Full Length Research Paper

Prebiotic effects of *Vernonia amygdalina* and *Ocimum gratissimum* aqueous leaf extracts in rabbit (*Oryctolagus cuniculus*)

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The prebiotic potentials of crude extracts of *Vernonia amygdalina* and *Ocimum gratissimum* in rabbit (*Oryctolagus cuniculus*) were studied. The aqueous leaf extracts of both plants, taken orally, were evaluated for their effects on the composition and relative abundance of the rabbit gastrointestinal microflora as well as their protective roles against gastrointestinal infection by selected test organisms. The crude extracts were administered to eight-week old rabbits with average weight of 0.85 ± 0.14 kg, at a dosage of 0.1 g/day. Species of *Corynebacterium, Enterococcus, Bacteroides, Lactobacillus* and *Actinomyces* were identified as resident bacteria in the rabbit gastrointestinal tract (GIT). Consumption of *Vernonia amygdalina* extract selectively and significantly ($p < 0.05$) increased the numbers of *Enterococcus* and *Actinomyces* species recovered from the animals faecal specimens, while *O. gratissimum* increased only the *Actinomyces* species. In contrast, the numbers of *Bacteriodes* species were significantly decreased by both extracts. Consumption of *V. amygdalina* extract also reduced colonization and damage of the animals GIT by *Escherichia coli* and *Staphylococcus aureus* as evidenced by restricted shedding of the organisms in faeces and histopathological examination of colon tissues. The results from this study showed that the *V. amygdalina* leaf extract has prebiotic effects in the rabbit GIT including protection of the animals against some GIT pathogens.

Key words: Prebiotics, *Vernonia amygdalina*, *Ocimum gratissimum,* gastrointestinal tract, rabbit.

INTRODUCTION

Prebiotics are non-digestible substances that when consumed provide a beneficial physiological effect on the host, by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria (Roberfroid, 2007; Mitchell, 2010). These prebiotic substances survive digestion in the stomach and reach the colon where they are metabolized by the bacteria, thereby directly providing the host with energy and metabolic substrates (Wang and Gibson, 1993; Cummings et al., 2001). Efficient prebiotics usually have a specific fermentation in the colon and have the ability to alter the faecal microflora composition towards a more beneficial community structure (Kolida et al., 2002; Chakraborti, 2011). Thus, prebiotics exert their beneficial effects on the host indirectly, by stimulating the beneficial functions of the intestinal microflora.

The microflora of the intestinal microenvironment, as a unit, has important protective, metabolic and trophic functions (Canny and McCormick, 2008). Among several functions, these commensal bacteria compete for available nutrients in ecological niches and, in doing so, maintain the collective microenvironment by administering and consuming all resources. The host also benefits from the ability of the intestinal microflora to synthesize vitamins, exert trophic effects on intestinal epithelial cells, and salvage energy from unabsorbed food by producing short-chain fatty acids. Furthermore, these beneficial bacteria inhibit the growth of pathogens, sustain intestinal barrier integrity and maintain mucosal

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Plate 1. Photograph of *Vernonia amygdalina* leaves.

immune homeostasis (Ewaschuk and Dieleman, 2006; Canny and McCormick, 2008).

Prebiotics have recently come into use as an alternative to probiotics. While probiotics are meant to bring beneficial microorganisms to the gut, prebiotics selectively stimulate the beneficial microorganisms that already live there. Thus, prebiotics have two clear advantages relative to probiotics: firstly, there are no critical problems of inability of the ingested probiotic to survive the acid conditions of the stomach and secondly, there is no introduction of foreign microbial species into the gut (Macfarlane et al., 2006; Falcao-e-Cunha et al., 2007). In addition to these, prebiotics also have the advantage of relative ease of manufacture because they can be either directly extracted from natural sources or be produced by partial acid or enzymatic hydrolysis of polysaccharides or by transglycosylation reactions (Macfarlane et al., 2006; Falcao-e-Cunha et al., 2007).

The role of prebiotics in modulation of bowel function has been widely studied and reported (Gibson and Roberfroid, 1995; Hamilton-Miller, 2004; Lomax and Calder, 2009), but more recent studies have focused on their protective role against infections and diseases (Lomax and Calder, 2009; Chakraborti, 2011; Licht et al., 2011). The most widely studied prebiotics are inulin and non-digestible oligosaccharides such as oligofructose (Watzl et al., 2005; Leenen and Dieleman, 2007; Guarner, 2007; Lomax and Calder, 2009). However, recent studies show that there may be other candidate-prebiotics such as xylitol, sorbitol, mannitol and lactulose (Chakraborti, 2011).

V. amygdalina (bitter leaf) is a popular African vegetable. It is used in various food preparations and in ethnomedicine for the treatment of malaria and gastrointestinal infections. The aqueous leaf extract has also been shown to have blood sugar and lipid-lowering effects in experimental animals (Akah and Okafor, 1992; Adaramoye et al., 2008). The prebiotic potentials of *V. amydgalina* leaf extract and its effect on bowel function in humans has previously been reported (Ezeonu and Ukwah, 2009). The ability of this extract to act as carbon and energy source for some intestinal bacteria, *in vitro* has also been reported (Ukwah and Ezeonu, 2008). This present study, using rabbit models, focuses on the protective role of the leaf extract against selected intestinal pathogens.

