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**Original Article****Open Access*****In vivo* assessment of antibacterial activity of *Cassia sieberiana* stem bark extracts on enterohaemorrhagic *Escherichia coli* infection in Wister rats**¹Usanga, V. U., ¹Ukwah, B. N., ²William, O., *¹Kalu, M. E., ³Akpan, J. L., ¹Azi, O. S., and ¹Ude, U. A.¹Department of Medical Laboratory Science, Faculty of Health Science and Technology, Ebonyi State University, Abakaliki, Nigeria²Department of Laboratory Services, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria³Department of Pharmacology, University of Calabar, Cross River State, Nigeria*Correspondence to: kalu.erem@ebsu.edu.ng; +234806 402 8592**Abstract:**

Background: The acceptance of traditional medicine as an alternative form of health care has led researchers to further investigate the antimicrobial and other health benefits of medicinal plants including *Cassia sieberiana*. The objective of this study is to assess the *in vivo* antibacterial effects of *C. sieberiana* stem bark extracts on infections caused by human and animal isolates of enterohaemorrhagic *Escherichia coli* (EHEC) in Wister rats.

Methodology: This *in vivo* study was designed for 21 days in 3 phases of 7 days each; adaptation, infection and treatment. *Escherichia coli* were isolated from aerobic cultures of human and cattle faecal samples and EHEC 0157 identified by serological typing using latex agglutination method. Aqueous and ethanol extracts of authenticated *C. sieberiana* stem bark, were prepared using standard method. Forty-five Wister rats were randomly divided into 9 groups (A-I) of 5 rats each. Rats in group A (uninfected with human or animal EHEC isolate and untreated) served as negative control while rats in group B (infected with EHEC animal isolate and untreated) and group C (infected with EHEC human isolate and untreated) served as positive controls. Rats in group C through group I were experimental groups that were either infected with human or animal EHEC isolate and treated, or uninfected but treated with ethanol and aqueous extracts of *C. sieberiana*. During each of the study phase, faecal samples were collected from the rats and processed for evaluation of EHEC count and to determine faecal occult blood. Data were analyzed using the Statistical Package for Social Sciences, version 20.0 and categorical variables were compared with Pearson's Chi-square, with significant value taken as $p < 0.05$

Results: Three EHEC isolates (2 from cattle and 1 from human) were identified from 22 *E. coli* isolates cultured from cattle and human faecal samples. Rats in group A (negative control) and those in group G (infected with EHEC human isolate and treated with ethanol extract), group H (not infected but treated with aqueous extract) and group I (not infected but treated with ethanol extract) were faecal occult blood negative throughout the study period. Rats in group B (infected with animal isolate of EHEC without treatment) were occult blood negative after infection on day 14 but positive on day 21, while rats in group C (infected with human isolate of EHEC without treatment) were occult blood positive on day 14 but negative on day 21. Rats in groups D, E and F infected with human and cattle EHEC isolates and treated, were faecal occult blood positive on day 14 but negative on day 21, with high colony counts recorded, cleared within 7 days of treatment by both aqueous and ethanolic extracts of *C. sieberiana*.

Conclusion: The findings of this study confirmed the antibacterial potentials of *C. sieberiana* stem bark against EHEC. The beneficial effects of this plant extract should be exploited for commercial medicinal purposes.

Keywords: *Cassia sieberiana*; Enterohemorrhagic *Escherichia coli*; Wister rats; Antibacterial; *In vivo* assessment

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Évaluation *in vivo* de l'activité antibactérienne d'extraits d'écorce de tige de *Cassia sieberiana* sur l'infection entérohémorragique à *Escherichia coli* chez des rats Wister

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Résumé:

Contexte: L'acceptation de la médecine traditionnelle comme une forme alternative de soins de santé a conduit les chercheurs à étudier plus avant les avantages antimicrobiens et autres pour la santé des plantes médicinales, y compris *Cassia sieberiana*. L'objectif de cette étude est d'évaluer les effets antibactériens *in vivo* des extraits d'écorce de tige de *C. sieberiana* sur les infections causées par des isolats humains et animaux d'*Escherichia coli* entérohémorragique (EHEC) chez des rats Wister.

Méthodologie: Cette étude *in vivo* a été conçue sur 21 jours en 3 phases de 7 jours chacune ; adaptation, infection et traitement. *Escherichia coli* a été isolé à partir de cultures aérobies d'échantillons fécaux humains et bovins et EHEC 0157 identifié par typage sérologique à l'aide de la méthode d'agglutination au latex. Des extraits aqueux et à l'éthanol d'écorce de tige de *C. sieberiana* authentifiée ont été préparés en utilisant la méthode standard. Quarante-cinq rats Wister ont été répartis au hasard en 9 groupes (A-I) de 5 rats chacun. Les rats du groupe A (non infectés par l'isolat EHEC humain ou animal et non traités) ont servi de contrôle négatif tandis que les rats du groupe B (infectés par l'isolat animal EHEC et non traité) et du groupe C (infectés par l'isolat humain EHEC et non traité) ont servi de contrôle positif. Les rats du groupe C au groupe I étaient des groupes expérimentaux qui étaient infectés avec un isolat EHEC humain ou animal et traités, soit non infectés mais traités avec de l'éthanol et des extraits aqueux de *C. sieberiana*. Au cours de chacune des phases de l'étude, des échantillons fécaux ont été prélevés sur les rats et traités pour l'évaluation du nombre d'EHEC et pour déterminer le sang occulte fécal. Les données ont été analysées à l'aide du package statistique pour les sciences sociales, version 20.0 et les variables catégorielles ont été comparées au chi carré de Pearson, avec une valeur significative prise comme $p < 0,05$.

