

# Original Research Article

## Effects of *Moringa oleifera* lam. leaf powder on *Bifidobacteria* and *Escherichia coli* in the gut of albino rats

### ABSTRACT

The *Moringa oleifera* leaf alters the microbiota in the gut, a situation which sends impulses to the brain. This study was carried out to determine the effect of dried *Moringa oleifera* leaves on *Bifidobacteria* and *Escherichia coli* in albino rats at 2.5%, 5% and 10%. In this experimental analytic study, there were five Groups in all. Thein which 5-to 6 weeks old rats were habituated under laboratory conditions at the Animal house of the Department of Zoology, Faculty of Science, University of Ibadan, for two weeks. These rats in other to were adapt to the environmental conditions during the experiment before exposing them to the moringa Moringa feed for four weeks. Each group had 5 animals. No supplement of Moringa feed was administered to Group A, while Group B received streptomycin antibiotics. Groups C, D and E received dried leaf supplement of *Moringa oleifera* (DMO) 1.25 g/kg body weight, 2.5 g/kg body weight and 5.0 g/kg body weight respectively. *E.coli* counts increased only by 13% in group E, 26% in group B; but reduced by 9.7% in group D and 27.7% in group C between day 20 and day 28. The rate of *Bifidobacteria* viable counts increase in group E is was expressed as  $P = 0.05$  at the beginning of the experiment unlike *E.coli* counts where there was a decrease. Thus, the *Moringa oleifera* leaf powder is a potential prebiotic for probiotics like *Bifidobacteria*, and as well induce changes in the gut-brain axis.

**Keywords:** — *Moringa oleifera*, *Escherichia coli*, *Bifidobacteria*, prebiotic, gut.

### INTRODUCTION

In the sub-Himalayan areas of Afghanistan, India, Pakistan, Bangladesh, *Moringa oleifera* is known found to be widely grown, likewise in the tropics. The leaves, bark, flowers, fruit, seeds, and root are used to make medicine. "Tired blood", also known as anemia, is treated using *Moringa oleifera*. Other diseases like arthritis and rheumatism are also treated using *Moringa oleifera* [19, 20]. Myriads of infections, ailments and diseases are also treated by the use of *Moringa oleifera*. These ailments and diseases include constipation, epilepsy, stomach pain, stomach and intestinal ulcers, asthma. Others are cancer, intestinal spasms, headache, heart problems, high blood pressure, diabetes, diarrhea, kidney stones, fluid retention, thyroid disorders. Furthermore, bacterial, fungal, viral, and parasitic infections are not excluded [12, 13]. *Moringa oleifera* contains various nutrients and important classes of food such as proteins, vitamins, and minerals. A very good characteristic of *Moringa oleifera* is its antioxidant ability because it protects cells from damage. Interestingly, all parts of the *Moringa oleifera* tree are edible. Over the years, humans of been consuming all parts of the *Moringa oleifera* tree. Various chemicals like namely alkaloids, proanthocyanidins, cinnamates, flavonoids and anthocyanins have been reported to be found in the *Moringa oleifera* tree [8, 14].

Thus, the experimental effect of *Moringa oleifera* leaf powder on albino rats could involve actions against oxidants and inflammations with probable mechanisms of action which will be evaluated in this study. *Moringa oleifera* is potentially active against free radicals [7]. A laboratory albino rat is a rat of the species *Rattus norvegicus* (brown rat) which is bred and kept for laboratory analysis and research in numerous fields across the medical and health sciences [17]. Arguably, Wistar rats were the first set of rats to be developed for the purpose of research and stand as model organisms. Distinct characteristics such as high activity rate, long ears for hearing sensitivity, makes it preferable than other type of rats for research.

*Bifidobacterium* is a genus of gram-positive Gram-positive, non-motile, often branched anaerobic bacteria. They are ubiquitous and inhabit major areas in the gut and tissues of humans and animals. Some of the major areas they inhabit are the gastrointestinal tract, vagina, mouth of mammals, including humans; in an endo-symbiotic relationship. *Bifidobacteria* are one of the common probiotics and major genera of bacteria that constitute a good fraction the colon flora in mammals [6, 18]. In the gut of humans, there exists a microbiota of organisms which include beneficial organisms like a few strains of *Escherichia coli* (*E.coli*), a type of coliform bacteria which has a few species that act in the synthesis of some vitamins. However, some strains of *E.coli* produce toxins and cause diarrhea in humans. An example is the O157:H7 strain.

