World Journal of Microbiology
Vol. 1(1), pp. 002-009, May, 2014. © www.premierpublishers.org, ISSN: 2141-5032

WJM

Research Article

In vivo antimicrobial activity of plant species on *Escherichia coli O157:H7* inoculated into albino rats

[*1]J.U. Itelima and [2]S.E. Agina

[*1,2]Department of Plant Science and Technology, Faculty of Natural Sciences, University of Jos, Nigeria.

Escherichia coli O157:H7 **is an enteric bacterium that has been implicated in outbreaks of disease worldwide and is currently considered an emerging pathogen. An investigation was carried out to determine the *In vivo* antimicrobial activity of 5 plant species (Allium sativum, Mangifera indica, Psidium guajava, Vernonia amgygdalina and Zingiber officinale) on *E. coli O157:H7* inoculated into albino rats. The plant extracts were prepared according to standard method using ethanol as a solvent. An antibiotic (ciprofloxacin) served as a positive control. Five rats from each group were challenged with 1.0 ml of 10^9cfu/ml *E. coli O157:H7* and simultaneously administered 3.0 mg extract of the plant species and the antibiotic drug per kg of rat body weight orally. The numbers of the pathogen shed in rat faeces were determined. The result revealed that there was a lot of variation in the percentage of the albino rats that shed the organism during the experiment. There was a significant interaction between treatment and time ($p<0.05$) over the course of the study. However, when comparing treatment groups at specific sampling days, the proportion of albino rats shedding faecal *E. coli O157:H7* in the infected antibiotic-treated group was significantly higher ($p<0.05$) than infected non-treated group only on days 4 days. The present study has revealed that the ethanolic extracts of the plant species not only prevented the development of diarrhoea in rats treated with the plants but inhibited the growth of *E. coli O157:H7* in them and thus have the ability to fight the pathogen as antimicrobial as well as anti-diarrhoeal agents.**

Keywords: *In vivo*, antimicrobial activity, plant species, *Escherichia coli O157:H7*, albino rats.

INTRODUCTION

Many works have been done which aimed at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant (Akinpelu and Onakoya, 2006). It has been reported by Edeoga *et al.* (2005) that the pace of development of new antimicrobial drugs has slowed down; while the prevalence of resistance (especially multiple resistances) has increased astronomically The increase in number of antibiotic resistant bacteria is no longer matched by expansion in the arsenal of agents available to treat infections. Literature reports and ethno-botanical records suggest that plants are the sleeping giant of pharmaceutical industry (Akinpelu and Onakoya, 2006). They may provide natural source of antimicrobial drugs that will provide novel or lead compounds that may be employed in controlling some infections globally.

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body.

*Corresponding author: Itelima U. Janet, Department of Plant Science and Technology, Faculty of Natural Sciences, University of Jos, Nigeria. E-mail: Itelimajanet@unijos.edu.ng

The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, saponin, cardiac glycosides and phenolic compounds (Edeoga *et al.,* 2005; Jung *et al*., 2009). Many photochemical compounds have been shown to be bioactive, that is they exhibit remarkable biological activity in other living organism (Jin-Hyung *et al*., 2011). Many workers have demonstrated the antidiarrhoeal activity of phytochemical compounds such as tannin (Mukherjee *et al*., 1998), flavonoids (Galvez *et al*., 1993a), alkanoids (Gricilda and Molly, 2001), saponins, sterols and terpenes (Otshudi *et al*., 2000). The phytochemical research based on ethno-pharmacological information is generally considered as effective approach in the discovery of new antimicrobial agents from higher plants (Kloucek *et al.,* 2005).The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erodgrul, 2002). Several scientific studies carried out on plant species such as garlic (*Allium sativum),* Mango *(Mangifera indica)*, guava (*Psidium guajava)*, bitter leaf (*Vernonia amgygdalina)* and ginger (*Zingiber officinale)* have confirmed the traditional claims of their effectiveness in treating diarrhoea related infection (Tona, 1999; Abdelrahim, 2002; Nwokedi *et al*., 2003; Akinpelu and Onakoya, 2006; Okorondu *et al*., 2006; Eman and Hoda, 2008).

