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 Bacteroides 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...
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. amygdalina 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 Arhogho et al. (2009). Briefly, 200 g of washed and air-dried leaves of each plant were macerated 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.
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 typhimurium (S57), originally obtained from Engeline Van Duijkeren, Department of Infectious Diseases and Immunology, 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 occurrence 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...
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 repeated 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*,

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Table 1. Animal treatment and challenge groups.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Animal group</th>
<th>Treatment</th>
<th>Challenge organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group A</td>
<td>Distilled water</td>
<td><em>Salmonella typhimurium</em> (S58)</td>
</tr>
<tr>
<td>1</td>
<td>Group B</td>
<td>Distilled water</td>
<td><em>Escherichia coli</em> (ATCC 11775)</td>
</tr>
<tr>
<td>1</td>
<td>Group C</td>
<td>Distilled water</td>
<td><em>Staphylococcus aureus</em> (ATCC 10145)</td>
</tr>
<tr>
<td>2</td>
<td>Group D</td>
<td>Vernonía amygdalina</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Group E</td>
<td>Ocuimum gratissimum</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Group F</td>
<td>Ocuimum gratissimum (OG)</td>
<td><em>Salmonella typhimurium</em> (S58)</td>
</tr>
<tr>
<td>3</td>
<td>Group G</td>
<td>Vernonía amygdalina (VA)</td>
<td><em>Salmonella typhimurium</em> (S58)</td>
</tr>
<tr>
<td>3</td>
<td>Group H</td>
<td>Vernonía amygdalina</td>
<td><em>Escherichia coli</em> (ATCC 11775)</td>
</tr>
<tr>
<td>3</td>
<td>Group I</td>
<td>Vernonía amygdalina</td>
<td><em>Staphylococcus aureus</em>(ATCC 10145)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Period of Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>+</td>
</tr>
<tr>
<td><em>Bacillus</em> spp</td>
<td>+/-</td>
</tr>
<tr>
<td><em>Bacteroides</em> spp</td>
<td>+</td>
</tr>
<tr>
<td><em>Lactobacillus</em> spp</td>
<td>+</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> spp</td>
<td>+/-</td>
</tr>
<tr>
<td><em>Actinomyces</em> spp</td>
<td>+</td>
</tr>
<tr>
<td><em>Staph saccharolyticus</em></td>
<td>+</td>
</tr>
<tr>
<td>Coagulase negative <em>Staphylococcus</em> spp</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp</td>
<td>+/-</td>
</tr>
<tr>
<td><em>Clostridium</em> spp</td>
<td>+</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp</td>
<td>+</td>
</tr>
</tbody>
</table>

+/-: occurrence of organism in all animals; -: non-occurrence of organism in all animals; +/-: occurrence of organism in some animals.

**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 significant (*p > 0.05*) stimulation only with *O. gratissimum* (Figure 5).
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 $0.85 \pm 0.14$ kg to $0.42 \pm 0.16$ kg) in the treated animals but not in the control animals. Furthermore, death occurred in the treated animals, from the fifth day post...
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.

<table>
<thead>
<tr>
<th>Day post infection</th>
<th>Number of animals shedding the organism (%)</th>
<th>Group</th>
<th>E. coli</th>
<th>VA</th>
<th>CL</th>
<th>S. aureus</th>
<th>VA</th>
<th>CL</th>
<th>S. typhimurium</th>
<th>VA</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 (66.7)</td>
<td>CL</td>
<td>2 (66.7)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>2 (66.7)</td>
<td>3 (100)</td>
<td>2 (66.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 (66.7)</td>
<td>CL</td>
<td>2 (66.7)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>2 (66.7)</td>
<td>3 (100)</td>
<td>2 (66.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 (33.3)</td>
<td>CL</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
<td>2 (66.7)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0 (0)</td>
<td>CL</td>
<td>0 (0)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0 (0)</td>
<td>CL</td>
<td>0 (0)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0 (0)</td>
<td>CL</td>
<td>0 (0)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0 (0)</td>
<td>CL</td>
<td>0 (0)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0 (0)</td>
<td>CL</td>
<td>0 (0)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0 (0)</td>
<td>CL</td>
<td>0 (0)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td>3 (100)</td>
<td>1 (33.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 throughout 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, Lactobacillus* 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...
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
Robberfroid, 1995; Gibson, 1999; Robberfroid et al., 1998;
Menne et al., 2000). In this study, V. amygdalina 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 pro-
tection 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. Exami-
nation 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,
dietary 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.

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