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The gastrointestinal micro-ecosystem is always fluctuating leading to an altered microbiota which disrupts the intestinal microbial balance exposing the compromised host to opportunistic infections [17]. The *Moringa oleifera* leaf has since become an important food supplement worldwide because it possesses anti-inflammatory and antioxidant properties [2]. The *Moringa oleifera* leaf powder's beneficial and ~~bacteriocidal~~bactericidal effect on organisms in the gut representative of *Bifidobacteria*, a Gram positive anaerobe as well as a probiotic and *Escherichia coli*, a Gram negative facultative aerobe as well as a prominent coliform respectively explains the need for this study. The aim of this study is to examine and analyze the effect of *Moringa oleifera* leaf powder on the population of *Bifidobacteria* spp. and *Escherichia coli* in the gut of Wistar Albino rats.

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## METHODS

### Moringa leaf powder preparation:

Approximately 500 g of fresh tender leaves of *Moringa oleifera* were washed in water to remove dirt and later washed in 1% saline solution to remove microbes and ~~washed~~ again with fresh water [4]. Water was allowed to drain for about 15 minutes. The *Moringa oleifera* leaves were dried in air at 25-~~to~~ 28°C, turned over at intervals with gloves and kept away from sun rays for 7 days. The *Moringa oleifera* dried leaves were processed into powder form and kept in well-covered containers to prevent air [9]. One hundred grams of the dry powder was obtained ~~and???????~~

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### Animal Grouping:

~~Five g~~ Groups of five (n=5) *Rattus norvegicus* albino rats of weight range 170-~~to~~ 230 g were used and named as follows:

- Group A (normal control) – fed with normal feed diet (50 g/kg body weight per day per rat)
- Group B (experimental control) – received streptomycin 40 mg/kg body weight/day per rat
- Group C – received ~~Dried-dried~~ leaf supplement of *Moringa oleifera* (DMO) 1.25 g/kg body weight/day per rat (2.5% moringa feed)
- Group D – received Dried leaf supplement of *Moringa oleifera* (DMO) 2.5 g/kg body weight/day per rat (5% moringa feed)
- Group E- received Dried leaf supplement of *Moringa oleifera* (DMO) 5.0 g/kg body weight/day per rat (10% moringa feed).

Exposure to these treatments was done after the acclimatization period. Thorough close physical examination as well as temperature reading was done to ensure the rats were healthy.

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### Bacteria counts:

The determination of bacteria counts in the ~~faeces~~feces was performed. Viable fecal bacteria counts were determined before exposure and determined at 4-day intervals up to the 28<sup>th</sup> day of exposure. ~~Faeces~~Feces were collected in sterile containers, weighed and suspended in 10 ml of 0.9% saline solution. This was shaken vigorously for 10-~~to~~ 20 minutes to allow the larger particles to settle below. 1ml of the suspensions were serially diluted 10-fold and appropriate dilutions were plated in duplicates on ~~Nutrient-nutrient~~ agar and incubated at 37°C for 24-~~to~~ 48 hrs both aerobically and anaerobically.

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On the 28th day of exposure, the gastrointestinal tract of animals from each group was cut open and samples were taken from the duodenum and ileum of the small intestine, as well as the ascending colon and descending colon of the large intestine including the control groups to analyze the tissue samples from the duodenum and ileum of the small intestine and from the ascending and descending colon of the large intestine in order to know the quantity of *Bifidobacteria* spp. and *E. coli* strains in the different intestinal parts from animals of each group in comparison with the control group(s). Swab of the intestinal parts were taken after they were cut open.

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### Animal housing and feeding:

The ~~Rattus norvegicus~~ rats (aged between 5-~~to~~ 7 weeks) were housed five per cage. Cages (24x18x12 cm) were made of plastic and metal ~~guazegauze~~ cover. The animals were habituated under laboratory conditions at the ~~Animal-animal~~ house of the Department of Zoology, Faculty of Science, University of Ibadan, for two weeks in order to adapt to the environmental conditions during the experiment. They were fed with standard diet 50 g/kg body weight per day per rat, and water was provided *ad libitum* (without measurement) [15].