E. coli O157:H7 is a leading food borne enteric pathogen associated with human illness including hemorrhagic colitis and hemolytic uremic syndrome (HUS) the leading cause of acute renal failure in children (CDC, 1995). In addition, the infection with this organism is known to be a common cause of bloody and non bloody diarrhoea (Bell *et al*., 1994; Thomas *et al*., 1995). *E. coli O157:H7* is usually the most common bacterial pathogen isolated from bloody stools and has been isolated from as many as 40% of all bloody stools in United States of America (Griffin and Tauxe, 1991). Other symptoms related to *E. coli O157:H7* infection include severe abdominal cramps, vomiting, nausea and mild fever (Ostroff *et al.,* 1990). *E. coli O157:H7* infection is generally found to affect both male and female and also people of all age groups, but has a more devastating effects on the young and elderly (Thomas *et al*., 1995). Although it has been over 30 years since the discovery of *E. coli O157:H7* as an enteric pathogen and despite the recent increase in the rate of severe disease associated with infection by the organism, no treatment yet exists (Krystle and Alison, 2011). Antibiotics and anti-mobility agents are not recommended as they increase the risk of developing HUS. Treatment of the infection is limited to supportive care (CDC, 1995). A variety of treatment and prevention strategies to protect against *E. coil* O157:H7 are currently in development. These include toxin receptor analogs, passive antibody therapy and vaccines to protect human against the systemic effects of the toxin produced by the pathogen. Since an *E. coil* O157:H7 vaccine has not been developed and licensed for immunization of humans, two vaccines are currently in use in cattle (Fox *et al*., 2009; Moxley *et al*., 2009; Smith and Ravdin, 2009). The most promising prevention strategies for *E. coli O157:H7* focus on minimizing expose to this pathogen. Recently, some researchers used bioactive ingredients to treat *E. coli O157:H7* (Vikram *et al*., 2010; Jin-Hyung *et al*., 2011). The use of human subjects to investigate the steps required for *E. coli O157:H7* to evoke intestinal pathology is considered unethical because of the possibility that a volunteer could develop hemolytic uremic syndrome (HUS). Thus, *in vitro* assays and animal models have been developed to demonstrate various aspects of *E. coli O157:H7*. Many plants conveniently available in Nigeria and other countries are used in traditional folklore medicine for the treatment of diarrhoea dysentery and other gastrointestinal diseases. Several studies have also shown that prior administration with some plants extract had a protective effect on intestinal tract (Rani *et al*, 1999; Majumdar *et al*., 2000; Kumar *et al*., 2001). The present study was undertaking to determine the *In vivo* antimicrobial activity of extracts of 5 plant species which include garlic bulb (*Allium sativum),* Mango leaf *(Mangifera indica)*, guava leaf (*Psidium guajava)*, bitter leaf (*Vernonia amgygdalina)* and ginger rhizome (*Zingiber officinale) on Escherichia coli O157:H7* inoculated into albino rats.

MATERIALS AND METHODS

Preparation of Inoculum

The inoculum was prepared from a stock culture of *E. coli O157:H7* isolated from human stool sample. A loopful of the organism stored in nutrient agar slant in a refrigerator was transferred onto test tubes containing 10 mls of sterilized peptone water and incubated at 37^{O}C for 18-24 hrs. In order to activate the cells further, two successive transfers of the organism unto TSB and incubation at 37^{O}C for 18-24 hrs were made. The activated culture was serially diluted in test tubes with TSB until a cell concentration of 1.0×10^{9} cfu/ml was obtained by pour plate technique (Eman and Hoda, 2008). A high concentration of the inoculums was prepared in order to increase the probability of establishing the disease condition in the experimental animals (Griffin, 1995).

Experimental Animals

Forty healthy albino rats (*Rattus norvegicus*) of both

sexes, aged 3 months and weighing between 200–250g were used for this experiment. The rats were obtained from Animal House of the University of Jos, Nigeria. The rats were assigned randomly and individually in micro-isolated cages in the same room on a 12/12 light-dark cycle. The rats were allowed to acclimatize to their new environment for 2 weeks before inoculation and were tested four times over the 2-week period to ensure that they were negative for *E. coli* O157: H7 (Alali *et al*., 2004). Food and deionized water were autoclaved and provided *ad libitum* from the day the rats were procured until the completion of the experiment.

Source of Plant Materials and Antibiotic Drug

The plant used for this experiment included garlic bulb (*Allium sativum),* Mango leaf *(Mangifera indica)*, guava leaf (*Psidium guajava)*, bitter leaf (*Vernonia amgygdalina)* and ginger rhizome (*Zingiber officinale).* All plants were obtained from Jos North Local Government Area of Plateau State, Nigeria and authenticated in the Department of Plant Science and Technology of University of Jos, Nigeria by Prof. S.W. H. Hussaini, a plant taxonomist. The antibiotic drug (ciprofloxacin) was purchased from a pharmaceutical shop located in Jos metropolise.

Preparation of Plant Extracts

Plant extracts were prepared by cold percolation method described by Akinpelu and Onakoya (2006). The various test plant species were well dried under the shade and then ground into fine powder using an electrical blender. A portion of 250g each of the plants powder was separately soaked in 300ml of 95% ethanol in glass containers and covered with their lids. The plants soaked in ethanol were kept at room temperature and allowed to stand for7 days to permit full extraction of the active ingredients or the chemical components. The fluids were then filtered using whatman No 1 filter paper into beakers. The extracts were obtained by oven drying the filtrate at 50^{O}C and then kept in refrigerator before use.

Inoculation of *E. coli O157:H7* and Administration of Plant Extracts and Antibiotic to Albino Rats

Rats were divided into 8 groups of 5 replicates (n=5). The doses of the plant extracts and antibiotic (ciprofloxacin) administered to the rats was according to the prescription of Venkatesan *et al*. (2005). The volume of the inoculum introduced into each rat as prescribed by (Eman and Hoda, 2008). The doses of the extracts and the antibiotic Group (I): 5 rats were orally challenged with 1.0 ml of *E. coli O157:H7* inoculum at a dose of 1.0×10^{9} cfu/ml (infected-non treated group).Groups (II-VII): 5 rats from each group were challenged with 1.0 ml of 1.0×10^{9}cfu/ml *E. coli O157:H7* and simultaneously administered 3.0 mg extracts of *A. sativum, M. indica, P. guajava, V. amgygdalina, Z. officinale* and standard antibiotic (ciprofloxacin) per kg of rat body weight orally (infected treated group).Group (VIII): 5 rats were not infected with *E. coli O157:H7* and were not given any treatment (Control). The rats were held firmly by the scruff of the neck in a vertical position before they were orally inoculated with the inoculum and the different plant extracts using a disposable sterile syringe without needle.