Résultats: Trois isolats d'EHEC (2 bovins et 1 humain) ont été identifiés à partir de 22 isolats d'*E. coli* cultivés à partir d'échantillons fécaux bovins et humains. Les rats du groupe A (témoin négatif) et ceux du groupe G (infectés avec l'isolat humain EHEC et traités avec de l'extrait à l'éthanol), du groupe H (non infectés mais traités avec de l'extrait aqueux) et du groupe I (non infectés mais traités avec de l'extrait à l'éthanol) ont été sang occulte fécal négatif pendant toute la période d'étude. Les rats du groupe B (infectés par l'isolat animal d'EHEC sans traitement) étaient négatifs au sang occulte après l'infection au jour 14 mais positifs au jour 21, tandis que les rats du groupe C (infectés par l'isolat humain d'EHEC sans traitement) étaient positifs au sang occulte le jour 14 mais négatif au jour 21. Les rats des groupes D, E et F infectés par des isolats EHEC humains et bovins et traités, étaient positifs au sang occulte fécal au jour 14 mais négatifs au jour 21, avec un nombre élevé de colonies enregistrées éliminées dans les 7 jours suivant le traitement par des extraits aqueux et éthanoliques de *C. sieberiana*.

Conclusion: Les résultats de cette étude ont confirmé les potentiels antibactériens de l'écorce de tige de *C. sieberiana* contre l'EHEC. Les effets bénéfiques de cet extrait de plante devraient être exploités à des fins médicales commerciales.

Mots clés: *Cassia sieberiana*; *Escherichia coli* entérohémorragique; Wister rats; Antibactérien; Évaluation *in vivo*

Introduction:

The genus "*Cassia*" is a member of the Fabaceae family (Leguminosae family) in the major group angiosperms (flowering plants), commonly known as the legume, pea or bean family, and is a large and economically important family of flowering plants (1). The name "*Cassia*" means Cinnamon-like bark (2). Plants of this family are found throughout the world, growing in many different environments and

climates (3). The plants ranged from giant trees to small annual herbs with the majority being herbaceous perennials, and have indeterminate inflorescences, which are sometimes reduced to single flower. The flowers have short hypanthium and single carpel with short gynophores, and after fertilization produce fruits that that are legumes (1). The leaves are usually alternate compounds and are even- or odd-pinnate.

The entire plants have numerous food,

medicinal and non-medicinal uses (4). The medicinal value of this plant lies in some chemical substances that produce a definite physiological effect and these substances are alkaloids, flavonoids, glycosides, tannin oils, phenols and many others (5). Many plants in the genus are used extensively in traditional medicine in tropical and warm sub-tropical countries (6). It is believed to possess laxative effect and its extract is reported to be beneficial in treating many skin diseases such as eczema, rashes and ringworms. These plants have also been reported to be effective in the treatment of constipation, common colds, fevers, intestinal disorders, and for wound healing (7).

Alkaloids are very important in medicine and constitute most of the valuable drugs used locally. They have marked physiological effects on animals and show considerable pharmacological activity (8). Alkaloids are stimulants that act by prolonging actions of several hormones which require phosphodiesterase (7, 8), though are poisonous to cattle (9). Tannins are useful in medicine because of their astringent properties. Tannins and alkaloids are also known to have anti-herbivore defense functions in plants (5,9). Thus, the presence of tannins and alkaloids in this medicinal plant could be serving as a deterrent to grazers (8). Herbs that contain tannins are recommended for a wide range of treatments including inflammation, liver injury, kidney problems, arteriosclerosis, hypertension, stomach problems and inhibition of active oxygen, and are commonly recommended as diuretics, anti-diarrheal and haemostatics (10).

The recognition of enterohaemorrhagic *Escherichia coli* (EHEC) as an aetiological agent of diarrhea with life-threatening complications has made this type of infection a public health challenge of serious concern. The pioneering work leading to the discovery of *E. coli* verocytotoxins (VTs) was carried out by Konowalchuk et al., (11) and O'Brien and LaVeck (12) in the late 1970s. They soon purified it and found that it had similar structure and biological activity to the shiga toxin produced by *Shigella dysenteriae* type1 (11,12). The verotoxigenic *E. coli* belonged to a previously rare serogroup O157:H7 (13) that has been most commonly associated with large outbreaks (14).

The justification for continued interest in EHEC infections from a clinical perspective is the high rate of serious complications associated with this infection especially in children. Haemolytic uraemic syndrome (HUS) is defined by a triad of features; acute renal failure, thrombocytopenia and microangiopathic haem-

olytic anaemia, which occurs in 2-15% of cases of EHEC infections (15-17). Mortality from HUS is high between 3% and 17% (18) and a significant number (approximately 30%) suffer a range of permanent disabilities, including chronic renal insufficiency, hypertension and neurological deficits (19,20).

The pathogenesis of EHEC infection involves the establishment of the organism in the gut where it has to compete for space and nutrients with other microorganisms of the normal intestinal microflora (21,22). Local intestinal effects cause the development of bloody diarrhea as elaborated toxin internalizes in the cells of the gut where it blocks cellular protein synthesis, and may lead to apoptosis. HUS results from microvascular disease when the toxins enter the bloodstream and bind to receptors on endothelial cells that are abundant in kidneys and brain (22). The capacity to control EHEC infection in humans and to the scale off outbreaks is dependent upon rapid and accurate diagnosis and identification of the source of infection. Molecular biological techniques have been tested and shown to be very useful in this regard.

According to Anon (4), there is a need to evaluate local herbs for phytochemicals so as to determine the potential of these indigenous sources of medicines. The acceptance of traditional medicine as an alternative form of healthcare has led researchers to further investigate the antimicrobial and other health benefits of these medicinal plants. Medicinal plants are the richest bio-resources of drugs of traditional systems of modern medicine, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Extracting the relatively complex mixtures of metabolites is achieved by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (23).

