was corroborated by an important decreasing of faecal *E. coli* ESBL load during the experiment that may be ascribed to the healing power of the extract. After the treatment, the bacterial load of the infected and treated Lots decreased significantly ($p < 0.001$) as during the treatment and dose-dependent manner compared to the untreated infected Lot. Similar results were obtained by Niehaus et al. (2011) and Ibrahim and Sarhan (2015) when enumerating *E. coli* from diarrheal rat faeces. These results highlighted the therapeutic potentialities of the ethanolic fraction of *A. leiocapa.*

The antibacterial effect of *A. leiocarpa* extracts could be attributed to the bioactive compounds present in the extracts such as phenolic compounds (flavonoids, tannins, leucoanthocyanins and polyphenols), and saponins and sterols (Moronkola and Kunle, 2014). It has been suggested that bioactive compounds act through two main mechanisms of action. The first is related to their hydrophobic property, which facilitates their adhesion to the surface of bacteria, causing their instability (Jongbloed et al., 2007). The second was the inactivation of different bacterial molecules such as enzymes or receptors, following the binding of bioactive compounds (Pandey and Kumar, 2013). Some of these compounds

could lead to cell membrane perturbations and exert a β-lactam action on the transpeptidation of the cell wall. Other compounds could interact with the lipid bilayers in cell membranes, leading to the separation of the two membranes, thus leading to cellular swelling and cell death (Tshingani et al., 2017).

The reduction in the bacterial burden of faeces in infected and untreated rats may be explained by the protective action of their immune system against the pathogens (Lunga et al., 2014). The *in vitro* and *in vivo* analyses of antibacterial activity revealed the antibacterial and therapeutic potential of *A. leiocarpa*.

Conclusion

This study showed that root barks of *A. leiocarpa* extracts possessed antibacterial activity by inhibiting *in vitro* bacterial growth. Its therapeutic activity was carried out against *E. coli* in experimentally infected rats by the significant reduction of defecation frequency, water content of faeces, weight and volume of diarrheic faeces, and *E. coli* load of faeces. The present results demonstrated that *A. leiocarpa* should be used in the traditional therapeutic arsenal against resistant infectious germs.

Abbreviations

ETHA, Ethanol fraction of *Anogeissus leiocarpa*; **ETAA,** total aqueous extract of *Anogeissus leiocarpa*; **EDMA,** dichloromethane fraction of *Anogeissus leiocarpa*; **EAA,** ethyl acetate fraction of *Anogeissus leiocarpa.*

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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