### Isolation of *E. coli*:

Thirty-seven grams of Eosin ~~Methylene-methylene~~ Blue-~~lue~~ (EMB) Agar (HMK Ltd), ~~fifty-two grams~~ 52 g of MacConkey agar (Biotec Ltd) and ~~twenty-eight grams~~ 28 g of ~~Nutrient-nutrient~~ agar (HMK Ltd) were dissolved in ~~one liter~~ 1 l of distilled water, swirled and ~~sterilised~~sterilized by autoclaving for 15 minutes at 121°C. The prepared media was allowed to cool to

116 about 45°C and 20 ml volumes of the liquid medium was poured aseptically into sterilized petri dishes and allowed to cool  
117 before inoculation with suspected colonies of *E. coli*.

#### 118 Isolation of *Bifidobacteria*

119 -Three selectively modified *Bifidobacteria* media (BFM), selective media recommended for the isolation of the  
120 *Bifidobacterium* spp. from tissues, *faeces/feeces* or stool specimens were used for the isolation and identification of  
121 *Bifidobacteria* spp.: BFM 1, BFM 2 and BFM 3.

122 ***Bifidobacterium* Medium (BFM 1)** was specially composed with the following ingredients in Grams-grams per Litre/Liter:  
123 *Peptone-peptone* special (23.0), *Sodium-sodium* chloride (5.0), *Glucose-glucose* (5.0), L-*Cysteine-cysteine* hydrochloride  
124 (0.3), *Starch-starch* soluble (1.0), *Agar-agar* (15.0), with a final pH of 5.5±0.2 (at 25°C) [6].

125 ***Bifidobacterium* Medium (BFM 2)** was specially/especially composed with the following ingredients in Grams-grams per  
126 Litre/Liter: MRS Agar (25.0), L-*Cysteine-cysteine* hydrochloride (5.0) [6]

127 ***Bifidobacterium* Medium (BFM 3)** was specially composed with the following ingredients in Grams-grams per Litre/Liter:  
128 *Peptone-peptone* (5.0), *Sodium-sodium* chloride (5.0), *Lactulose-lactulose* (5.0), L-*Cysteine-cysteine* hydrochloride (0.5),  
129 *Starch-starch* soluble (2.0), *Tryptone-tryptone* (15.0), *Meat-meat Extract-extract* (2.0), *Yeast-yeast Extract-extract* (7.0),  
130 *Peptone-peptone* (5.0), *Riboflavin-riboflavin* (0.001), *Thiamine-thiamine Chloride-chloride HCl* (0.001), *Methylene*  
131 *methylene* blue (0.016), *Lithium-lithium* chloride (2.0), *Propionic-propionic* acid (5 ml) (added after sterilization at 55°C) at  
132 a final pH (at 25°C) of 5.5 (with 10\_N NaOH).

133 NB: Lactulose is the main carbon source.

134 Methylene blue, propionic acid and lithium chloride are inhibitors of other bacteria.

135 The low pH inhibits *Enterobacter*. **BFM 3 is a novel media composition specifically used for this study.**

#### 136 Sub-culturing:

137 The distinct colonies from agar plates were cultured on freshly prepared agar plates using proper streaking techniques.  
138 Pure isolates were sub-cultured on prepared nutrient agar slants in McCartney bottles at 37°C overnight

#### 139 Cultural characteristics of organisms

140 Distinct colonies from the plates were observed and classified based on the cultural characteristics such as shape,  
141 surface, elevation, colour, opacity, consistency and edges on the agar plate.

#### 142 Biochemical tests

143 This/The tests involved the use of the indole, methyl red, Voges-Proskauer and citrate (IMViC) tests as well as catalase,  
144 urease and sugar fermentation tests. The test required to identify *Bifidobacteria* is the *Fructose/fructose*-6-  
145 phosphate/phospho-ketolase (F6PPK). The F6PPK detection was used for the identification of *Bifidobacteria* were the  
146 isolates were grown anaerobically for 42 hours in a 20 ml broth culture at 37°C. The broth was centrifuged for 3 minutes to  
147 harvest the cells at 14 000 × g. The centrifuged broth formed a pellet of harvested cells which was washed twice with a  
148 phosphate buffer (0.05\_M, pH 6.5, cysteine 500 mg/l) and ruptured in ice for 2 minutes and mixed with 0.25 ml each of  
149 *Sodium-sodium* fluoro-iodoacetate and 7 fructose-6-phosphate solution. The reaction was incubated for 30 minutes at  
150 37°C and 1.5 ml of *Hydroxymine-hydroxymine Chloride-chloride* (pH 6.5) was added. At room temperature, 1 ml each of  
151 15% *Tricarboxylic-tricarboxylic Acid/acid*, 4\_M *Hydrochloric-hydrochloric Acid-acid* and Iron III *Chloride-chloride*  
152 *Hexahydrate-hexahydrate* was added. The tube was shaken vigorously after the addition of each solution. A reddish-violet  
153 colour indicated the presence of fructose-6- phosphate phosphoketolase characteristic of *Bifidobacteria* spp. The result  
154 was negative if the colour remained yellow [6].