Stem barks are very important to nutrition (4). *Cassia sieberiana* nuts are edible and the majority of human calories come from stem barks, especially from cereals, legumes and nuts. Seeds of *C. sieberiana* provide most cooking oils, many beverages and spices and some important food additives (3,5). These stem barks range in colors from greenish-brown to dark brown with smooth surfaces, and may have small bright-colored bands on the outer surface. It is used for multiple medical purposes in Africa (4). The objective of this study is to assess the *in vivo* antibacterial efficacy of *C. sieberiana* stem bark extracts on experimental EHEC infection in Wister rats.

Materials and method:

Test plants

Cassia sieberiana was obtained from Uyo, Akwa Ibom State, Nigeria, and authenticated by a botanist in the herbarium section of the Department of Botany, University of Nigeria, Nsukka. The stem bark of *C. sieberiana* was used for the study

Collection and preparation of plant material

The stem bark of *C. sieberiana* was rinsed in clean water, cut into smaller pieces and air-dried at room temperature. The dried stem bark was pulverized to homogeneous powder using mortar and pestle.

Extraction of plant material and preparation of extracts for administration

One hundred grams (100 g) each of the powder was soaked in 1 litre of hot water and ethanol separately for 24 hours with intermittent stirring. A 2-mm pore size mesh filter paper was used to filter the extract and the filtrate was air-dried into concentrate. For the aqueous extract, 1g of the concentrate was dissolved in 4 ml of distilled water, while for ethanol extract, 1g of the concentrate was dissolved in 10% v/v of Tween 20 in distilled water.

Source of EHEC

Faecal samples were collected from cattle and humans at a slaughter house located in Gariki, Ebonyi State, Nigeria for isolation of *Escherichia coli* on MacConkey agar plates following aerobic incubation of the culture plates at 37°C for 24 hours. Identification of *E. coli* isolates on MacConkey agar plates was done by conventional microbiological methods including colony morphology (lactose fermentation), Gram stain reaction, and biochemical tests such as indole, citrate utilization and urease tests (24). Presumptive EHEC isolates were inoculated on blood agar and incubated for 24 hours at 37°C to observe for β -haemolytic colonies. The colonies were also tested for O157 antigen by the latex bead agglutination assay (Oxoid, UK). Confirmed EHEC isolates were used for the *in vivo* study

Test animals and ethical issues

A total of 45 healthy Wister rats, weighing between 150 and 170 grams were supplied by an animal breeder in Abakaliki, Nigeria. The rats were housed in standard cages for 7 days for acclimatization (adaptation) during which they were confirmed not to be pre-infected by EHEC. All experimental procedures were

performed with strict adherence to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised in 1996).

Phases of the experimental study

The study was carried out in three phases of 7 days each, over a period of 21 days. The first 7 days was acclimatization (adaptation) period during which the Wister rats were observed, and faecal samples collected for screening to ensure that the rats had no EHEC. In the second phase (infection period), rats were infected with human and animal EHEC isolates, and faecal samples were collected and processed for identification and enumeration of EHEC as well as for occult blood, as evidence of successful infection of the rats. In the third phase (treatment period), the extracts (aqueous and ethanol) were administered orally to the Wister rats, and faecal samples were collected and cultured for identification and enumeration of EHEC, and for detection of faecal occult blood.

Experimental design

After the acclimatization (adaptation) period, rats were randomly divided into 9 groups of 5 rats each; group A contains healthy Wister rats not infected with human or animal EHEC isolates, and not treated with the extracts of *C. sieberiana* (negative control); group B contains Wister rats infected with animal EHEC isolate without treatment with *C. sieberiana* extract (positive control 1); group C contains Wister rats infected with EHEC human isolate without treatment with *C. sieberiana* extract (positive control 2); group D contains Wister rats infected with EHEC animal isolate and treated with aqueous extract of *C. sieberiana*; group E contains Wister rats infected with EHEC animal isolate and treated with ethanol extract of *C. sieberiana*; group F contains Wister rats infected with EHEC human isolate and treated with aqueous extract of *C. sieberiana*; group G contains Wister rats infected with EHEC human isolate and treated with ethanol extract of *C. sieberiana*; group H contains Wister rats not infected with EHEC but treated with aqueous extract of *C. sieberiana*, while group I contains Wister rats not infected with EHEC but treated with ethanol extract of *C. sieberiana*.

Infection of the Wister rats

The Wister rats in groups B, C, D, and E, were infected on day 8 by oral gavage with 1.0×10^8 CFU/ml of each of EHEC human and animal isolates, which were prepared from ino-

culum of each isolate on nutrient broth and standardized by comparing with 0.5 McFarland standards.

Administration of the extract

The Wistar rats in groups D, E, F, and G, were treated with 0.25 ml of the extracts per kg body weight (250 mg/kg body weight) by oral gavage daily for 7 days from day 15. Extract of *C. sieberiana* has previously been reported to be safe at a dosage of 500 mg/kg body weight (4).

Faecal sample collection and determination of EHEC counts

Fresh stool samples were collected from the rats at an interval of 3 days (2 times a week) through the entire study period using sterile containers. All stool samples were processed within 3 hours of collection. Briefly, 0.5 g of faecal sample was homogenized in 5 ml of phosphate buffered saline (PBS) containing 0.8% NaCl; 0.2% KH₂PO₄; and 0.115% Na₂HPO₄ at pH 7.4, which had been sterilized by autoclaving at 121°C for 15 minutes and 15 psi pressure. The emulsified faecal samples were serially diluted 10-folds in the sterile PBS, then, 0.1 ml of the 10⁵ dilution was inoculated onto two sets of replicate MacConkey agar plates, which had been prepared according to manufacturer's instructions. The agar plates were incubated aerobically at 37°C for 24 hours, and the mean EHEC count was determined on the agar plates (24).

