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ANTIBACTERIAL ACTIVITIES AND SYNERGISTIC EFFECT OF THE BIOACTIVE COMPOUNDS OF SELECTED MEDICINAL PLANTS AGAINST DIARRHOEA-CAUSING PATHOGENS

Murtala Saadu[1], Nasiru Usman Adabara[2], Faruk Abubakar Kuta[3], Hadiza Lami Muhammed[4], Mohamed Wuna[5]

[1]Kebbi State University of Science and Technology, Aliero, Nigeria

[2,3,4,5]Federal University of Technology, Minna, Nigeria

murtalas123@gmail.com

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Abstract

In this study, the antimicrobial properties and toxicological effects of Anogeissus leiocarpus and Khaya senegalensis, two traditional medicinal plants from West Africa, were investigated against diarrhea-causing pathogens. Cold maceration was used to prepare extracts from the plants. Anogeissus leiocarpus yielded 22.87g of extract, while Khaya senegalensis yielded 13.94g. Both plant crude extracts exhibited varying degrees of antibacterial activity against Vibrio cholerae, Klebsiella pneumoniae, and Salmonella enterica serovar at different concentrations. A. leiocarpus and K. senegalensis showed the highest antibacterial activity, with significantly higher zones of inhibition at all concentrations against all test organisms. The Minimum Inhibitory Concentration (MIC) for A. leiocarpus ranged from 0.10 to 0.96 mg/mL, while the Minimum Bactericidal Concentration (MBC) ranged from 0.10 to 1.09 mg/mL. For K. senegalensis, MIC ranged from 0.96 to 1.80 mg/mL, and MBC ranged from 1.02 to 1.92 mg/mL. Fractionation of the most active crude extracts resulted in the highest yields in the n-Hexane fractions for both A. leiocarpus and K. senegalensis. Significant differences were observed in the

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antibacterial activity of these fractions. K. senegalensis fractions and A. leiocarpus n-Hexane fraction showed the highest activity against V. cholerae, while the ethyl acetate fraction of K. senegalensis exhibited significant activity against K. pneumoniae. The aqueous fraction of A. leiocarpus displayed the highest activity against Salmonella, whereas none of the K. senegalensis fractions were active against Salmonella. Antibacterial activity of K. senegalensis ethyl acetate and A. leiocarpus n-Hexane and aqueous column chromatography fractions against the test organisms was concentration-dependent, with the highest antimicrobial activity observed at 200 mg/mL concentration. Importantly, there were no significant differences in the body weights of experimental animals across all groups. In conclusion, A. leiocarpus and K. senegalensis extracts and their fractions demonstrated promising antimicrobial properties against diarrhea-causing pathogens. These findings support their traditional medicinal use in West Africa and suggest their potential as natural remedies for combating bacterial infections.

Keywords: Medicinal Plants, Antimicrobial Activity, Diarrhea Pathogens, Anogeissus Leiocarpus, Khaya

INTRODUCTION

Diarrhea is one of the leading causes of morbidity and mortality worldwide, especially in developing countries in sub-Saharan Africa and Southeast Asia (Cohen et al., 2022). According to the report of the global enteric multicenter study, secretory diarrhea is a historically known serious health problem around the world that primarily originates through pathogenic microorganisms including *Escherichia coli, Shigella* species*, Salmonella* species*, Proteus* species*, Yersinia* species*, Vibrio cholerae and Campylobacter* species. These are some of the common infectious agents which cause enteric disease rather than immunological or genetic disorders, (WHO, 2015). Most diarrhoea-associated morbidities and mortalities occur in low-income and medium-income countries, usually in rural areas as well as in the suburbs and slums of urban areas (Mohammed et al., 2014). In these settings, the incidence is further fuelled by the vicious cycles of poverty, ignorance, malnutrition, and endemic infectious diseases. Issues directly or remotely connected to socio-environmental factors such as sanitation and quality of water, unhygienic feeding practices (including hand hygiene), suboptimum breastfeeding, zinc deficiency, and barriers to appropriate and affordable health care exist as catalysts for diarrheal disease burden among under-fives in these parts of the world (WHO, 2018). Large numbers of plant