## 155 RESULTS

156 The Gram-staining reaction revealed short Gram-negative rods which were further identified as *E. coli*. Indole,  
157 *Methyl/methyl*-red, citrate (IMViC) biochemical and sugar-fermentation tests results confirmed the presence of *E. coli*. The  
158 Gram-staining reaction also revealed Gram-positive rods of various sizes and shapes, in single and chains further  
159 identified as *Bifidobacteria* of characteristic V-shape and Y-shape otherwise known as 'palisade' arrangements. *Fructoso*-  
160 6-phosphate phospho-ketolase test results for *Bifidobacteria*  
161 The F6PPK test result for *Bifidobacteria* was positive if there is a reddish-violet colour immediately after shaking the tube  
162 which indicates the presence of the fructose-6-phosphate phospho-ketolase enzyme characteristic of *Bifidobacteria* spp.  
163 The F6PPK result was negative if the colour does not change from yellow to reddish-violet [6].

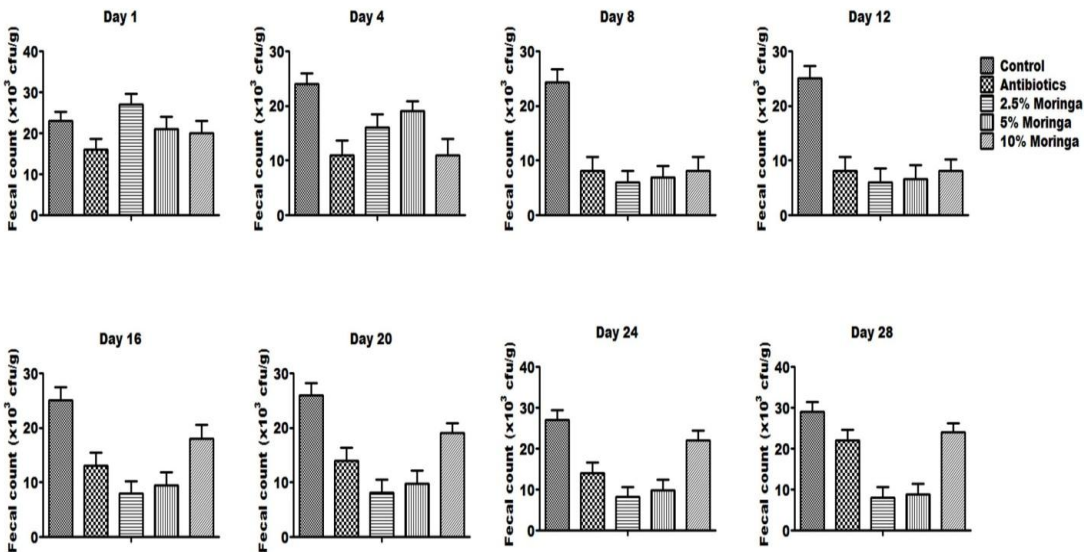
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I	R	R	R	18
Ac	R	R	R	16
Dc	R	R	R	20

N-Negligible<6, R-Resistant,Du-Duodenum, I-Ileum,Ac-Ascending colon,Dc-Descending colon.

However, **Figures 1 (one)** and **2 (two)** shows the comparative variation in counts of *Bifidobacteria* and *E. coli* respectively in faecal samples between days 1 to 28 which reveals a decrease in the *E. coli* and *Bifidobacteria* viable counts up to day 8 in groups C, D and E. *Bifidobacteria* counts increased by more than 50% between day 12 and day 16 with group E having the highest percentage increase but became relatively stable between day 20 and 28. *E. coli* counts increased only by 13% in group E, by 26% in group B; but reduced by 9.7% in group D and 27.7% in group C between day 20 and day 28.