MATERIALS AND METHODS

Collection of plant materials and preparation of aqueous plant-leaf extracts

Leaves of *V. amygdalina* and *O. gratissimum* were collected from private farms in Nsukka town. The plants were authenticated by a taxonomist in the Department of Botany, University of Nigeria, Nsukka. The pictures of the plants are shown in Plates 1 and 2. The aqueous extracts of the plants were prepared by modification of the methods of Arhoghro et al. (2009). Briefly, 200 g of washed and air-dried leaves of each plant were marcerated in 1 L of sterile distilled water for 24 h and then filtered through Whatman No. 1 filter paper. The resulting filtrate was evaporated to a sticky paste under a constant stream of cool air. Working solutions of the concentrated extracts were obtained by dissolving 1 g portions of each in 1 L of sterile distilled water.

Plate 2. A photograph of *Ocimum gratissimum* leaves.

Collection of animals

Twenty-seven 8-week old, New Zealand breed rabbits of either sex were purchased from a commercial supplier. The rabbits, with average weight of 0.85 ± 0.14 kg, were maintained three to a cage, in the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were allowed free access to feed (growers guinea feed) and clean water according to the guidelines of the National Institute of Health (NIH) publications for laboratory animal care and use (NIH, 1985). The animals were acclimatized to the housing and feeding conditions for a period of ten days.

Test organisms

Type strains of *E. coli* (ATCC 11775) and *S. aureus* (ATCC 10145) were obtained from the laboratory of Prof. C. U. Iroegbu of the Department of Microbiology, University of Nigeria, Nsukka, while *Salmonella typhimirium* (S57), originally obtained from Engeline Van Duijkeren, Department of Infectious Diseases and Immu-nology, Faculty of Veterinary Medicine, Utrecht University, Netherlands was generously provided by Dr. K. F. Chah of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The strains were maintained in normal nutrient broth (Oxoid) at 37°C.

Screening of animals for colonization by E. coli, S. aureus and Salmonella

At the beginning of the experiment, the animals used in this study were all screened to ensure that they were free from *E. coli, S. aureus* and *Salmonella* infections prior to treatment and subsequent challenge with the test organisms. Screening for *E. coli* infection was carried out by culturing rectal swabs from each animal on freshly prepared MacConkey agar (Oxoid). For *Staphylococcal* infection, rectal swabs were cultured unto freshly prepared blood

agar medium, while *Salmonella* infection was screened by pre-enriching the rectal swab samples in Selenite F broth and incubating at 37°C for 18 to 24 h before subculturing unto freshly prepared MacConkey agar. All agar plates were incubated at 37°C for 24 to 48 h.

Determination of rabbit gastrointestinal microflora

The animals were monitored for a period of 11 days, during which there was repeated isolation of organisms from the animals faecal samples at three-day intervals. All samples were collected directly from the animals rectum into sterile containers and weighed. Analyses of samples were done within one hour of collection, and those that could not be analysed within that time were held at 4°C until analysis for no longer than 24 h.

One gram of each faecal sample was homogenized in 10 ml of freshly prepared sterile 0.1 M phosphate buffer (pH 7) to obtain a 0.1 g/ml faecal suspension. Serial 10-fold dilutions were prepared using half-strength sterile peptone water. Samples (0.1 ml) of the faecal suspensions were inoculated onto different bacteriological media [MacConkey agar, Blood agar and Man Rogosa Sharpe (MRS) agar] (Oxoid), in duplicates, using the spread plate method. Plates were incubated at 37°C for 24 h for coliforms, 4 days for bifidobacteria, and 3 days for lactobacilli. Isolated colonies were picked and identified by the standard methods of Cheesebrough (2000). The consistency of occurence of each of the isolates was noted. Counts of the different organisms were also taken during each of the repeated isolations and expressed as colony forming units per gram (cfu/g) of faeces.

Administration of extracts to animals

Extracts were administered to the animals at the dosage of 100 mg/day; given in two doses, morning and evening. Control animals

Table 1. Animal treatment and challenge groups.

Treatment group	Animal group	Treatment	Challenge organism
1	Group A	Distilled water	*Salmonella typhimurium* (S58)
1	Group B	Distilled water	*Escherichia coli* (ATCC 11775)
1	Group C	Distilled water	*Staphylococcus aureus*(ATCC 10145)
2	Group D	*Vernonia amygdalina*	None
2	Group E	*Ocimum gratissimum*	None
3	Group F	*Ocimum gratissimum* (OG)	*Salmonella typhimurium* (S58)
3	Group G	*Vernonia amygdalina* (VA)	*Salmonella typhimurium* (S58)
3	Group H	*Vernonia amygdalina*	*Escherichia coli* (ATCC 11775)
3	Group I	*Vernonia amygdalina*	*Staphylococcus aureus*(ATCC 10145)

In all, twelve animals were treated with *V. amygdalina,* six with *O. gratissimum* and nine with distilled water. Animals given no extracts (given distilled water) and later challenged, as well as the animals given extracts but not challenged with any test pathogens all served as controls.

not receiving extracts were given a corresponding volume of distilled water. The extracts were administered orally using 200 ml baby feeding bottles, one for each extract group.

Determination of the effect of extracts on composition of microflora

While the animals were on treatment, faecal samples were taken at three-day intervals and analysed as previously described. The microbial counts obtained during this period for each of the isolates were compared with that obtained for the same isolates at the pre-treatment phase.

Experimental infection of animals with test organisms

At the end of the 16-day treatment period, with the animals still on the extracts, animals were orally infected with different test pathogens, *S. typhimurium* (S58), *E. coli* (ATCC 11775) and *S. aureus* (ATCC 10145) according to the groupings as shown in Table 1.