species have been documented for the treatment of various ailments and serve as remedies for human diseases because they contain chemical components of therapeutic value (Ibrahim et al., 2016). Nigeria is blessed with a large number of plant species such as *Acacia nitolitica, Carica papaya, Khaya senegalenses, Ficus sycomorus, and Piliostigma thonningii,* some of which have been in use for centuries to diagnose, prevent, and treat various ailments. The exploration of newer antimicrobial agents in plants brings about a different approach to minimizing antibiotic resistance and thus offers potential benefits (Kowero et al., 2015). The medicine quest focuses on the drug of the future that will be derived from natural products (Eng et al., 2015). The search for unfamiliar plants in the wild with potential value as human and animal food as well as curative medicine is gathering momentum (Okorondu et al., 2015). The derivatives of these plants are claimed to have several medicinal and other desirable properties (Chomini et al., 2021). Furthermore, the nontoxic nature of most chemicals in plants, positive healthy properties, consumer perception, and acceptance of their use have been well demonstrated. There are an estimated 250,000–500,000 species of plants on Earth. A relatively small percentage (1–10%) of these is consumed as food by both humans and animal species. A greater number may be used for medicinal purposes. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants. Currently, antimicrobial plant extracts are of special interest to chemists and microbiologists due to growing public awareness of the negative effects of the overuse of antibiotics and disinfectants. Therefore, the research aims to determine the antibacterial activities and synergistic effect of the bioactive compounds of selected medicinal plants against diarrhoea-causing pathogens.

METHODS

Study Area

The study was carried out in General Hospital Minna, Niger State, Nigeria. Which is the major hospital attended by the populace. Minna is a city in Middle Belt Nigeria, consisting of 2 Major ethnic groups: Nupe and Gwari. Minna Lies between Latitude 9.58360 N and Longitude 6.54630 E at an altitude of 256m above sea level and has a land area of about 88 km2 (www.minna.climatetemps.com/map.php).

Sample Collection

Stool samples from diarrhea patients were collected in sterile sample bottles, packaged in icepacks, and transported to the microbiology laboratory at the Center for Genetic Engineering and Biotechnology, Federal University of Technology, Minna.

Isolation of Diarrhoea Causing Pathogens

Samples collected were inoculated onto MacConkey agar, Eosine methylene blue (EMB) agar, and Thiosulfate Citrate Bile-salts Sucrose (TCBS) agar and incubated at 37^0C for 24 hours. Distinct colonies were sub-cultured repeatedly to obtain pure cultures of diarrhoea-causing pathogens, which were stored on nutrient agar slant for further use.

Plant Sample Collection and Identification

Fresh leaves of five medicinal plants Anogeissus leiocarpus (Marke) (KSUSTA/PSB/H/84D), Khaya senegalenses (Madaci) (KSUSTA/PSB/H/61A) were collected in Minna, Niger State. The plants were identified at the Department of Plant Biology School of Life Sciences, Federal University of Technology Minna, Niger State.

Extraction of the Crude Extracts

The plant extraction procedure was carried out according to the method described by AOAC (2010). The different parts of the plant were dried under shade at room temperature for at least 7 days, segregated, and pulverized by a mechanical grinder to form a coarse powder. 100g of powder samples were weighed and macerated into 500 ml of methanol for 72 h in the ratio of 1:5 (w/v) respectively. The supernatant obtained was filtered using Whatman No. 1 filter paper and evaporated until dried under reduced pressure (204 mbar) at the temperature of 400C.

Column Chromatography

Ten grams (10g) of aqueous 5g of n-hexane of *Anogessus leocarpus* and 5g of Ethyl acetate fraction of *khaya senegalensis* were subjected to column chromatography to separate the extract into its component fractions. A 120g Silica gel for column chromatography (60-120 mesh) was used as the stationary phase, and the solvent system, n-hexane: ethyl acetate: methanol as the mobile phase. The plant extracts were loaded on top of the packed column. The elution of the extract was done using the solvent system; hexane: ethyl acetate: methanol (100:0:0 % v/v) to (0:80:20 % v/v) respectively 100% each. The eluent was collected into sterile sample bottles (Doughari, 2012).

UV Analysis

After column chromatography the eluates were scanned using Double beam Shimadzu UV visible spectrophotometer (UV-1800 series) to ascertain the eluates with the same compounds, as to bulk them together (Sarker et al.,2006) The eluates bulked together were properly labeled as fractions. The fractions were air-dried using a water bath at 45 C, and the dried fractions were weighed, and later screened against the test bacteria compounds responsible for the activities confirmed using GC-FID.