Enumeration and /or detection of *E. coli O157:H7*

The faeces of the test animals were collected from transparent plastic dishes placed beneath the individual rat cages 4 times daily until 2 weeks after inoculation to determine the number of rats shedding the pathogen and the faecal counts shed (Aranda-Michel and Gianella, 1999). *E. coli O157:H7* in each faecal sample was quantified as follows: 1.0 g of faeces was added to 9ml of TSB, vortexed and incubated at 37^{O}C for 2 hours, after which the suspension was serially diluted (10^{-1} to 10^{-5}) in TSB. Aliquots (0.lml) from each dilution were plated in triplicate by the spread-plate method onto SMAC agar. After incubation at 37^{0}C for 8-24 hrs, sorbitol negative colourless colonies were counted. Ten colonies were randomly selected from each plate and confirmed as *E. coli O157:H7* by biochemical and latex agglutination test.

Mortality Rate and Pathological Manifestations

The mortality rate of the rats in the different groups was calculated as numbers of the rats that died during the course of the experiment in relation to all rats used in each group (Eman and Hoda, 2008). The animals were observed for consistency of faecal material. The frequency of defaecation was noted from the transparent plastic dishes placed beneath the individual rat cages for up to 4 hours. Diarrhoea was noted and scored based on consistency, colour and the number of defaecation. A daily score of watery stool that was >2 was considered proof of diarrhoea, while a score that was = or <2 was not. Cages and bedding were changed on a daily basis during collection of faecal samples to avoid cross-contamination. The animals were also observed for any abnormalities and pathological manifestation during the period of the experiment. At the end of the study (2 weeks after inoculation and treatment), the infected rats were killed using sodium pentobarbital (l ml/4.5kg) to prevent the spread of the infection associated with *E. coli O157:H7* in the environment (Alali *et al*., 2004).

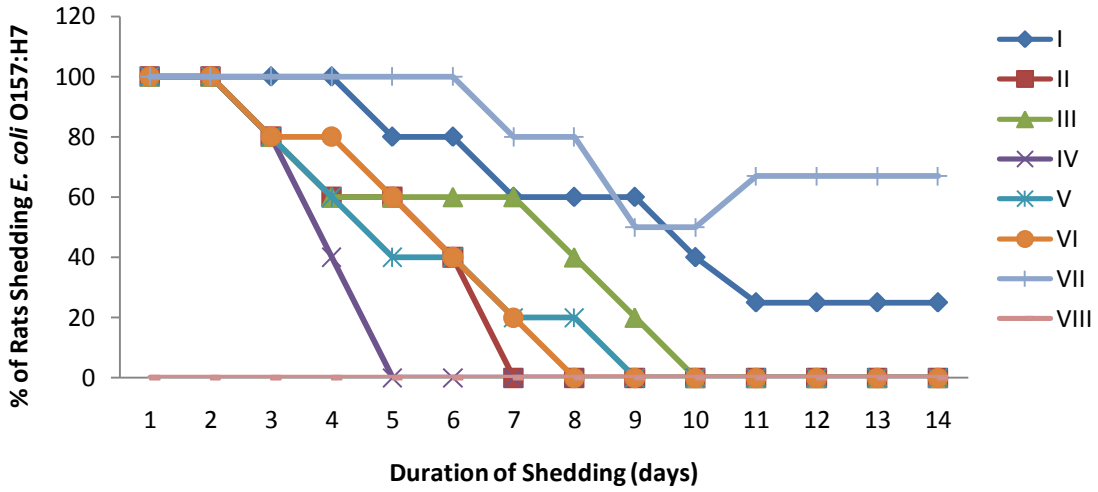

Figure 1. The Percentage of Rats that Shed *E. coli O157:H7* in their Faeces after Inoculation and Treatment with or without Plant Extracts and Antibiotic (Ciprofloxacin)

I = Rat group infected but not treated, II = Rat group infected and treated with extract of *A. sativum*, III = Rat group infected and treated with extract of *M .indica*, IV= Rat group infected and treated with extract of *P. guajava*, V= Rat group infected and treated with extract of *V. amygdalina*, VI= Rat group infected and treated with extract of *Z. officinale*, VII = Rat group infected and treated with antibiotic (ciprofloxacin) and VIII = Rat group not infected and not treated (Control)

RESULTS

All the rats were found negative for *E. coli O157:H7* in faeces before inoculation and treatment with plant extracts and an antibiotic drug (Ciprofloxacin). The percentage of albino rats that shed *E. coli O157:H7* in their faeces after inoculation and treatment with antimicrobial agents was presented in Figure 1. The result revealed that there was a lot of variation in the percentage of the albino rats that shed the organism during the experiment. There was a significant interaction between treatment and time ($p<0.05$) over the course of the study. However, when comparing treatment groups at specific sampling days, the proportion of albino rats shedding faecal *E. coli O157:H7* in the infected antibiotic-treated group was significantly higher ($p<0.05$) than infected non-treated group only on days 11,12,13 and 14.