**Figure 1:** — *Bifidobacteria* fecal counts in all groups between days 1 and 28.

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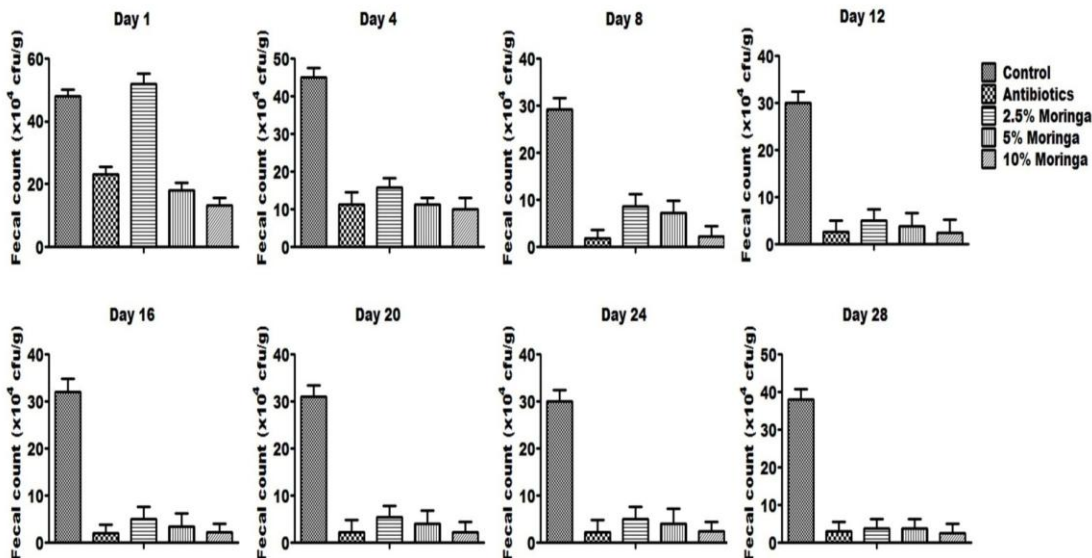
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Figure 2: *E. coli* fecal counts in all groups between days 1 and 28.

The *E. coli* and *Bifidobacteria* counts in faecal samples in all groups between days 1 to 28 is shown in Table 3 (three). Group A without moringa-Moringa feed supplement is the control group. Group B were given with normal rat feed and streptomycin 40 mg/kg body weight.

Table 3: Antimicrobial activity of *Moringa oleifera* and streptomycin on faecal isolates of *E.coli* and *Bifidobacteria*.

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	ZONES OF INHIBITION (mm)																			
	Group A (Control)		Group B (Antibiotics)				Group C (2.5% Moringa)				Group D (5% Moringa)				Group E (10% Moringa)					
	<i>E. coli</i>		Bfd		<i>E.coli</i>		Bfd		<i>E.coli</i>		Bfd		<i>E. coli</i>		Bfd		<i>E. coli</i>		Bfd	
	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M
Day 1	14	8	6	6	14	8	6	6	14	10	8	6	16	10	6	6	16	8	6	6
Day 4	14	8	8	6	16	8	6	R	14	6	6	R	16	6	6	R	18	6	6	6
Day 8	20	8	6	R	18	18	R	R	16	R	R	R	18	6	R	R	18	6	R	R
Day 12	20	6	6	6	20	R	R	6	20	R	R	R	18	R	R	R	24	R	R	R
Day 16	18	8	6	6	12	R	R	R	14	R	R	R	14	R	R	R	14	R	R	R
Day 20	18	6	6	6	12	R	R	R	14	R	R	R	20	R	R	R	14	R	R	R

Day 24	20	6	N	N	12	R	R	R	16	R	R	R	20	R	R	R	20	R	R	R
Day 28	20	N	N	N	10	R	R	R	14	R	R	R	20	R	R	R	16	R	R	R

*E.coli*-Escherichia coli, Bfd-Bifidobacteria, M-Moringa oleifera, S-Streptomycin, R-Resistant, N-Negligible(< 6)

Table 4 (four) reveals that at 50 mg/l for *Moringa oleifera* and 0.64 mg/l for streptomycin, *E. coli* is strongly inhibited while *Bifidobacteria* is slightly inhibited.