Evaluation of faecal occult blood

Faecal occult blood test kit (QUICK VUE CLIA, Waive Inc. Ohio, USA) was used to determine the presence of blood in the faecal samples.

Statistical analysis:

The data generated were analyzed using the Statistical Package for the Social Sci-

ences version 20.0 (SPSS Inc. Chicago, IL). Results were presented in percentages. Categorical variables were compared with Pearson's chi-square, and significant value was taken as $p < 0.05$.

Results:

As shown in Table 1, 22 *Escherichia coli* isolates were recovered from 25 faecal samples collected from cattle and humans in the slaughter slabs, 3 of which were identified by serotyping as EHEC pathotype, while the remaining 19 were other (non-EHEC) pathotypes. Two EHEC pathotypes were from cattle, while 1 was from human.

Table 2 shows that Wistar rats in group A (not infected and not treated, negative control), group G (infected with EHEC human isolate and treated with ethanol extract of *C. sieberiana*), group H (not infected but treated with aqueous extract of *C. sieberiana*), and group I (not infected but treated with ethanol extract of *C. sieberiana*) were faecal occult blood negative throughout the study period.

Wistar rats in group B (infected with EHEC animal isolate without treatment, positive control 1) were faecal occult blood negative on day 14 but became positive on day 21, while rats in group C (infected with EHEC human isolate without treatment, positive control 2) were occult positive on day 14 but became negative on day 21.

Rats in group D (infected with EHEC animal isolate and treated with aqueous extract of *C. sieberiana*), group E (infected with EHEC animal isolate and treated with ethanol extract of *C. sieberiana*), and group F (infected with EHEC human isolate and treated with aqueous extract), were occult blood positive on day 14, but became negative on day 21.

Table 1: Source of Enterohaemorrhagic *Escherichia coli* (EHEC) used for the *in vivo* experiment

Source of faecal sample	Number examined	Number (%) positive of other strains of <i>E. coli</i>	Number (%) positive for EHEC
Human	8	7 (87.5)	1 (12.5)
Cattle	17	15 (88.2)	2 (11.8)
Total	25	22 (88)	3 (12)

Table 2: Detection of faecal occult blood in Wistar rats during the study period

Wistar rat group	Day 8-14 (Infection period)	Day 15-21 (Treatment period)
A (Not infected, not treated, negative control)	Negative	Negative
B (Infected with EHEC animal isolate, not treated, positive control 1)	Negative	Positive
C (Infected with EHEC human isolate, not treated, positive control 2)	Positive	Positive
D (Infected with EHEC animal isolate, treated with aqueous extract)	Positive	Negative
E (Infected with EHEC animal isolate, treated with ethanol extract)	Positive	Negative
F (Infected with EHEC human isolate, treated with aqueous extract)	Positive	Negative
G (Infected with EHEC human isolate, treated with ethanol extract)	Negative	Negative
H (Not infected, treated with aqueous extract)	Negative	Negative
I (Not infected, treated with ethanol extract)	Negative	Negative

Fig 1 shows the effects of *C. sieberiana* extracts on the EHEC animal isolate counts. Wistar rats in group B (infected with EHEC animal isolate without treatment, positive control 1) had the highest EHEC count (10,000 CFU/ml) on day 14, which remained at < 5,000 CFU/ml on day 21, whereas rats in groups D and E (infected with EHEC animal isolate but treated with *C. sieberiana* extracts) had EHEC count of > 5,000 CFU/ml each on day 14, but these reduced to 0 CFU/ml on day 21.

Fig 2 shows the effects of *C. sieberiana* extracts on the EHEC human isolate counts. Wistar rats in group C (infected with EHEC human isolate without treatment, positive control 2) had the highest EHEC count (10,000 CFU/ml) on day 14, which remained at >5,000 CFU/ml on day 21, while rats in groups F and G (infected with EHEC human isolate and treated with *C. sieberiana* extracts), had EHEC count of > 5,000 CFU/ml on day 14 but these reduced to 0 CFU/ml on day 21.

Fig 3 shows the comparative effects of *C. sieberiana* aqueous extracts on both human and animal EHEC isolates. The EHEC counts for

both isolates were > 5,000 CFU/ml on day 14 but these reduced to 0 CFU/ml on day 21 following the 7-day treatment with *C. sieberiana* aqueous extracts, while the EHEC count for the positive control 1 rats (infected with EHEC animal isolate without treatment) was > 5,000 CFU/ml on day 14 and <5,000 CFU/ml on day 21, and the count for the positive control 2 rats (infected with EHEC human isolate without treatment) was > 5000 CFU/ml on day 14, and was still > 5000 CFU/ml on day 21.

Fig 4 shows the comparative effects of *C. sieberiana* ethanolic extracts on both human and animal EHEC isolates. The EHEC counts for both isolates were > 5,000 CFU/ml on day 14 but reduced to 0 CFU/ml on day 21 following the 7-day treatment with *C. sieberiana* ethanol extracts, while the EHEC count for the positive control 1 rats (infected with EHEC animal isolate without treatment) was >5,000 CFU/ml on day 14 and < 5,000 CFU/ml on day 21, and the count for the positive control 2 rats (infected with EHEC human isolate without treatment) was > 5,000 CFU/ml on day 14, which was still > 5,000 CFU/ml on day 21.