The infecting dose of each test organism was prepared by resuscitating a frozen stock culture of the organism in freshly prepared peptone water and incubating for 48 h at 37°C. Following incubation, the culture was adjusted to a concentration of 10^8 cfu/ml. Then a 1 in 10 dilution was made (1 ml of culture into 9 ml of sterile normal saline) to obtain the challenge dose of 10^7 cells. Oral infection was accomplished by direct inoculation of the culture suspension using a sterile 1 ml syringe. This concentration of *Salmonella* has been shown by Hanes et al. (2001) to establish diarrhoeal disease in adult rabbits.

Evaluation of the protective role of extract consumption against infection

Animals infected as described above were monitored for progression of gastrointestinal infection and disease or otherwise, by monitoring the shedding of the organisms in faecal specimens, monitoring body weight differences, observing the animals for clinical signs of illness and histopathological examination of organs following sacrifice. Rectal swabs were collected daily from each of the animals and cultured on MacConkey agar, following 18 h of pre-enrichment in selenite-F broth for *Salmonella*. Examination for the three test organisms commenced 24 h post-infection and continued

till the animals were either dead or sacrificed. Body weights of the animals were recorded and animals were monitored for signs of diarrhoea. After ten days observation or immediately following death, when death occurred, representative animals were sacrificed and tissues taken for histopathological studies.

The colon tissues were cut into small pieces and fixed in 10% formal saline for 24 to 48 h. Then, the tissues were dehydrated in a series of ascending ethanol concentrations (70, 80, 90, and 95%, and absolute) and embedded in paraffin. Sections were cut with a microtome, at a thickness of 5-6 µm. The cut sections were stained with haematoxylin and eosin stain (H and E stain) according to the methods of Harris et al. (1993). The stained sections were examined under a microscope and photographed with a Motic cam digital camera (Motic China Group Co Ltd).

Statistical analysis

The 2-tailed student's t test was used to assess the statistical significance of the differences between the pre-treatment counts and the treatment counts, while the differences in mean body weight of the animals were analysed by least Significant difference (LSD). Significance was taken at the 95% confidence limit.

RESULTS

Precolonization of animals by E. coli, S. aureus and Salmonella

All animals used in this study were found to be free of colonization by the three test organisms before the start of the experiment.

Identification of rabbit intestinal microflora

Up to eleven different bacteria were identified from re-peated examination of faecal samples of the test animals over a period of 16 days. The identified organisms and their patterns of occurrence are shown in Table 2. Organisms appearing consistently through the monitoring period were considered members of the resident microflora. These included species of *Corynebacterium,*

Table 2. Suspected bacteria isolated from faecal samples of animals before treatment.

Organism	Period of Isolation					
	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16
Corynebacterium spp.	+	+	+	+	+	+
Bacillus spp	+/-	+/-	+	-	+	-
Bacteroides spp	+	+	+	+	+	+
Lactobacillus spp	+	+	+	+	+	+
Bifidobacterium spp	+/-	+/-	+/-	+	+/-	+/-
Actinomyces spp	+	+	+	+	+	+
Staph saccharolyticus	+	-	-	+/-	-	-
Coagulase negative *Staphylococcus* spp	-	-	-	+/-	+	+
Pseudomonas spp	+/-	-	+/-	-	-	-
Clostridium spp	+	-	+/-	-	-	-
Enterococcus spp	+	+/-	+	+	+	+

+: occurrence of organism in all animals; -: non-occurrence of organism in all animals; +/- : occurrence of organism in some animals.
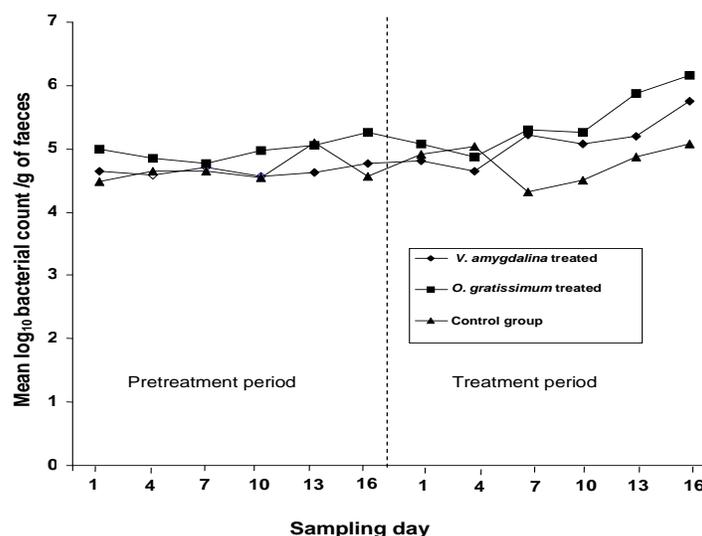
Figure 1. Effect of extract consumption on counts of *Corynebacterium* spp in animals faecal samples.

Enterococcus, Bacteroides Lactobacillus and *Actinomyces.* Other organisms were considered transient flora. Bifidobacteria were isolated throughout the monitoring period, but only in low numbers.