In vivo Toxicity Studies

An acute oral toxicity test was carried out according to Lawal et al method (2017). It involved two phases (Phase 1 and 2) of three stages each. The first phase involved the oral administration of the extract with the highest activity, to the three groups of the Wistar albino rats, each group comprising three Wistar albino rats, group one received 10ml/kg bodyweight 100mg/kg body weight and 1000ml/kg bodyweight after an overnight fasting of the animals and control group received 1ml respectively. Phase 2 animals in their respective group receive doses of 1600, 2000, and 5000, depending on the result obtained from the Phase 1 experiment. The side cage observation was done within the first 6 hours and the mortality rate was monitored for 48 hours.

Sub-Acute Toxicity Studies

The sub-acute toxicity studies were carried out according to Dekant and Vanvakas (2005), the dose of the extract with the highest activity was to be administered in sub-acute toxicity studies and this depended on the result obtained from the acute toxicity test. However, rats were grouped into six, each Group having four rats, Group four and five was negative and positive control while Group six was normal respectively, the extracts with the highest activity was administered (after overnight fasting) to the remaining group once in a day for twenty-eight (28) days. All clinical signs, mortality, and morbidity were recorded. At the end of the experiment the animals were euthanized under s mild chloroform and the blood samples were collected from the jugular vein for hematological and biochemical analysis using EDTA and sample bottles respectively.

Experimental Animals

Adult Wistar rats of both sexes with weights ranging between 120 and 130 g were bought from Olatunji farms in Ogbomosho, Oyo State Nigeria and were used for the study. The

rats were kept in plastic cages in a conventional laboratory setting of 37 ^{0}C and relative humidity of 40-45 ^{0}C. The animals were fed with finisher (Vital Feeds Nigeria) and tap water for three weeks as they were allowed to acclimatize to the settings above ahead of the research.

Animal Grouping

The rats were divided into ten (10) groups each with three rats and were administered with various proportions of the plant fractions.

Infection of Animals

The three rats from each group were infected with the test organisms using the method described by Pan et al. (2014). Two milliliters (2ml) of saline solution (0.9% NaCl) containing 1.5x10^8 CFU of the test organism was injected into the rats in each group, injection of the solution was done intrapritoneally After 72 hours blood culture was collected through the tail (jugular method) from each group into already prepared 5 ml nutrient broth in test tubes which was then inoculated unto Petri dishes containing nutrient agar and incubated for 24 hours. After this the growth of microorganisms was observed, immense of microorganism was indicated on the plates that the animals was infected.

Administration of the Plant Fractions

Aqueous and ethyl acetate plant fractions of 100 and 300mg/kg BW were used to treat the animals for seven days (7) the concentrated fractions were chosen based on the previous literature Muhammad et al. (2015). The fractions were administered twice a day, one 1ml in the and 1ml in the evening alongside feeding but the animal in group ten (10) received no treatment, they were only fed and given water, During treatment the weight of the animals was taken each day, their feces observed daily and behavior toward eating was observed on daily basis.

Animal Sacrifices and Blood Culture

Microbial blood culture was carried out on each of the animals before they were sacrificed to ascertain the level of activity of the plant fractions in treating their infections. Blood samples were collected using jugular methods, dropped on Petri dish soap, and mixed with 20ml of nutrient agar immediately. This was incubated at 37^0 C for 24 hours and the colonies on the plates were identified and counted with the compared result of the blood

culture done before the treatment, which indicated the efficacy of plant fractions after the treatment.

Statistical Analysis

The data was evaluated using analysis of variance (ANOVA) and was presented as the mean value ± SEM (standard error of the mean). Differences among the means for the groups were assessed using Duncan's Multiple Range Test to determine which mean values were significantly different at $p<0.05$.

RESULTS

Extraction Yields of Plants

The highest extract yield was obtained from *Anogeissus leiocarpus* (22.87g) followed by *Khaya senegalensis* (13.94g).

Table 1: Yield of Extract from Plants used in this study

Plants	Extraction Yield (g)
Anogeissus leiocarpus	22.87
Khaya senegalensis	13.94

The MIC and MBC of the crude extracts of *Anogeissus leiocarpus* and *Khaya senegalensis* are shown in Table 1. The MIC of *A. leiocarpus* against *Vibrio cholera, Klebsiella pneumoniaee*, and *Salmonella enterica* serovar *Kentucky* were observed at 0.96±0.03, 0.19±0.00 and 0.96±0.06mg/mL respectively, while the MBC was observed at 1.09±0.87, 0.10±0.05 and 1.04±0.17mg/mL respectively. For *K. senegalensis,* MIC was observed at 0.96±0.04, 0.96±0.05 and 1.80±0.17mg/mL, while MBC was recorded at 1.02±0.12, 1.04±0.29 and 1.92±0.23 mg/mL concentrations.