One rat in the infected non-treated group and two rats in the infected antibiotic treated group shed the organism throughout experiment. The groups of the infected animals (II-VI) treated with *P. guajava*, *A. sativum*, *Z. officinale*, *V. amgydalina* and *M. indica* stopped shedding *E. coli O157:H7* at quantifiable concentration levels at days 5,7,8,9 and 10 respectively (Figure 1).

The mean concentrations of *E. coli O157:H7* in faeces from positive samples quantifiable by direct plating in each treatment group are shown in Figure 2. Variation was also apparent in the amount of *E. coli O157:H7* shed in faeces among the various rat groups. Thus, during the course of the experiment the concentrations of the organism in faeces of the positive animals in groups I to VII ranged between 2.8×10^3 - 7.9×10^3 cfu/g, 1.2×10^3 - 6.4×10^3 cfu/g, 1.7×10^3 - 6.7×10^3 cfu/g, 1.4×10^3- 6.3×10^3 cfu/g, 1.6×10^3 -7.0×10^3 cfu/g, 1.3×10^3 - 6.5×10^3 cfu/g and 3.0×10^3 - 7.8×10^3 cfu/g respectively. Statistical analysis of the results showed that there was a significant time effect ($p< 0.05$), but no significant treatment effect ($p>0.05$) among some of the infected rat groups treated with the different plant extracts. However, significant difference was observed in treatment effects among the infected non-treated group of animals and those treated with plant extracts ($p<0.05$). Analysis of variance also revealed that no significant treatment effect existed between infected non-treated groups and the infected antibiotic treated group.

The results in Table 1 show the mortality rate and pathological manifestation observed in the different rat groups respectively. Mortality rates in groups I and VII were 20% and 40% respectively, while zero mortality rates was recorded among rats of the other groups of the experiment. None of the rat group suffered from bloody diarrhoea. However 100% of the infected non treated group (group I) and 60% of the infected antibiotic treated group (group VII) manifested the symptom of watery diarrhoea a day after inoculation with *E. coli O157:H7* cells (Table 1). It was observed that there was more reduction in the number of rats defaecating watery stool over time among the infected

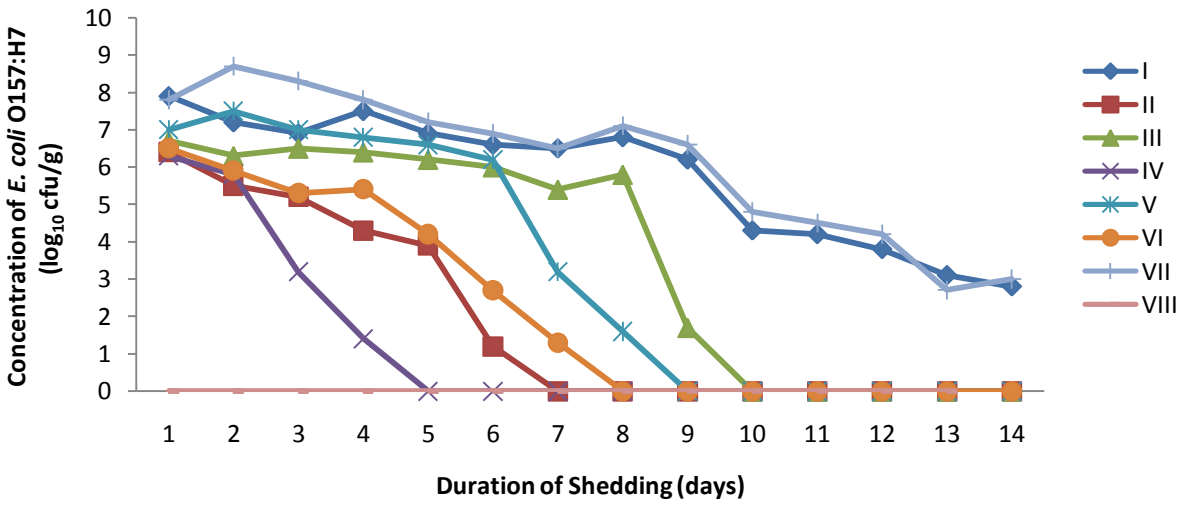

Fig. 2. The Mean Concentration of *E. coli O157:H7* $\log_{10}$ cfu/g in Faeces from Positive Samples from Albino Rats Treated with or without Plant Extracts and Antibiotic (Ciprofloxacin).

I = Rat group infected but not treated, II = Rat group infected and treated with extract of *A. sativum*, III = Rat group infected and treated with extract of *M .indica*, IV= Rat group infected and treated with extract of *P. guajava*, V= Rat group infected and treated with extract of *V. amygdalina*, VI= Rat group infected and treated with extract of *Z. officinale*, VII = Rat group infected and treated with antibiotic (ciprofloxacin) and VIII = Rat group not infected and not treated (Control)

Table 1. Mortality Rate/Pathological Manifestations observed in Rat Groups during the Course of the Experiment

Mortality rate/ Clinical symptoms	Number of rats affected / total number of rat in each group							
	I	II	III	IV	V	VI	VII	VIII
Mortality rate	1/5(20)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	2/5(40)	0/5(0)
Watery diarrhoea	5/5(100)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	3/5(60)	0/5(0)
Loss of appetite	5/5(100)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	3/5(60)	0/5(0)
Loss of weight	5/5(100)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	3/5(60)	0/5(0)
Body weakness /slow movement	5/5(100)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	0/5(0)	3/5(60)	0/5(0)

Figures inside the parenthesis stand for the percentage of the rat affected in each group during the experiment.

non treated group of rats than the infected antibiotic treated group. Thus, the defaecation of watery diarrhoea by the rats lasted between 4 to 5 days in group I and 6 to 8 days in group VII. Among the rats that suffered from diarrhoea and abnormalities such as general weakness with slow movement, loss of appetite and loss of weight were observed in them. No pathological changes were observed in other rat groups all through the course of the experiment.