Effect of oral administration of the aqueous leaf extracts on the composition of the rabbit intestinal microflora

The effects of oral administration of crude extracts of *V. amygdalina* and *O. gratissimum* on the relative abundance of the rabbit intestinal microflora are shown in Figures 1 to 5. Both extracts did not significantly alter the composition of the rabbit gastrointestinal microflora as most of the organisms encountered in the pre-treatment phase were still present during the treatment phase. However, there were significant effects of consumption of the extracts on the relative abundance of the organisms. The extracts had no significant effects on the counts of *Corynebacterium* species (Figure 1). *V. amygdalina* extracts significantly ($p < 0.05$) increased the numbers of *Enterococcus* and *Actinomyces* from 24 h post treatment, while *O. gratissimum* significantly ($p < 0.05$) increased only numbers of *Actinomyces* spp. (Figures 2 and 3). The numbers of *Bacteroides* spp. were significantly ($p < 0.05$) reduced by both extracts (Figure 4) and for *Lactobacillus* there was significantl ($p > 0.05$) stimulation only with *O. gratissimum* (Figure 5).
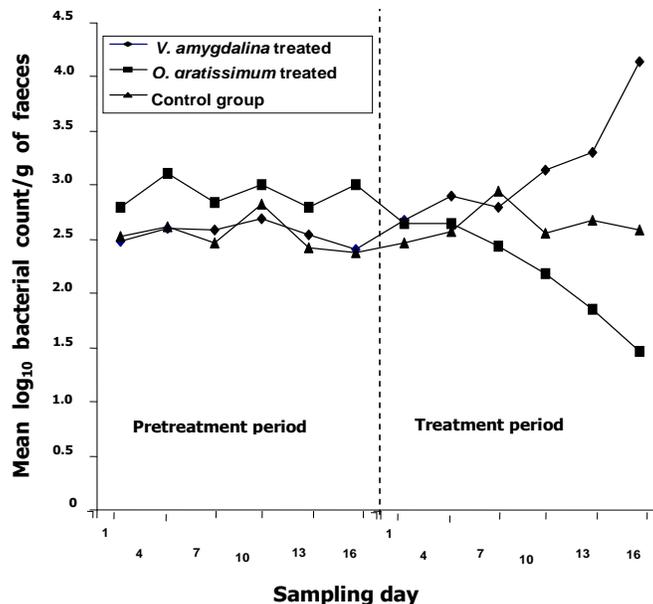
Figure 2. Effect of extract consumption on counts of *Enterococcus* spp in animals faecal samples
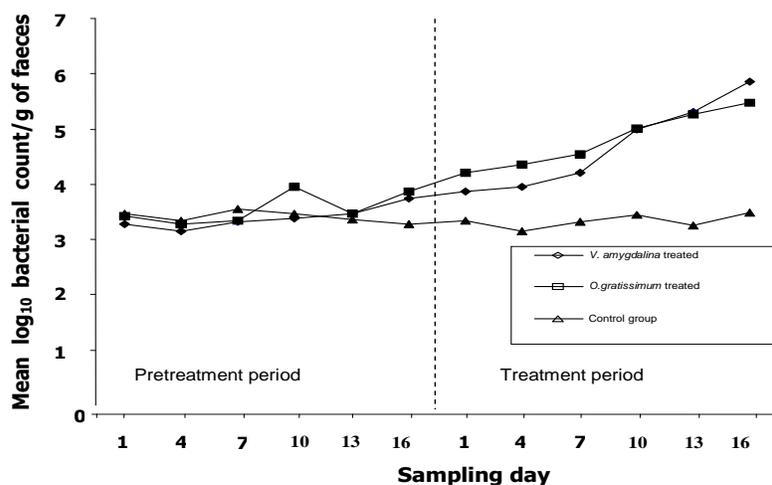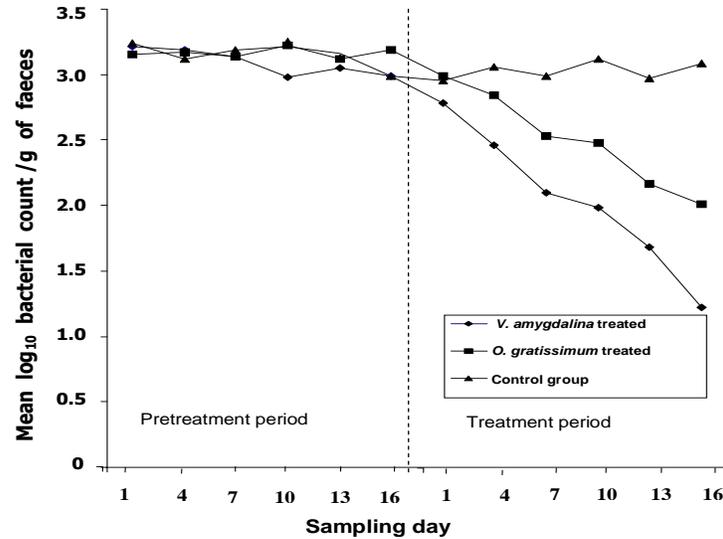

Figure 3. Effect of extract consumption on counts of *Actinomyces* spp in animals faecal samples.

## Protective role of extracts against infection by test organisms

Following infection with the test organisms, the animals were found to shed the organisms in their faeces from 24 h post infection. For animals treated with *E. coli* and *S. aureus,* shedding occurred only for three and four days, respectively in the treated animals but continued for up to seven days or more in the control animals. For animals treated with *S. typhimurium*, on the other hand, shedding of the organisms occurred more in the treated than the control animals (Table 3).