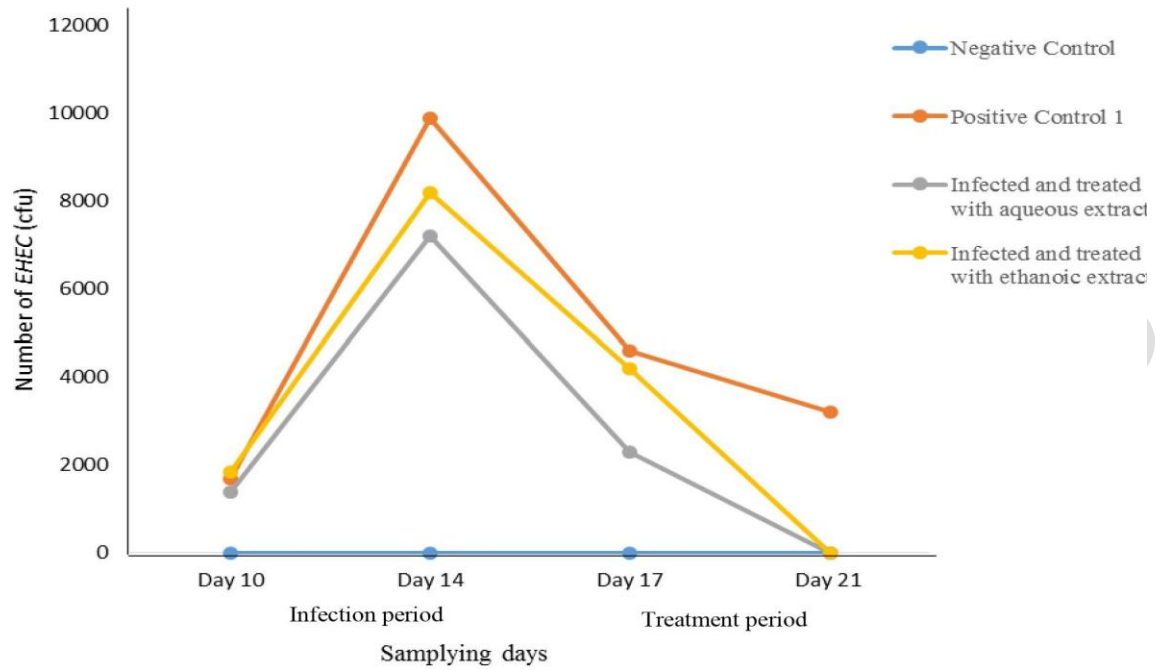


Fig 1: Antibacterial effects of *Cassia sieberiana* extracts on the EHEC animal isolate counts

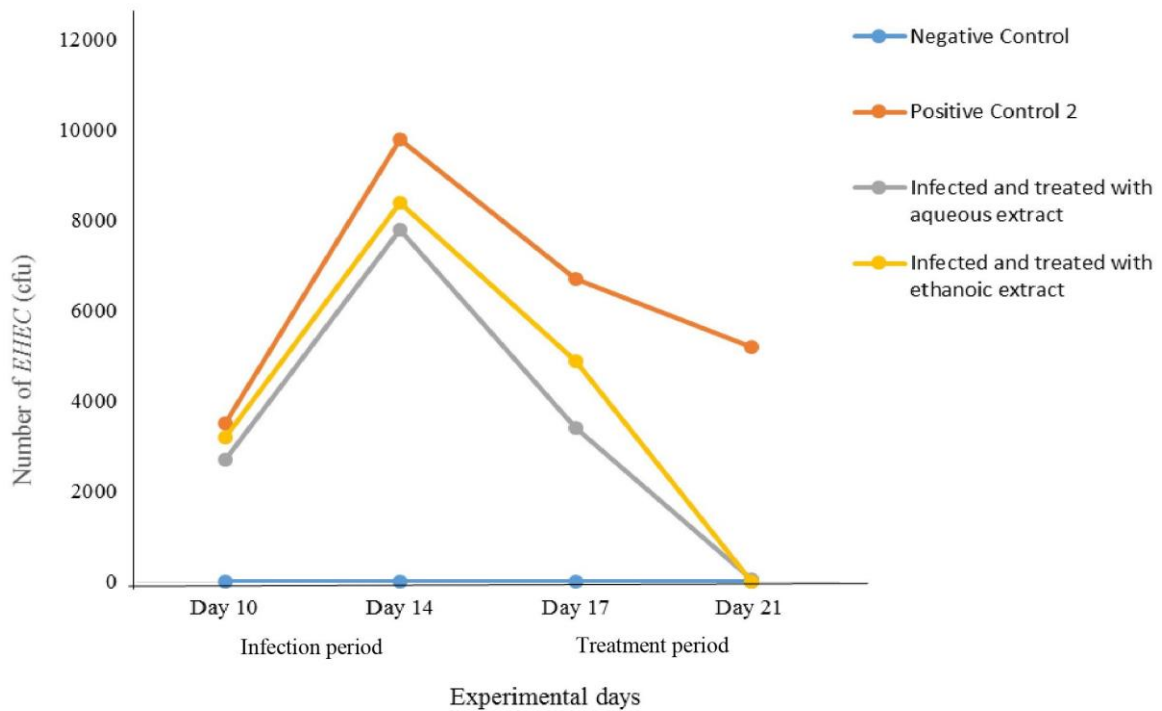


Fig 2: Antibacterial effects of *Cassia sieberiana* extracts on the EHEC human isolate counts