Table 4: Determination of minimum inhibitory concentration of *Moringa oleifera* and streptomycin

	<i>Moringa oleifera</i> (mg/l)			
	50	25	12.5	6.25
<i>E. coli</i>	24_mm	16_mm	8_mm	0_mm
<i>Bifidobacteria</i>	6_mm	0_mm	0_mm	0_mm
	<i>Streptomycin</i> (mg/l)			
	0.64	0.32	0.16	0.08
<i>E. coli</i>	32_mm	20_mm	12_mm	0_mm
<i>Bifidobacteria</i>	6_mm	0_mm	0_mm	0_mm

Figure 3 (three) shows the effect of the *Moringa oleifera* leaf powder on *Bifidobacteria* and *E. coli* in mean and standard deviation form.

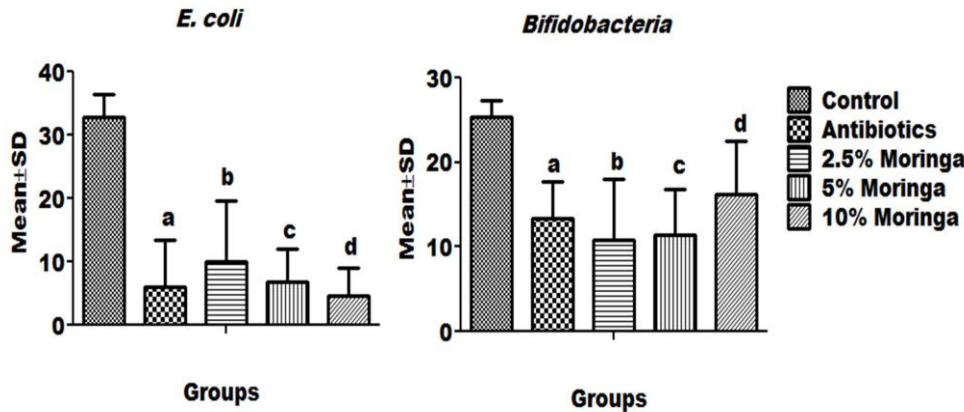


Figure 3: Effect Of *Moringa Oleifera oleifera* Leaf Powder On *Bifidobacteria* And *E. Coli coli* Counts In All Groups In "Mean ± Sd" Form.

- a= Statistically significant compared to the control group.
- b= Statistically significant compared to the control and antibiotics group.
- c= Statistically significant compared to the control, antibiotics and 2.5% Moringa group.
- d= Statistically significant compared to the control, antibiotics, 2.5% and 5% Moringa group.

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241 | Table 5 (five) reveals a significant increase in both *Bifidobacteria* in group E and *E. coli* in group C with high maximum  
242 | limits of the organisms in the respective groups using the Dunnet's test.  
243

244 | **Table 5:** — Comparison ratio of *Bifidobacteria* and *E. coli* counts after various treatments of *Moringa oleifera* leaf  
245 | powder using Dunnett's test.

Group	<i>Bifidobacteria</i>		<i>E. coli</i>	
	Min. limit	Max. limit	Min. limit	Max. limit
C	-8.4	3.6	-3.17	11.27
D	-7.85	4.15	-6.28	8.16
E	-3.02	8.98	-8.58	5.86

246 | NB: Groups A and B are not included because they are negative and positive controls respectively.  
247