In animals treated with *E. coli* and *S. aureus*, there was no episode of diarrhoea and no significant weight change in either the treated or control animals. In *Salmonella* treated animals, however, 2 (66.7%) of the animals developed severe diarrhoea by four days postinfection, as indicated by loose stool. By 8 days post-infection, all the animals in the group had developed diarrhoea while no animal in the control group had diarrhoea. There was a significant ($p < 0.05$) drop in body weight (from about 0.85 ± 0.14 kg to 0.42 ± 0.16 kg) in the treated animals but not in the control animals. Furthermore, death occurred in the treated animals, from the fifth day post

Figure 4. Effect of extract consumption on counts of *Bacteroides* spp in animals faecal samples.
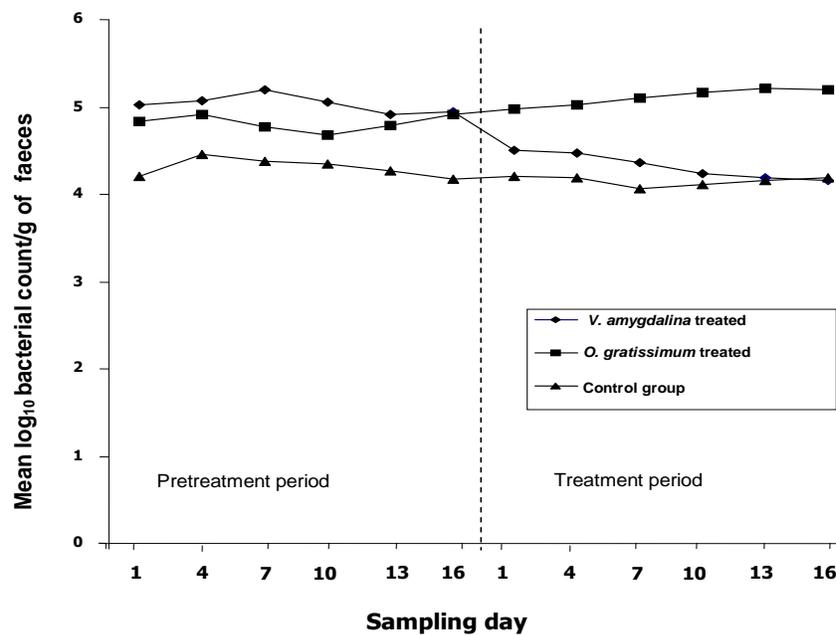
Figure 5. Effect of extract consumption on counts of *Lactobacillus* spp in animals faecal samples.

infection and by 14 days post infection, all the animals were dead. No death occurred in the control animals.

Histopathological examination of the colon tissues of treated and untreated animals showed that in animals treated with extract and then infected with *E. coli* and *S. aureus*, there was only mild pathological changes in the intestinal tissues compared with untreated animals (Figures 6 and 8). However, for animals treated and infected with *Salmonella*, there was gross damage to

tissues of the colon and other organs as well, including liver, kidney and spleen (pictures not shown) (Figure 7).

DISCUSSION

Bacteria from eleven different genera, yeasts and *Mucor* spp were isolated from faecal samples of the rabbits. Of these, only five, namely *Corynebacterium* spp.,

Table 3. Occurrence of *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium* shedding over a 21-day post- infection monitoring period**.**

| Day post infection | Number of animals shedding the organism (%)/Group | | | | | |
| | *E. coli* | | *S. aureus* | | *S.   typhimurium* | |
	VA	CL	VA	CL	VA	CL
1	2 (66.7)	3 (100)	2 (66.7)	3 (100)	3 (100)	2 (66.7)
2	2 (66.7)	3 (100)	2 (66.7)	3 (100)	3 (100)	2 (66.7)
3	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7)	3 (100)	2 (66.7)
4	0 (0)	2 (66.7)	1 (33.3)	2 (66.7)	3 (100)	1 (33.3)
5	0 (0)	1 (33.3)	0 (0)	1 (33.3)	3 (100)	1 (33.3)
6	0 (0)	1 (33.3)	0 (0)	1 (33.3)	3 (100)	1 (33.3)
7	0 (0)	1 (33.3)	0 (0)	1 (33.3)	3 (100)	1 (33.3)
14	0 (0)	1 (33.3)	0 (0)	0 (0)	3 (100)	0 (0)
21	0 (0)	1 (33.3)	0 (0)	0 (0)	3 (100)	0 (0)