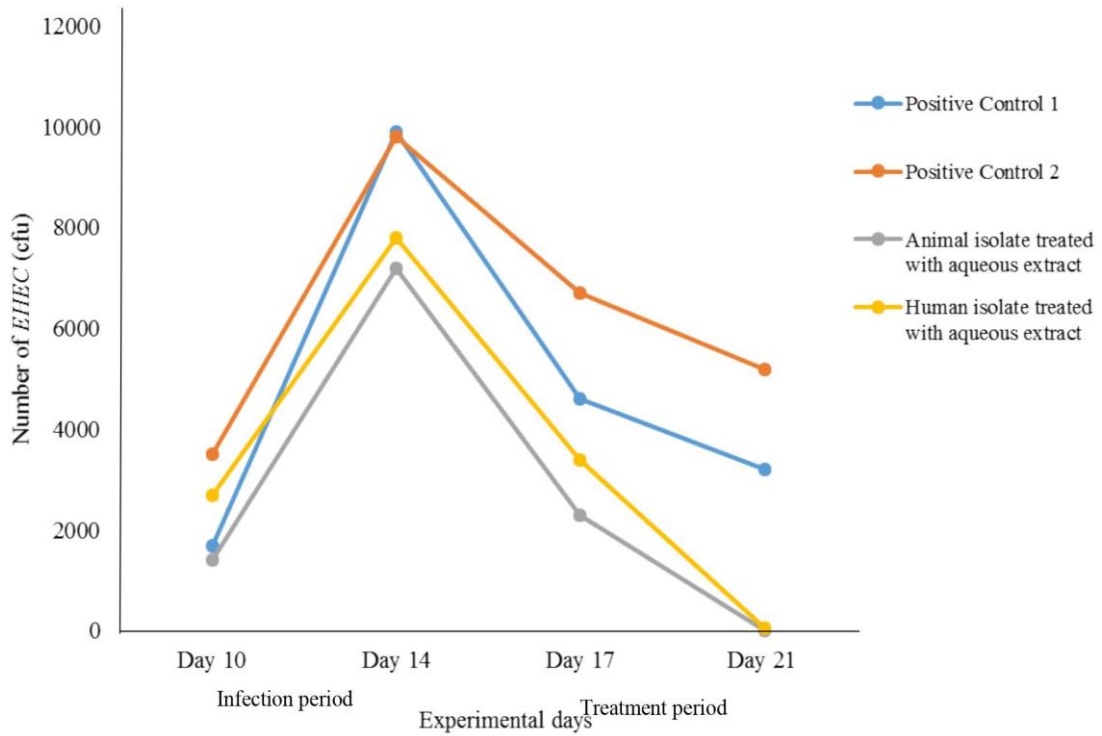


Fig 3: Comparative antibacterial effects of *Cassia sieberiana* aqueous extracts on human and animal EHEC isolates

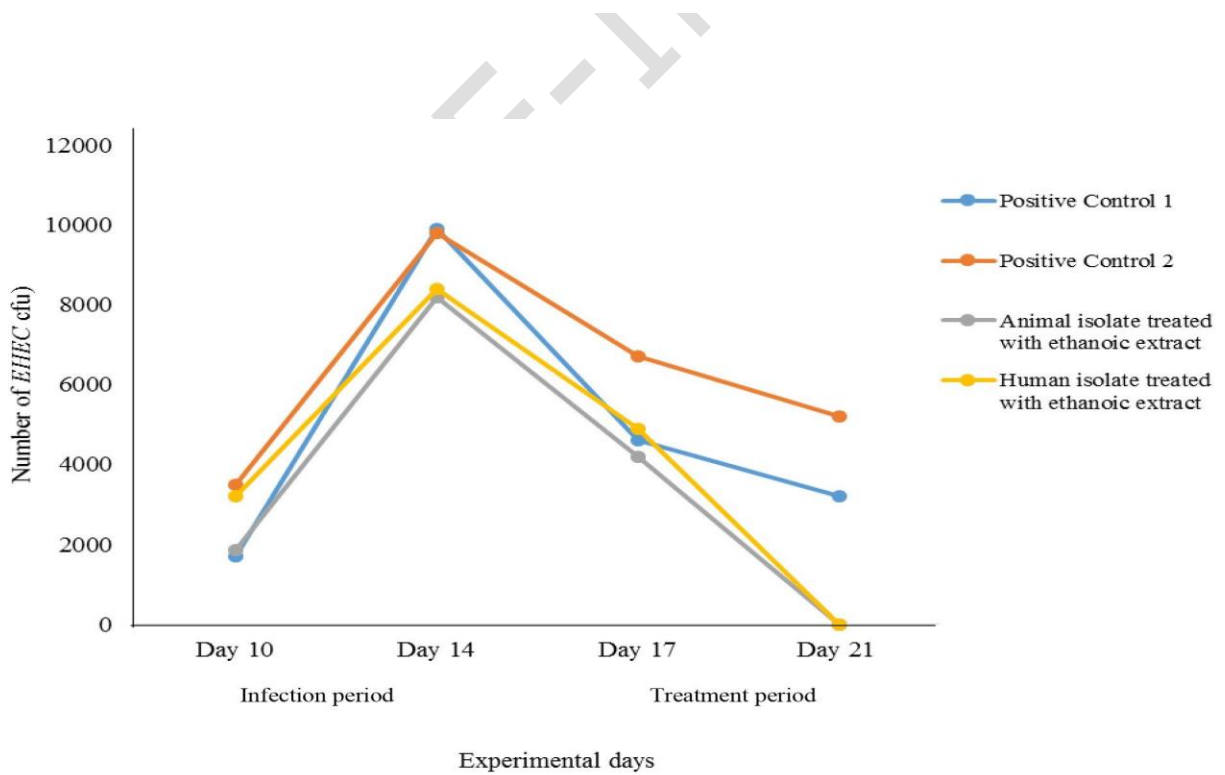


Fig 4: Comparative antibacterial effects of *Cassia sieberiana* ethanolic extracts on human and animal EHEC isolates

Discussion:

The findings of this study confirmed the presence of EHEC in humans and cattle. This is in conformity with previous studies conducted by Cornick and Vukhac (25) and Okere et al., (26), who reported that cattle are the major reservoir hosts of EHEC. They tend to be infected asymptotically and shed the bacteria in faeces. Other animals, including rabbits and pigs, have also been reported to be carrier of EHEC (26). Humans acquire EHEC O157:H7 by direct contact with animal carriers, their faeces and contaminated soil or water, or via ingestion of undercooked beef, other animal products, and contaminated vegetables and fruits (26). Although other pathotypes of *E. coli* were isolated in our study, EHEC was the pathotype of utmost priority but we confirmed that there are other pathotypes of *E. coli* in animals as reported by O'Brien and LaVeck (12).