DISCUSSION

The percentage faecal shedding of *E. coli O157:H7* following inoculation was highly variable among the individual rats, which is in agreement with previous studies (Cray and Moon, 1995; Hermon *et al*., 1999). The intermittent faecal-shedding patterns observed in the current study are also consistent with previous studies in calves (Cray and Moon, 1995; Alali *et al*.,

2004). The number of the albino rats shedding *E. coli O157:H7* was higher in the infected antibiotic treated group of rats (group VII) than the infected non treated group (group I) during some of the sampling periods in this study. This suggests that the antibiotic used may have enhanced the faecal shedding of the *E. coli O157:H7* in the rats. Price *et al*. (2002) also observed that treatment of calves experimentally inoculated with *E. coli O157:H7* with an antibiotic (tilmicosin) resulted in an increase in faecal shedding of the organism up to 5 days after inoculation, whereas treatment of the calves with another antibiotic (ceftifur) resulted in a decrease by the second day. A report by Elder *et al.* (2002) revealed that oral administration of neomycin sulphate at therapeutic doses to cattle that were naturally shedding *E. coli O157:H7* reduced their faecal shedding to undetectable concentrations compared with controls (non antibiotic treated cattle). The objective of the study by Elder *et al.* (2002) was to investigate short times intervention, and the study did not report result after day 7 post-treatment. The apparent difference between Elder *et al.* (2002) report and the present finding may be dose-related, or may be due to the different strain of *E. coli* O157: H7 that were used and / or the type of the experimental animals used for the experiment. According to Walterspiel *et al*. (1999), there is no evidence that antibiotics improve the course of disease caused by *E. coli O157:H7* and treatment with antibiotics may precipitate kidney complications. The result of the present study showed that some of the rats in the infected non treated group stopped shedding the organism before the end of the experiment. The reason for this could be because the rats developed protective immunity against the pathogen and was able to eliminate it before the end of the experiment. This result is similar to that of a human case in which some victims of *E. coli O157:H7* infection recovered without treatment within 5 to 10 days (CDC, 2006).

The ethanolic extracts of the 5 plant species employed in this study exhibited significant antimicrobial activity against *E. coli O157:H7* inoculated into the albino rats by reducing the concentration of the organism in their faeces to undetectable levels at different days after inoculation. It has been revealed that though there were no significant treatment effects among the rat groups treated with different plant extracts, there were differences in the time effect. This finding suggests that all the plants used in this experiment were effective against the test organism *In vivo* having almost the same potency. The differences in time effect exhibited by the plant extracts in reducing the concentration of *E. coli O157:H7* in the rats could be due to the type and the quantity bioactive ingredients present in the plants (Gricilda and Molly, 2001). The fact that *P. guajava* was the first to eliminate the pathogen from the faeces of the rats is in line with the report of Abdelrahim, (2002) that stated that the plant possesses not only antimicrobial properties but has been generally used for the treatment of diarrhoea, dysentery and many other ill health conditions. The present study has also revealed that the ethanolic extracts of the plant species prevented the development of diarrhoea in rats treated with the plants.

Thus all the rats treated in the plant species were protected against diarrhoea that is usually induced by *E. coli O157:H7* infection. This suggests that the extracts at a dose of 3 mg per kg of rat body weight suppressed the accumulation of fluid in the intestinal wall of the rats. Previous reports have demonstrated the anti-diarrhoeal activity of tannin (Mukherjee *et al*., 1998), flavonoids (Galvez *et al*., 1993a), alkaloids (Gricilda and Molly, 2001), Saponins, sterols, and terpenes (Otshudi *et al*., 2000) containing plant extracts. Preliminary phytochemical analyses of the plant extracts used in this experiment showed the presence of all these compounds. These constituents may be responsible for the anti-diarrhoeal activity of the plant extracts. Recently, several flavonoids have been shown to inhibit biofilm formation of *E. coli O157:H7* (Vikram *et al*., 2010). The hallmark of *E. coli* O157: H7 infection is attaching and effacing lesions, and the first step of infection involves adhesion of bacteria to host epithelial cells and the formation of microcolonies (biofilms). Jin-Hyung *et al*. (2011) reported that the treatment with phyloretin (54.8mg/ml) apparently reduced the attachment of *E. coli* O157: H7 cell to epithelial cells of mice. It has also been reported that phyloretin, a flavonoid, possesses anti-inflammatory effect against inflammatory bowel diseases (IBDs) *in vitro* (Jung *et al*., 2009). In addition, Jin-Hyung *et al*. (2011) reported that the effect of phloretin (20 mg/kg/day on mice) was more prominent than that of the conventional IBD drug 5-aminosalicylic acid (100mg/kg/day on mice) in every aspect of the inflammatory response: body weight, colon weight and myeloperoxidase (MPO) activity. Thus, suggesting that phloretin can be a potent therapeutic agent for IBD. The anti-diarrhoeal activity of the flavonoid has been ascribed to their ability to inhibit intestinal mobility and hydro electrolytic secretion (Dicarlo *et al*., 1993). *In vivo* experiment has shown that flavonoids are able to inhibit the intestinal secretory response (Sanchezde *et al*., 1997). Flavonoids also possess antioxidant properties (Su *et al*., 2000), which are presumed to be responsible for inhibitory effect exerted upon several enzymes including those involve in the arachidonic acid metabolism (Mora *et al*., 1990). As a consequence it is possible to suggest that the antibiofilm formation, anti-inflammatory, antisecretory and antioxidant properties of flavonoid could be contributory to the observed antidiarrhoeal effect of the extracts of the plant employed in this study.