VA: *Vernonia amygdalina* treated group*;* CL: Control group. *Note that in the groups treated with extract before infection (VA), shedding of the pathogens ceased after 3 and 4 days for *E. coli* and *Staphylococcus aureus,* respectively.
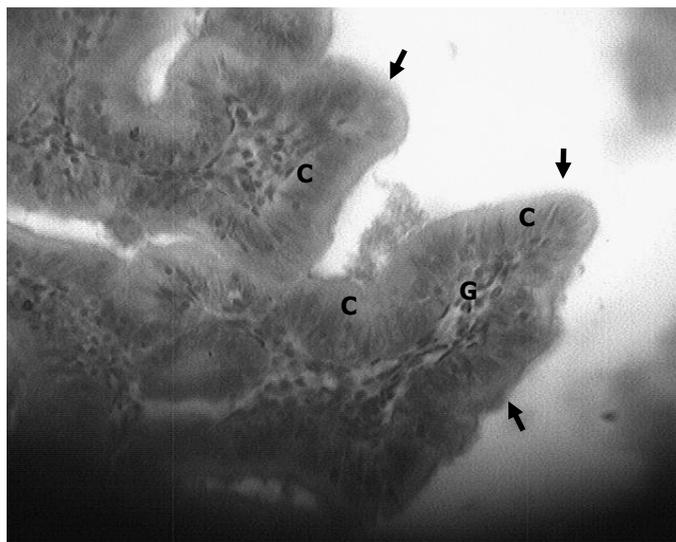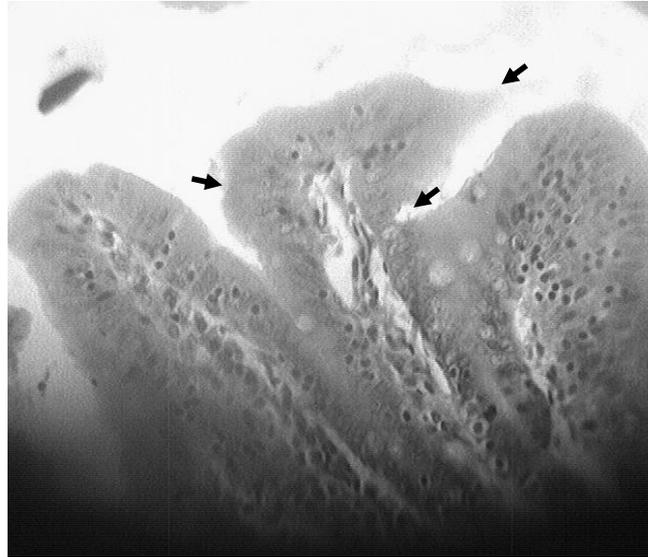

Figure 6. Photomicrograph of section of colon of rabbit treated with *Vernonia amygdalina* leaf extract and experimentally infected with *Escherichia coli*. Note relatively normal villi architecture (arrows) with columnar epithelium (C) and goblet cells (G). H and E stain. X400.

Enterococcus spp., *Bacteroides* spp., *Lactobacillus* spp. and *Actinomyces* spp. were consistently isolated through-out the sixteen-day monitoring period. These were therefore considered to be the resident microflora of the rabbit GIT, while the other bacteria were considered to be transient. *Bifidobacterium* species were among the least isolated organisms.

Although this finding appeared to be contrary to expectation as bifidobacteria are known to play important roles in maintaining microbial balance of a healthy intestinal tract in many animals (Cummings et al*.,* 2004), it is consistent with the findings of Straw (1989) that these

bacteria never dominated in the rabbit intestinal tract. Reports of use of probiotics in rabbits have also shown that microorganisms used in such probiotics are mostly strains of *Bacillus, Enterococcus, Lactobacillis* and *Saccharomyces* (Amber et al*.,* 2004; Falcao-e-Cunha et al., 2007), suggesting that these may be the more important beneficial organisms for rabbit.

The consumption of the extracts by the rabbits did not cause any observable changes in the composition of the microflora. The organisms encountered before the administration of the extracts were the same organisms encountered after treatment. However, there were effects

Figure 7. Photomicrograph of section of colon of rabbit treated with *Vernonia amygdalina* leaf extract and experimentally infected with *Staphylococcus aureus.* Note damaged and degenerated villi epithelium (arrow) and mild infiltration of neutrophils. H and E stain. X400.
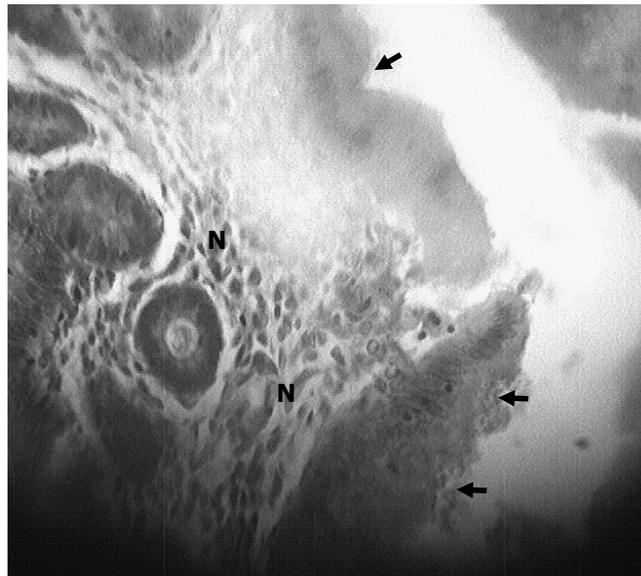
Figure 8. Photomicrograph of section of colon of rabbit not treated with extract and infected with *Escherichia coli.* Note severe destruction of the villi (arrow) and dense infiltration of the neutrophils (N). H and E stain. X400.

on the relative abundance of the different bacterial species. *V. amygdalina* extracts significantly (p < 0.05) increased the numbers of *Enterococcus* and *Actinomyces* from 24 h post treatment, while *O. gratissimum* significantly (p < 0.05) increased only numbers of *Actinomyces*

spp. The numbers of *Bacteroides* spp. were significantly (p < 0.05) reduced by both extracts while *Corynebacterium* and *Lactobacillus* were not significantly (p > 0.05) affected.