Table 2: Minimum Inhibitory and Minimum Bactericidal Concentrations

Plant Extract	MIC (mg/mL)			MBC (mg/mL)		
	*Vibrio cholerae*e	*Klebsiella pneumoniae*e	Salmonella enterica serovar Kentucky	*Vibrio cholerae*e	*Klebsiella pneumoniae*e	Salmonella enterica serovar Kentucky
Anogeissus leiocarpus	0.96±0.03	0.19±0.00	0.96±0.06	1.09±0.87	0.20±0.05	1.04±0.17
Khaya senegalensis	0.96±0.04	0.96±0.05	1.80±0.17	1.20±0.12	1.40±0.29	1.920±0.23
Control	0.005	0.005	0.020	0,005	0.005	0.020

Values are presented as mean ± standard error of the mean (SEM) of triplicates
MIC = Minimum Inhibitory; MBC = Minimum Bactericidal Concentrations

Table 3 presents the Column Chromatography yields of *Khaya senegalensis* and *Anogeissus leiocarpus* fractions. UV spectroscopy of fractions *of Anogeissus leiocarpus* and *Khaya senegalensis* was done at a range of 200 to 700nm.

Table 3: Column Chromatography yields of *Khaya senegalensis and Anogeissus leiocarpus* fractions

Fractions	***K. senegalensis***	***A. leiocarpus***
	Ethyl acetate	**Aqueous**
1	0.0407	0.551
2	0.7610	0.076
3	0.1442	0.044
4	0.02367	0.976
5	0.5616	0.394
6	0.23965	1.237

7	0.8987	0.552
8	-	1.368
9	-	2.235

In-vivo Antibacterial Susceptibility of Aqueous *Anogeissus leiocarpus* and Ethyl Acetate *Khaya senegalensis* Fractions

In-vivo Antibacterial activity of the Aqueous *Anogeissus leiocarpus* and Ethyl acetate *Khaya senegalensis* fractions is shown in Table 4. The in vivo assay showed that the Ethyl Acetate fraction of *Khaya senegalensis* had higher activity than the aqueous fraction of *Anogeissus leiocarpus*. The in vivo activity was dose concentration dependent with 300mg/ml having the highest colonies reduction for all the test organisms.

Table 4: In-vivo Antibacterial Susceptibility

Extracts	**Concentrations (mg/mL)**	***V. choleraee*** **(±SD) Cfu/p**	***K. pneumonia*** **(±SD) Cfu/p**	***S. enterica*** **serovar Kentucky (±SD) Cfu/p**
Ethyl acetate *K. senegalensis* fraction	100	59.00±4.62	24.00±3.46	12.10±1.54
	300	48.00±3.46	21.33±1.86	8.00±1.43
Aqueous *Anogeissus leiocarpus* fraction	100	71.33±1.86	68.67±2.33	25.50±3.14
	300	47.00±4.04	50.00±1.73	17.00±2.32
Positive control	100	29.33±3.18	12.00±2.56	43.00±4.62
Negative control	NS	180.67±4.41	124.67±5.87	157.67±6.39

Key: AS: *Anogeissus leiocarpus;* KS: *Khaya senegalensis*

Effect of Plant Fractions on Body Weight Gain of infected mice

The effect of plant fractions on the body weight gain of infected mice is shown in Table 5. There was no significant difference between the bodyweights of all experimental animals from all groups when compared to the control.