The present study has demonstrated that the inhibitory effect of the antibiotic drug against the organism *In vivo* seemed to be less effective than the effect of the plant extract; hence, 60% of the rat had the symptoms of diarrhoea. The reason for the less effectiveness of the antibiotic as compared to the plant extracts could be attributed to the fact that the antibiotic drug inhibited the competitive microorganism in the gut more than *E. coli* O157: H7 strain. This condition enables the proliferation of *E. coli* O157: H7 in the gut and also enhances the development of disease condition such as watery diarrhoea. This suggestion was supported by Jin-Hyung *et al.* (2011) who reported that most antibiotics often eradicate intestinal commensal bacterial more than the pathogenic bacteria. Hence most antibiotics that primarily aim to inhibit cell growth may result in bacterial drug resistance. Meanwhile, biofilm inhibitors such as flavonoids do not affect cell growth and there is less of a chance of resistance development (Hentzer, 2002). Due to increased resistance to antibiotic treatment, biofilms formed by pathogenic bacteria pose a serious problem to human health (Costerton *et al*., 1999). In contrast some commensal bacterial cells are crucial for nutrient assimilation and beneficial to human immune system (Hopper and Gordon, 2001). The result of this study revealed that 100% of the rats infected with *E. coli* O157: H7 had symptoms of diarrhoea. The present study confirms the work of (Alali *et al*., 2004), where the 10 rats challenged with *E. coli O157:H7* at a dose of 1×10^9 cfu/ml all had symptoms of *E. coli O157:H7* infection such as watery diarrhoea and bloody diarrhoea. Robinson *et al*. (2006) reported that infection by *E. coli O157:H7* caused non bloody diarrhoea in some cases of infected calves. Alali *et al*. (2004) also reported that *E. coli* O157: H7 was isolated from 60% of diarrheoic lambs. In this study, bloody diarrhoea was not established in any of the rat infected with *E. coli O157:H7*. According to Alali *et al*. (2004) *E. coli O157:H7* infection can manifest in variety of ways, thus in human clinical condition, some individuals who were infected with the microbe remain asymptomatic, others experienced diarrhoea, but most developed bloody diarrhoea (Nataro and Kaper, 1998). The ability of *E. coli O157:H7* to cause diarrhoea in all the infected non treated rats and in some of the infected antibiotic group of rats and the consequent pathological manifestation such as general body weakness, loss of appetite, loss of body weight could have led to the death of the rats that died during the experiment. Many authors have ruled out the use of antibiotics and favoured the use of phytochemical compounds as they exhibited strong antimicrobial activity against a wide range of Gram positive and negative bacteria without mutagenicity (Galvez *et al*., 1993b; Jin-Hyung *et al*., 2011). Thus, there is a growing interest in using herbs both in animal production and in treatment of various diseases of man and animals. It is clear that the re-isolation of *E. coli* O157H7 from the faeces of the rats treated with 5 plant extracts was 0% up to the end of the experiment. This may be as a result of the medicinal potency of these plants against *E. coli O157:H7* cells in the gut of the rats. It is also evident that the dose of the plant extracts (3.0 mg/kg) used in this study was effective in preventing the development of symptoms of *E. coli O157:H7* infection such as watery diarrhoea and bloody diarrhoea. Hence, at such low dose, the plant extracts had the ability to destroy the pathogen but not to kill the experimental animals. Rats of infected non-treated group and those of infected antibiotic- treated group that survived at the end of the experiment may be attributed to individual host immune status (Girard *et al*., 2005).

CONCLUSION

From this study, it is concluded that *E. coli* O157: H7 is a zoonotic and virulent microorganism which causes pathological symptoms such as diarrhoea and other abnormalities and can lead to death of animals experimentally infected with the organism. All the test plant extracts have the ability to fight *E. coli O157:H7*, as antimicrobial and anti-diarrhoeal agents. However, among the extracts of various plants, that of *P. guajava* can be the most potent therapeutic agent for the treatment of *E. coli O157:H7* infection as the rat group treated with it stopped shedding the pathogen in their faeces at quantifiable concentration levels before the rat groups treated with the extracts of other plants.
The plant species employed in this study are available in our environment and at a low dosage can protect animals suspected to have *E. coli O157:H7* infection.

ACKNOWLEDGEMENT

We thank the University of Jos, Nigeria administration for sponsoring this research.

REFERENCES

Abdelrahim SI, (2002). Antimicrobial activity of *Psidium guajava* L. *Fitoterapia*, 73(7 – 8): 713 – 715.