The prebiotic concept dictates that the prebiotic

ingredient should selectively stimulate the growth of a particular or limited number of potentially beneficial bacteria in the complex intestinal microflora, following its consumption. The number (for example, expressed as $\log_{10}$ cfu/g of faeces) of the stimulated organism(s) in that particular population should change significantly while others remain unchanged or even decrease (Gibson and Roberfroid, 1995; Gibson, 1999; Roberfroid et al., 1998; Menne et al., 2000). In this study, V. amygadalina extract significantly increased the counts of Enterococcus which has previously been listed as one of the probiotic organisms active in the gut (Macfarlane and Cummings, 1999; Crittenden et al., 2003; Linaje et al., 2004; Chakraborti, 2011). O. gratissimum, on the other hand, decreased the counts of Enterococcus and did not significantly affect Lactobacillus. Thus, V. amygdalina but not O. gratissimum was established by these findings as having prebiotic potentials.

The stimulation of Enterococcus is consistent with the findings of a previous study, in which V. amygdalina extract significantly enhanced the growth of enterococci in human GIT (Ezeonu and Ukwah, 2009). Reduction in the counts of Bacteroides following consumption of the extract, is also consistent with the action of a true prebiotic, according to Burns and Rowland (2000) and Chakraborti (2011), who stated that prebiotics increase the number of beneficial bacteria at the expense of other microflora components such as Bacteroides, clostridia and E. coli. These results with Lactobacillus was somewhat surprising, considering that lactobacilli are the most widely reported probiotic bacteria. However, an earlier study had shown that the numbers of lactobacilli increased only after the peak of enterococci (Ezeonu and Ukwah, 2009). The authors suggested that metabolic activities of the enterococci may have provided some essential requirements of the lactobacilli, for example folate. The lack of stimulation of lactobacilli observed in this study, could therefore be attributed to the lack of extended monitoring.

One of the major roles of the commensal bacteria in the GIT is to act as a barrier against colonization of the GIT by pathogenic bacteria. This protective role is also ascribed to prebiotics, which stimulate the beneficial bacteria. In this study, the protective role of the V. amygdalina extract against experimental infections with three test organisms was evaluated. Rabbits treated with extract for 21 days before experimentally infecting with the organisms were monitored for signs of illness, weight loss, shedding of organisms in faeces, diarrhoea and death. These results show that following infection with E. coli and S. aureus, shedding of the organisms in the faeces lasted for only 3 and 4 days, respectively while shedding continued for 21 and 14 days, respectively in the control animals. This finding suggests that consumption of the V. amygdalina extract protected the animals against sustained colonization by the test organisms. Also, histological examination of sections of

the colon of these animals after sacrifice, showed gross pathological changes in the intestinal architecture of the control animals, which were less evident in the treated animals. These results are therefore consistent with what is expected of a prebiotic; to stimulate probiotic bacteria in the gut, which in turn provide the protective effect (Kolida et al., 2002; Licht et al., 2011; Chakraborti, 2011). Although, previous studies on effects of prebiotics on rabbit performances have been inconsistent (Falcao-e-Cunha et al., 2007). Studies in other animal models, using known prebiotics like inulin and oligofructose, have established the role of prebiotics in restoring microbial balance and inhibiting the progression of disease in various forms of gastrointestinal illness (Ewaschuk and Dieleman, 2006; Bosscher et al., 2006).

Contrary to these results obtained with E. coli and S. aureus, consumption of the extract did not offer any protection to the animals against S. typhimurium. Shedding of the organism was evident in the animals and by 5 days post-infection, the animals developed severe diarrhoea with significant weight loss. On examination of the organs after sacrifice, the colon showed severe damage. Examination of other organs showed severe histopathologic changes in the liver and kidneys as well.

It is interesting that while treatment with V. amygdalina extract could protect the animals against infections with E. coli and S. aureus, the same protection was not offered against Salmonella. These findings suggest that protection conferred by probiotic organisms in the gut could vary with the type of infection or pathogen. The inability of the extract and the stimulated probiotic organisms to protect against Salmonella is however consistent with the findings of Bovee-Oudenhoven (2003), in a study in which is contrary to expectations, dietry fructo-oligosaccharides and lactulose impaired the resistance of the rats to Salmonella infection. The findings are also in agreement with other studies in which inclusion of fructo-oligosaccharide prebiotics in water and feed of animals protected against E. coli but not S. typhimurium infection (Lomax and Calder, 2009; Licth et al., 2011).

This study has confirmed the prebiotic nature of V. amygdalina leaves and also provided evidence that these leaves can be of use in preventing gastrointestinal disease and maintaining gastrointestinal health.

ACKNOWLEDGMENTS

REFERENCES

Adaramoye OA, Akintayo O, Achem J, Fafunso M (2008). Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. Vascular Health and Risk Management, 4: 235-241.

Amber KH, Yakout HM, Hamed Rawya S (2004). Effect of feeding diet containing yucca extract or probiotic on growth, digestibility, nitrogen balance and caecall microbial activity of growing New Zealand white rabbits. In Proc.: 8th World Rabbit Congress, Puebla, Mexico, pp.737-741.

Akah PA, Okafor CL (1992). Blood sugar lowering effect of *Vernonia amygdalina Del.* in an experimental rabbit model. Phytotherapy Res. 6: 171-173.

Arhoghro EM, Ekpo KE, Anosike EO, Ibeh GO (2009). Effect of aqueous extract of bitter leaf (*Vernonia amygdalina* Del) on carbon tetrachloride (CCl_4) induced liver damage in albino rats. Eur. J .Sci. Res. 26: 122-130.

Bosscher D, Van LI, Franck A (2006). Inulin and oligofructose as prebiotics in the prevention of intestinal infections and diseases. Nutr. Res. Rev. 18: 216-222.

Bovee-Oudenhoven IMJ, Bruggencate SJM, Lettink-Wissink MLG, Van der Meer R (2003). Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonization but stimulate translocation of *Salmonella* in rats. Gut. 52: 1572-1578.