The current study demonstrated *in vivo* efficacy of *C. sieberiana* in treatment of experimentally induced EHEC infections in Wistar rats that manifested as haemorrhagic colitis, as all the rats which tested positive to faecal occult blood before treatment, became negative after 7 days treatment with *C. sieberiana* stem bark extracts. The gastro-protective and anti-ulcer property of *C. sieberiana* could be attributed to the high content of alkaloids (27), which is in line with similar ethnopharmacological studies conducted by other researchers in Nigeria (28-32).

Our study showed that positive control rats (group B rats infected with EHEC animal isolate without treatment and group C rats infected with EHEC human isolate without treatment) had the highest EHEC counts (~10,000 CFU/ml) as expected, but infected rats treated with aqueous extract of *C. sieberiana* showed higher CFU/ml on day 17 than those treated with ethanol extract, indicating a slightly higher anti-bacterial activity of ethanol over aqueous extract. However, in all the infected rats, treatment with either aqueous or ethanolic extract reduced EHEC counts to zero level after 7 days of extract administration. Our findings agree with reports of previous studies on similar plant extracts (27,33).

Apart from the documented antimicrobial activity of alkaloids, a major phytochemical constituent of *C. sieberiana* stem bark (27), against bacterial infections, flavonoids, tannins and saponins present in the stem are also known to possess antimicrobial potential by either altering the bacterial cell membrane or

inhibiting the synthesis of nuclei acid and proteins (33). *Cassia sieberiana* has been shown to possess both bacteriostatic and bactericidal effects against *E. coli*, *Staphylococcus aureus*, *Pseudomonas* spp. and other pathogens (34, 35). In another study, Ulanowska and co (36) attributed the antimicrobial activity of *C. sieberiana* to the action of the phytochemical compounds on the RNA, DNA and protein synthesis apparatus of the bacteria.

Conclusion:

The findings of this study confirmed *in vivo* anti-EHEC potential of *C. sieberiana*, which provide evidence for its use in traditional medicines for treatment of infectious diseases. This could serve as source of readily available and less expensive raw materials for production of novel antimicrobial drugs that are useful for treatment of infections caused by *E. coli* and other infective agents.

References:

- Schrire, B. A., Lavin, M., and Lewis, G. P. Global distribution patterns of the leguminosae: insights from recent phylogenies. In: Friis, I., and Basley, H. (ed). Plant diversity and complexity patterns: Local, regional and global dimension. Biol. 2005; 55: 375-378.
- Edeoga, H. O., Okwu, D. E., and Mbaebie, B. O. Minerals and nutritive values of some Nigeria medicinal plant. J Med Aromatic Plant Sci. 2003; 25: 1010-1015.
- Abdulrazak, N., Asiya, U. I., Usman, N. S., Unata, I. M., and Farida, A. Anti-plasmodial activity of ethanolic extract of root and stem bark of *Cassia sieberiana* DC on mice. J Int Ethnopharmacol. 2015; 4 (2): 96-101
- Anon, A. Stem bark of *Cassia* species. 2012. <http://en.wikipedia.org/wiki/seed>. Accessed 5/01/2022
- Omaye, S. T. Food and nutritional toxicology. C.R.S. Press, USA. 2004;184-215.
- Archer, M., Agyei, T. A., Mintah, S. O., Adejei, P. A., Kumadoh, D., and Asiedu-Larbi, J. Medicinal uses of *Cassia sieberiana*; a review. Int J Sci Basic Appl Res. 2019; 48 (2): 161-180
- Burkil, H. M. The useful plants of West Tropical Africa. (2nd edn) Families J-L. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 1995: 857.
- Edeoga, H. O., and Eriata, D. O. Alkaloids, tannin and saponin contents of some medicinal plants. J Med Aromatic Plant Sci. 2001; 22:344-349.
- Ambadiang, M. M. M., Atontsa, B. C. K., Tankeo, S. B., et al. Bark extract of *Cassia sieberiana* DC. (Caesalpinaceae) displayed good antibacterial activity against MDR gram-negative phenotypes in the presence of phenylalanine-arginine β -naphthylamide. BMC Complement Med Ther. 2020; 20: 342. doi.org/10.1186/s12906-020-03148-3
- Mohammed, Z., Bello, H., Abdullahi, Y. H., and Abdurrahman, E. M. Evaluation of antidiarrheal potential of methanolic root extract of *Cassia Sieberiana* Dc. (Fabaceae) in mice. Alt Med