Table 5: Effect of Plant Fractions on Body Weight Gain of infected mice

Treatment	Doses (mg/kg bw)	Body weight (g)			
		WEEK 0	WEEK 1	WEEK 2	WEEK 3
Aqueous (*Anogeissus leiocarpus*)	100	125.44±2.54[a]	134.81±2.67[a]	142.70±2.21[a]	149.59±2.00[a]
	200	123.41±3.82[a]	131.40±3.13[a]	139.66±3.32[a]	147.15±2.95[a]
	300	122.29±2.19[a]	130.36±2.42[a]	139.51±1.88[a]	147.27±1.37[a]
Ethyl acetate (*Khaya senegalensi*)	100	123.43±2.06[a]	132.30±1.42[a]	140.52±1.00[a]	148.11±0.90[a]
	200	124.17±3.85[a]	131.98±3.66[a]	139.87±4.68[a]	147.57±4.79[a]
	300	125.57±4.62[a]	134.48±3.78[a]	143.36±2.43[a]	150.58±2.49[a]
Hexane (*Anogeissus leiocarpus*)	100	122.82±1.57[a]	131.31±2.87[a]	138.46±2.97[a]	145.65±3.19[a]
	200	124.09±3.52[a]	131.49±2.64[a]	138.06±2.82[a]	145.63±2.68[a]
	300	124.46±2.94[a]	133.57±4.10[a]	140.72±3.79[a]	148.15±3.52[a]
control		123.38±3.76[a]	134.17±3.09[a]	143.35±3.38[a]	152.24±3.98[a]

Values are presented as mean ± standard error of the mean (SEM) of three replicates.

Values with different superscripts in a column are significantly different at $p < 0.05$

DISCUSSION

The minimum inhibitory concentration (MIC) is the smallest concentration that visibly inhibits growth. The MIC is useful in determining the smallest effective dosage of a substance against an organism (Kuta et al., 2015). The MIC of *Anogeissus leiocarpus* against *Vibrio choleraee, Klebsiella pneumoniaee*, and *Salmonella enterica* serovar *Kentucky* was observed at 0.96±0.03, 0.19±0.00 and 0.96±0.06mg/mL respectively, while the minimum bactericidal concentration (MBC) was observed at 24.00±0.87, 0.96±0.05 and 4.80±0.17mg/mL

respectively. For *Khaya senegalensis* MIC was detected at 0.96±0.04, 0.96±0.05 and 4.80±0.17 mg/mL concentration, while MBC was recorded at 4.80±0.12, 4.80±0.29 and 4.80±0.23 mg/mL concentration. Plant extract is considered to have a good inhibitory activity, if it presents MIC value ≤ 100mg/mL, a moderate inhibitory activity when MIC ranges between 100 to 500mg/mL, a weak inhibitory activity if the MIC value ranges between 500 to 1000mg/mL and no inhibitory activity when > 1000mg/mL.

Considering this report, the MIC and MBC values recorded from the antibacterial activity of the present study showed good inhibitory activity. The current findings lend credence to the traditional use of this plant as a medicine for infectious diseases particularly those caused by the test organisms susceptible to the extracts. However this result is contrary to the findings of Usman et al. (2014), Kuta et al. (2015), Abdallah et al. (2016), Ali et al. (2017), and Salih et al. (2020) who all reported much higher MIC and MBC values against various bacterial pathogens. Yuan et al. (2013) observed methanolic and ethanolic stem bark extracts of *Khaya senegalensis* to have an MIC value of 250 and 200mg/mL respectively against *Salmonella enterica* subsp. enterica serovar Typhi. MIC value of 25mg/ml was reported by Salih et al. (2020) for methanolic stem bark extract of *Khaya senegalensis* against both *Klebsiella pneumoniae* and a carbapenem-resistant strain of *Escherichia coli.*

For the aqueous extract, MIC was detected at 100, 200, and 100 mg/mL, while the ethanolic extract had MIC values at 200 400, and 200mg/mL against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus mutans* respectively. The MBC was detected at 400mg/mL for both aqueous and ethanolic extract against all the isolates. The variation in the MIC and MBC values reported in these studies could be due to the phytochemical composition of their respective extracts which is usually dependent on the polarity of the solvents used for extraction, as solvent type tends to influence the kind of bioactive compound released from the plant materials. Also, variation in results could be attributed to the genetic makeup of each test organism used. Different organisms have been shown to respond differently to different and same concentrations of a specific antimicrobial substance (Altemimi et al., 2017; Salih et al., 2020).

The antibacterial activity of *Khaya senegalensis* ethyl acetate column chromatography fractions against *Vibrio cholerae, Klebsiella pneumoniaee*, and *Salmonella enterica* serovar *Kentucky* was concentration-dependent as inhibition zones increased with increasing concentration of the extract. Significantly highest antimicrobial activity against *Vibrio choleraee* was observed in

fraction 7 (30.00±1.15) at 200mg/mL concentration, for *Klebsiella pneumoniaee* highest activity was observed at fraction 5 (24.00±1.73) at 200mg/mL concentrations while in the case of *Salmonella enterica* serovar *Kentucky* significant activity was observed in fraction 6 (32.00±2.31) 200mg/mL concentrations. The variation in the antibacterial activity of these fractions could be attributed to the differences in active components present in each fraction and the concentration of fractions. Also, the difference in susceptibility of each bacteria to the fractions could contribute to such variations (Angienda et al., 2010).