Akinpelu DA, Onakoya TM (2006). Antimicrobial activities of medicinal plants used in folklore remedies in South-Western States of Nigeria. *Afri. J. Biotechnol.*, 5(11): 1078 – 1081.

Alali WQ, Sargeant JM, Nagaraja TG, Debey BM (2004). Effect of antibiotics in milk replacer on faecal shedding of *Escherichia coli O157:H7* in calves. *J. Animal Sci.*, 82(7): 2148 – 2152.

Aranda-Michel J, Gianella RA (1999). Acute diarrhoea: A practical review. *Ame. J. Med.*, 106:670-676.

Bell BP, Goldoft M, Griffin PM, Davis MA, Gordon DC, Tarr PI, Bartleson CA, Lewis JH, Barret TJ, Well JG (1994). A multistate outbreak of *Escherichia coli O157:H7* associated bloody diarrhoea and haemolytic uraemic syndrome from hamburgers. The Washington Experience. *J. Ame. Med. Assoc.,* 272 (17):1349 – 1353.

Centres for Disease Control and Prevention (1995). *Escherichia coli O157:H7* outbreak linked to commercially distributed dry–cured salami Washington and California. 1994. *Morbidity and Mortality Weekly Report*, 44:154 – 160.

Centres for Disease Control and Prevention (2006). *Questions and Answers: Sickness caused by E. coli O157:H7*.http://www.cdc.gov/ncidod/dbmd/disease info/escherichia coli-g.htm. (Retrieved on 7/16/ 2007).

Costerton, JW, Stewart PS, Greenberg EP, (1999). Bacterial biofilms a common cause of persistent infections*. Science*, 284:1318-1322.

Cray WC, Moon HW (1995). Experimental infection of calves and adult cattle with *Escherichia coli O157:H7*. *Applied and Environmental Microbiology*, 61:1586-1590.

Dicarlo G, Autore G, Izzo AA, Maibline P, Mascolo N, Viola P, Diurno MV, Capasso F (1993). Inhibition of intestinal motility and secretion by flavonoids in mice and rats: Structure activity relationships. *J. Pharm. Pharmacol.*, 45:157-159.

Edeoga, HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afri. J. Biotechnol.*, 4:685 – 688.

Elder RO, Keen JE, Wittum TE, Callaway TR, Endrinton TS, Anderson RC, Nisbet DJ (2002). Intervention to reduce faecal shedding of enterohaemorrhagic *Escherichia coli O157:H7* in naturally infected cattle using neomycin sulfate*. J. Animal Sci,* 80(1):151.

Eman MA, Hoda MZ (2008). Studies on the effect of garlic preparation on *Escherichia coli O157:H7* causing enteritis in lambs. *Egy. J. Clinical Pathol.* 21(4):102-129.

Erodgrul OT (2002). Antibacterial activities of some plant extracts used in folk medicine. *Pharm. Biol.,* 40:269 – 273.

Fox JT, Thomson DU, Drouillard JS (2009). *Escherichia coli O157:H7* vaccine dose-effect in feedlot cattle. *Food Borne Pathogens and Disease*, 6(7): 893-899.

Galvez J, Crespo ME, Jimenez J, Suarez A, Zarzuelo A (1993a). Anti-diarrhoeic activity of quercitrin in mice and rats. *J. Pharmacol.*, 45:157-159.

Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocete MA, Jimenez J (1993b). Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. *Planta Medicine*, 59, 333-336.

Girard F, Oswald IP, Taranu I, Helie P, Appleyard GD, Harel J, Faibrother JM (2005). Host immune status influences the development of attaching and effacing lesions in weaned pigs. *Infection and Immunity,* 73(9): 5514 – 5523.

Gricilda SF, Molly T (2001). Study of anti-diarrhoeal activity of four medicinal plants in castor oil induced diarrhoea. *J. Ethanopharmacol.,* 76:73-76.

Griffin PM (1995). *Escherichia coli O157:H7 and other enterohaemorrhagic Escherichia coli.* In: Blazer M. J.,

Griffin PM, Tauxe RV (1991). The epidemiology of infections caused by *Escherichia coli O157:H7*, other enterohaemorrhagic *Escherichia coli*, and the associated haemolytic uraemic syndrome. *Epidemiologic Review*, 13:60 – 98.

Hentzer M (2002). Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology*, 148:87-2020.

Hermon BG, Brown CA, Tkalcic S, Mueller POE, Parks A, Jain AV, Zhao T, Doyle MP (1999). Faecal shedding and rumen growth of *Esherichia coli* in fasted calves. *J. Food Protection,* 62: 574-574.

Hopper LV, Gordon JI (2001). *Commensal host-bacterial relationships in the gut*. *Science*, 292:1115-1118.

Jin-Hyung L, Sushil CR, Jung-Ae K, Moo HC, Hyungdon Y, Chang-Soo L, Jintae L (2011). Apple flavonoid phloretin inhibits *Escherichia coli O157:H7*. Biofilm and ameliorates colon inflammation in rats. *Infection and immunity*, 79(12): 4819-4827.

Jung M, Triebel S, Anke T, Richling E, Erkel G (2009). Influence of apple polyphenols on inflammatory gene expression. *Mocular Nutritional Food Resources,* 53:1263-1280.