11. Chiropractic OAJ. 2019; 2 (2): 180015.
12. Konowalchuk, J., Speirs, J. I., and Starvic, S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immunol. 2010; 18: 1775-1979.
13. O'Brien, A. D., and LaVeck, G. D. Purification and characterization of a Shigella dysenteriae 1-like toxin produced by *Escherichia coli*. Infect Immunol. 2011; 40: 675- 683.
14. Riley, L. W., Remis, R. S., Helgerson, S. D., McGee, H. B., Wella, J. G., and Davis, B. R. Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med. 2013; 308: 681- 685.
15. Fukushima, H., Hashizume, T., Morita, Y., Tanaka, J., Azuma, K., and Mizumoto, Y. Clinical experiences in Sakai City Hospital during the massive outbreak of enterohaemorrhagic *Escherichia coli* 0157 infections in Sakai City. Paediatr Int. 2013; 41: 213-217.
16. Carter, A. O., Borczyk, A. A., Carlson, J. A., Harvey, B., Hockin, J. C., and Karmali, M. A. A severe outbreak of *Escherichia coli* 0157:H7-associated hemorrhagic colitis in a nursing home. N Engl J Med. 2013; 317: 1496-1500.
17. Slutsker, L., Ries, A. A., Greene, K. D., Wells, J. G., Hutwagner, L., and Griffin, P. M. *Escherichia coli* 0157:H7 diarrhea in the United States: Clinical and epidemiological features. Annu Int Med. 2012; 126: 505-513.
18. Rowe, P. C., Orrbine, E., Lior, H., Wells, G. A., Yetisir, E., Clulow, M., and McLaine, P. N. Risk of hemolytic uremic syndrome after sporadic *Escherichia coli* 0157:H7 infection: results of a Canadian collaborative study. Investigators of the Canadian Pediatric Kidney Disease Research Center. J Paediatr. 2013; 132: 777-782.
19. Griffin, P. M. and Tauxe, R. V. The epidemiology of infections caused by *Escherichia coli* 0157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uraemic syndrome. Epidemiol Rev. 2009; 13:60-98.
20. Karmali, M. A. Infection by verotoxin-producing *Escherichia coli*. Clin Microbiol Rev. 2013; 2:15-38.
21. Wellinder- Olsson, C., Stenqvist, K., Bandenfors, M., Brandberg, A., Floren, K., and Holm, M. EHEC outbreak among staff at a children's hospital: use of PCR for verocytotoxin detection and PFGE for epidemiological investigation. Epidemiol Infect. 2013; 132: 43-49.
22. Paton, J. C., and Paton, A. W. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin Microbiol Rev. 2012; 11: 450-479.
23. Afolayan, A. J., Aboyade, O. M., and Sofidiya, M. O. Total phenolic content and free radical scavenging and *Malva parviflora* L. (Malvaceae). J Biol Sci. 2008; 8: 945-949.
24. Nataro, J. P., and Kaper, J. B. Diarrhoeagenic *Escherichia coli*. Clin Microbiol Rev. 2009; 11: 142-201.
25. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Part 2, 2nd edition, Cambridge University Press, 2006
26. Cornick, N. A., and Vukhac, H. Indirect transmission of *Escherichia coli* 0157:H7 occurs readily among swine but not among sheep. Appl Environ Microbiol. 2008; 74 (8): 2488-2491.
27. Okere, O.S., Adeyomo, S. O., Adama, M. D. A., et al. Anti-dysentery effect of milled fermented maize ("Ogi") in *Escherichia coli* infected Albino rats. Int J Biochem. 2015; 197:455-460
28. Archer, M., Agyei T A, Mintah S O, Junior P A, Kumadoh D and Asiedu-Larbi. Medicinal Uses of *Cassia sieberiana*; A Review. Int J Sci Basic Appl Res. 2019; 48 (2): 161-180
29. Idowu, O. A., Soniran, O. T., Ajana, O. and Aworinde, D. O. Ethnobotanical survey of antimarial plants used in Ogun State, Southwest Nigeria. Afr J Pharm Pharmacol. 2010; 4: 55-60
30. Erinoso, S. M., and Aworinde, D. O. Ethnobotanical survey of some medicinal plants used in traditional health care in Abeokuta areas of Ogun State, Nigeria. Afr J Pharm Pharmacol. 2012; 6: 1352-1362
31. Soladoye, M. O., Chukwuma, E. C. and Owa, F. P. An 'Avalanche' of plant species for the traditional cure of Diabetes mellitus in South-Western Nigeria. J Nat Prod Plant Resour. 2012; 2: 60-72
32. Elufioye, T. O., Oladele, A. T., Cyril-Olutayo, C. M., Agbedahunsi, J. M., and Adesanya, S. A. Ethnomedicinal study and screening of plants used for memory enhancement and antiaging in Sagamu, Nigeria. Eur J Med Plants. 2012; 2: 262-275
33. Abolanle, A. A. K., Bilqis, A. L., Abdulrasheed, A. A., Mubo, A. S. and Jones, O. M. Medicinal Plants Used in the Treatment of Gastric Ulcer in Southwestern and North Central Nigeria. Res J Med Plants. 2019;13: 119-128
34. Ambadiang, M. M. M., Atontsa, Brice, C. K., Tankeo, S. B., et al. Bark extract of *Cassia sieberiana* DC. (Caesalpinaceae) displayed good antibacterial activity against MDR gram-negative phenotypes in the presence of phenylalanine-arginine β -naphthylamide. BMC Complementary Medicine and Therapies. 2020; 20: 342. <https://doi.org/10.1186/s12906-020-03148-3>
35. Halilu, A. M., Obtober, M. E. Balogun, N. Namrita, M. Studies of in vitro antioxidant and cytotoxic activities of extracts and isolated compounds from *Parinari curatellifolia* (Chrysobalanaceae). J Nat Sci Res. 2013; 3 (13): 149-154
36. Kpegba, K., Elo, K., Evennamede, K. S., et al. Comparative study of the chemical composition of extracts from leaves, stem bark and root bark of *Cassia sieberiana*: Antibacterial activities. Orient J Chem. 2019; 35 (6): doi.org/10.13005/ojc/350608
37. Ulanowska, K., Tkaczyk, A., Konopa, G., and Węgrzyn, G. Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains. Arch Microbiol. 2006; 184 (5): 271-278