Burns AJ, Rowland IR (2000). Anti-carcinogenicity of probiotics and prebiotics. Curr Iss Intestinal Microbiol. 1: 13-24.

Canny GO, McCormick BA (2008). Minireview: Bacteria in the intestine, helpful residents or enemies from within? Inf Immun. 76: 3360-3373.

Chakraborti CK (2011). The status of synbiotics in colorectal cancer. Life Sci Med Res. 2011: 1-13.

Cheesbrough M (2000). *District Laboratory Practice in Tropical Countries*. Tropical Health Technology and Butterworth – Heinemann Ltd Cambridge Low price ed. Vol. 2, pp 63-70.

Crittenden RG, Martinez NR, Playe MJ (2003). Synthesis and utilisation of folate by youghurt starter cultures and probiotic bacteria. Int .J. Food Microbiol. 80: 217-222.

Cummings JH, Macfarlane GT, Englyst HN (2001). Prebiotics digestion and fermentation. Am. J .Clin. Nutr. 73: 415-420.

Cummings JH, Antoine JM, Aspiroz F, Bourdet-Sicard R, Brandtzaeg P, Calder PC, Gibson GR, Guarner F, Isolauri E. (2004). PASSCLAIM-gut health and immunity. Eur .J .Nutr. 43: II118-II173.

Ewaschuk JB, Dieleman LA (2006). Probiotics and prebiotics in chronic inflammatory bowel diseases. World J Gastroenterol, 12: 5941-5950.

Ezeonu IM, Ukwah BN (2009). *In vivo* studies on the prebiotic effects of *Vernonia amygdalina* leaf extract. Bioresearch, 7: 396-401.

Falcao-e-Cunha L, Castro-Solla L, Maertens L, Marounek M, Pinheiro V, Freire J, Mourao JL (2007). Alternatives to antibiotic growth promoters in rabbit feeding: a review. World Rabbit Sci. 15: 127-140.

Gibson GR (1999). Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. J Nutr. 129: 1438S-1441S.

Gibson GR, Roberfroid MB (1995). Dietary modulation of the human colonic microflora: introducing the concept of prebiotics. J Nutr. 125: 1401-1412.

Guarner F (2007). Studies with inulin-type fructans on intestinal infections, permeability and inflammation. J Nutr. 137: 2568S-2571S.

Hamilton-Miller JM (2004). Probiotics and prebiotics in the elderly. Postgrad Med. J. 80: 447-451.

Hanes DE, Robl MG, Schneider CM, Burr DH (2001). New Zealand white rabbit as a nonsurgical experimental model for *Salmonella enterica g*astroenteritis. Inf Immun. 69: 6523-6526.

Harris TO, Grossman D, Kappler JW, Marrack P, Rich RR, Betley MJ (1993). Lack of complete correlation between emetic and T-cell-stimulatory activities of staphylococcal enterotoxins. Inf Immun. 61: 3175-3183.

Kolida S, Tuohy K, Gibson GR (2002). Review: Prebiotic effects of inulin and oligofructose. Bri .J Nutr . 87: S193-197.

Leenen CH, Dieleman LA (2007). Inulin and oligofructose in chronic inflammatory bowel disease. J. Nutr. 137: 2572S-2575S.

Licht TR, Ebersbach T, Frokiaer H (2011). Prebiotics for prevention of gut infections. Trends in Food Sci Tech xx: 1-13. [http://www.sciencedirect.com.]

Linaje R, Coloma MD, Perez-Martinez G, Zuniga M (2004). Characterization of faecal enterococci from rabbits for the selection of probiotic strains. J .Appl .Microbiol. 96: 761-771.

Lomax AR, Calder PC (2009). Prebiotics, immune function and inflammation: a review of the evidence. Br. J. Nutr. 101: 633-658.

Macfarlane GT, Cummings JH (1999). Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health? Br. Med .J. 318: 999-1003.

Macfarlane S, Macfarlane GT, Cummings JH (2006). Prebiotics in the gastrointestinal tract. Alimentary Pharmacol Therapy, 24: 701-714.

Menne E, Guggenbuhl N, Roberfroid M (2000). Fn-type chicory inulin-hydrolysate has a prebiotic effect in humans. J Nutr . 130: 1197-1199.

Mitchell D (2010). Prebiotics may help prevent colon cancer. [http://www.examhealth.com/1275/100/33330/prebiotics-mayhelp-prevent-colon-cancer.html]

National Institute of Health (NIH) (1985). Publications for laboratory animal care and use.

Roberfroid M (2007). Prebiotics: The concept revisited. J Clin Nutr 137: 830S-837S.

Roberfroid MB, Van Loo JAE, Gibson GR (1998). The bifidogenic nature of chicory inulin and its hydrolysis products. J Nutr. 128: 11-19.

Straw TE (1989). Bacteria of the rabbit gut and their role in the health of the rabbit. J Appl Rabbit Res. 11: 142-150.

Ukwah BN, Ezeonu IM (2008). *In vitro* stimulation of growth of intestinal bacteria by prebiotic components of *Vernonia amygdalina* leaf extracts. J. Appl. Nat Sci (JANS) 3: 2-7.

Wang X, Gibson GR (1993). Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J Appl Bacteriol 75: 373-380.

Watzl B, Girrbach S, Roller M (2005). Inulin, oligofructose and immunomodulation. Br J Nutr. 93: 549-555.