Antibacterial Activity of *Anogeissus* leiocarpus n-Hexane Column chromatography fractions against *Vibrio cholerae, Klebsiella pneumoniaee*, and *Salmonella enterica* serovar *Kentucky* showed significant differences in the zones of inhibition. Antibacterial activity was concentration-dependent as inhibition zones increased with increasing concentration of the extract. Significantly highest antimicrobial activity against *Vibrio choleraee* was observed in fraction 2 (22.67±0.75) at 200mg/mL concentration, for *Klebsiella pneumoniaee* highest activity was observed at fraction 2 (24.00±0.58), fraction 3 (25.67±1.11), and fraction 4 (24.00±1.73) all at 200mg/mL concentrations while in the case of *Salmonella enterica* serovar *Kentucky* significant activity was observed in fraction 1 (24.00±1.39, 24.00±0.75 and 26.00±1.33) at 50, 100 and 200mg/mL concentrations and fraction 4 (24.00±1.27) at 200ml/ml concentration. Variations in the antibacterial activity of these fractions could mostly be attributed to the differences in active components present in each fraction and the difference in susceptibility of each bacteria to the fractions (Angienda et al*.,* 2010).

A significant difference was observed in the zones of inhibition. Antibacterial activity was concentration-dependent as inhibition zones increased with increasing concentration of the extract. Fractions 1-4 showed no activity against *Vibrio cholerae*. Significantly highest activity against *V. cholerae* was observed in fraction 6 (42.00±1.27) at 200mg/mL concentration. Against *Klebsiella pneumoniaee* and *Salmonella enterica,* serovar *Kentucky* Fraction 1-5 showed no significant activity. The highest significant activity against *Klebsiella pneumoniaee* was observed in fraction 6 (32.00±1.15), fraction 7 (32.00±1.15), and fraction 9 (32.00±1.15) all at 200mg/mL concentration. While for *Salmonella enterica* fraction 8 (32.00±1.15) at 200mg/mL was the highest observed. Variations in the antibacterial activity of these fractions could mostly be attributed to the differences in active components present in each fraction and the difference in susceptibility of each bacteria to the fractions (Angienda et al., 2010).

The *in vivo* assay showed that the Ethyl Acetate fraction of *Khaya senegalensis* had higher activity than the aqueous fraction of *Anogeissus leiocarpus*. The dose concentration of 300mg/ml of the extracts had the highest colony reduction for all the test organisms, suggesting that as the dose of the extracts increases the number of colonies reduced. There was no significant difference when the concentrations used were compared, this could be attributed to the fact that antimicrobial activities of substances are a function of active compounds reaching an organism (Yunana et al., 2018). When treatment groups were compared with negative control, there was a significant difference between the treatment groups and the negative control suggesting, that plant extracts may have antibacterial activity and could be employed in the treatment of infectious diseases.

Change in body weight is a sensitive and predictive toxicity marker (Rao et al., 2016). Monitoring the body weight during treatment provides a fair index of the general health status of experimental animals (Dawkins et al. 2003). The obtained results for the effect of *Anogeissus leiocarpus* and *Khaya senegalensis* fractions on the weight of experimental animals indicate that there was no significant difference ($p < 0.05$) in body weight of all test animals when compared to the control after exposure to various doses of the extracts. Similar finding was observed by Onu et al. (2013), and Oyeleke et al. (2008). Changes in body weight have often been used as an indicator of toxicity of substances; an increase in body weight is an indication of inflammation, while the reduction in the same parameter can be attributed to cellular constriction (Agaie, et al., 2007). As such, the lack of any significant change in body weight as observed in this study means that the animals were in a good physical state which is a likely indication that the extracts were non-toxic.

CONCLUSION

In conclusion, this study highlights the significant antimicrobial potential of *Anogeissus leiocarpus* and *Khaya senegalensis*, two traditional medicinal plants from West Africa, against diarrhea-causing pathogens. The extracts and fractions of these plants demonstrated varying degrees of antibacterial activity, with *A. leiocarpus* and *K. senegalensis* exhibiting the highest efficacy. Importantly, the study also found no adverse effects on the body weights of experimental animals, suggesting the safety of these natural remedies.

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