Kloucek P, Polesny Z, Svobodova B, Vlkova E, Kokoska L (2005).Antibacterial screening of some Peruvian medicinal plants used in Calleria District. *J. Ethnopharmacol.*, 99:309 – 312.

Krystle LM, Alison DO (2011). Mouse Models of *Escherichia coli O157:H7* infection and Shiga toxin injection. *J. Biomed Biotechnol.,* 25:81-85.

Kumar S, Dewan S, Sangraula H, Kumar VL (2001). Anti-diarrhoeal activity of the latex of *Calotropis procera*. *J. Ethanopharmacol.,* 76:115-118.

Majumdar AM, Upadhye AS, Misar AV (2000). Studies on anti-diarrhoeal activity of *Jatropha curcus* root extract in albino mice. *J. Ethanopharmacol.*, 70: 183-187.

Mora A, Paya M, Rios JL, Alcaraz MJ (1990). Structure activity relationships of polymethoxy flavones and other flavonoids as inhibitors of non-enzymic lipid peroxiation. *Biochem. Pharmacol.*, 36:317-322.

Moxley RA, Smith DR, Luebbe M, Erickson GE, Klopfenstein TJ, Rogan.D (2009).*Escherichia coli O157:H7* vaccine dose-effect in feedlot cattle. *Food borne Pathogens and Disease*, 6(7) 879-884.

Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP (1998). Screening of anti-diarrheaol profle of some plant extracts of a specific region of

Wet Bengal, Indian. *J. Ethnopharmacol.*, 60:85-89.
Nataro JP, Kaper JB (1998). Diarrhoegenic *Escherichia coli*. *J. Clinical Microbiol. Review,* 11: 142 – 20.
Nwokedi VC, Itelima JU, Ogaraku AO (2003). The antimicrobial activities of extracts of *Veronia amydalina* and *Telfairia occidentalis* on some bacteria. *West Afri. J. Biol. Sci.*, 14:43 – 47.
Okorondu SI, Braide W, Ogbulie TE, Akujobi CO(2006). Antimicrobial and Phytochemical properties of some traditional species. *Nigeria J. Microbiol.*, 20(3): 1301 – 1308.
Ostroff SM, Griffin PM, Tauxe RV(1990). A state wide outbreak of *Escherichia coli O157:H7* infections in Washington State America. *J. Epidemiol.*, 132:239 – 247.
Otshudi AL, Foriers A, Vercruysse A, Van Zeebroeck A, Lauwers S (2000). *In vitro* antimicrobial activity of six medicinal plants traditionally used for treatment of dysentery and diarrhoea in Democratic Republic of Congo. *Phytomedicine*, 7.167-177.
Price SB, Wright JC, De Graves FJ (2002). Antibiotic-induced modulation of *Escherichia coli O157:H7* shedding in experimentally infected calves. Abstract in Proceeding 83rd Conference Resource. Workers of Animal Disease, St. Louis, Mo. USA, pp. 98.
Rani S, Ahamed N, Rajaram S, Saluja R, Thenmozhi S, Murugesan T (1999). Anti-diarrhoeal evaluation of *Clerodendrum phlomidis* Linn. Leaf extract in rats. *J. Ethanopharmacol.*, 68:315-319.
Robinson CM, Sinclair JF, Smith MJ, O' Brien AD (2006). *Shiga toxin of enterohaemorrhagic Escherichia coli type* O157:H7 promotes intestinal colonization. National Academe Science USA, 20103 (25):9667-9672.
Sanchezde MF, Galvez, J., Gonzalez, M.F., Melo, L.L, and Silveira, E.R. (1997). Effect of quercetin on epithelial cell secretion. *Life Science*, 61:2049-2055.
Smith, P. D and Ravdin JI (2009). I*nfection of the gastrointestinal tract*. Raven Press New York, pp. 739 – 761.
Su YL, Leung LK, Bi YR, Huang Y, Chen ZY (2000). Antioxidant activity of flavonoid isolated from *Scutellaria rehderiana. J. Ame. Chem. Soc.,* 77:807-812.
Thomas GB, David MD, Swerdlow MD, Patricia L, Griffin MD (1995). *Escherichia coli O157:H7* and haemolytic uraemic syndrome. *The New Eng. J. Med.*, 333(6): 364 – 368.
Tona L (1999). Biological screening of traditional preparation from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. *Phytomedicine*. 55(6): 34 – 40.
Venkatesan N, Vadivu T, Sathiya N, Arokya A, Sundararajan R, Sengodan G, Thandavarayan JB(2005). Anti-diarrhoea potential of *Asparagus racemosus* wild root extracts in laboratory animals. *J. Pharm. Pharm. Sci.*, 8(1):39-46.
Vikram A, Jayaprakasha GK., Jesudhasan PR, Pillai SD, Patil BS (2010). Suppression of bacterial cell-cell signaling, biofilm formation and type III secretion system by citrus flavonoids. *J. Applied Microbiol.,* 109:515-527.
Walterspiel JN, Ashkenazi S, Morrow AL, Cleary TG (1992). Effect of subinhibitory, concentrations of antibiotics on extracellular shiga-like toxin 1. *Infection*, 20 (1):25-9.

Accepted 2 March, 2014.

Citation: Itelima JU, Agina SE (2014). *In vivo* antimicrobial activity of plant species on *Escherichia coli O157:H7* inoculated into albino rats. World Journal of Microbiology, 1(1): 002-009.