## 248 | DISCUSSION

249 | The *Moringa oleifera* tree plant is a very important source of micro and macro nutrients, including dietary fiber. It has  
250 | respectable antioxidant and anti-inflammatory potency. As a single plant it contains almost all nutrients from all other  
251 | herbs combined. It is a potential source of tocopherols, pro-vitamin A, vitamin C, calcium, protein and minerals. The  
252 | *Moringa oleifera* plant leaf is the most nutritious part of the plant containing significant quantities of crude protein,  
253 | vitamins and minerals. The percentage dry weight of crude protein of the *Moringa oleifera* leaf powder used in this study  
254 | (31.5% Dry-dry Matter) is higher than that of other vegetable leaves. Abiodun *et al.* [2] reported that the *Moringa*  
255 | *oleifera* leaf powder has 25.29% protein dry weight of which no other vegetable has close to such amount.  
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258 | Over the years, people have made use of plants and herbs to cure several illnesses and diseases like fever, wounds and  
259 | bruises, constipation, weight management, cardiovascular diseases and many others. The effect of *Moringa oleifera*  
260 | leaves extract at different concentrations on growth of studied probiotic bacteria (i.e *Bifidobacteria* spp.) showed that the  
261 | growth of all studied probiotic bacteria was affected by the *Moringa oleifera* leaf extract [3]. Furthermore, Abeer, *et al.* [3]  
262 | reported that increasing the concentration of *Moringa oleifera* leaves extract from 0 to 8% led to increase in the probiotic  
263 | bacterial growth at 37°C for 24 hours of incubation time. The statement above is in tandem with the result obtained in this  
264 | research work where much increase in *Bifidobacteria* counts were observed at 10% *Moringa oleifera* concentration (in  
265 | group E) than all other groups between days 12 and 16. Abeer *et al.* [3] also stated that optimum growth was recorded  
266 | for all probiotic bacteria at 8% concentration of the *Moringa oleifera* leaves extract at 37°C for 24 hours incubation time.  
267 | *L. jonsonii* and *B. adolescentis* exhibited a higher growth than those of *L. casei* and *B. lactis*, respectively.  
268 | The presence of essential amino acids in the *Moringa oleifera* leaves improved the growth of the organisms [3]. The  
269 | antibacterial activity exhibited on some bacterial isolates by *Moringa oleifera* could be as a result of the presence of  
270 | flavonoids and tannins, since these phytochemicals are reported to confer antibacterial activity.  
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273 | Furthermore, this study reveals the *Moringa oleifera* leaf powder is being used as a modulatory tool against probable  
274 | disruption of gut microbiota by certain factors causing intestinal microbial imbalance which exposes the compromised  
275 | host to opportunistic infections. All animals in the groups fed with the *Moringa oleifera* leaf powder supplement were  
276 | found to be healthier and stronger throughout the days of the experiment. The animals in groups C, D, and E appeared  
277 | to be more active especially animals in group E with 10% *Moringa oleifera* leaf supplement.  
278 |

279 | The hyperactivity in these groups of animals exposed to the *Moringa oleifera* leaf powder could be linked to the  
280 | biochemical signals between the gastrointestinal tract (GIT) and the central nervous system (CNS) apparently described  
281 | as the gut-brain axis. This explains that changes in the microbiota of the GIT immediately triggers neurotransmitters to  
282 | the parts of the nervous system connected with the gut which includes the vagus nerve, CNS, enteric nervous system,  
283 | autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis. Drugs and food always cause microbiota  
284 | changes which further cause changes in the levels of cytokines, which further affect the brain functions [11].  
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the bacteria count? Agar plate?

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## CONCLUSION AND RECOMMENDATION

The addition of *Moringa oleifera* leaf supplement to animal food could help increase beneficial bacteria as well as inhibit harmful bacteria in the gut. However, the results of this present study could suggest that *Moringa oleifera* mechanism of action in *E. coli* involves anti-propulsive and anti-secretory effects. *Moringa oleifera* can be used to create high activated carbons which are able to sequester and remove cyanobacterial microcystin-LR quite effectively and the leaf extract also appears to be capable of suppressing cyanobacterial growth as 20 to 160 mg of *Moringa oleifera* extract per liter of water is able to suppress growth of *Microcystis aeruginosa* and cause the colony count to decline [7]. Therefore, moringa dried leaf supplement is suitable for the growth of beneficial organisms like *Bifidobacteria* in the gastrointestinal tract. Beneficial bacteria of intrinsic antibiotic resistance could also be boosted by *Moringa oleifera* plant leaf to restore the gut microbiota after antibiotic treatment. *Moringa oleifera* leaf supplement is suitable for improving the intestinal microbial balance thus could serve as a modulating tool for the immune system [4, 10].

*Bifidobacteria* has been known to prevent the incidence of inflammatory bowel diseases (IBD) like ulcerative colitis and Crohn's disease in the gut of humans and animals over the years [1, 16]. Thus, the increase in the load of *Bifidobacteria* in the gastrointestinal tract tends to reduce the rate of IBD occurrences [6].

Therefore, 10% moringa leaf in the powder form is recommended as a prebiotic for adults per meal per day for a substantial boost in the load of *Bifidobacteria* and other probiotics in the gastrointestinal tract and for an increase in activity as observed in this study.

Only at 10% Moringa powder concentration did *Bifidobacteria* grow maximally, while *E. coli* counts were low; but at 2.5% concentration, there were low *Bifidobacteria* counts but high *E. coli* counts. This typically reveals the importance of dosage and concentration in herbal therapy and medicinal plant intake as it were in pharmaceutical drugs administration. It also shows that the 2.5% moringa powder concentration was not effective enough in inhibiting the growth of *E. coli* and exhibiting the growth of *Bifidobacteria*, while the 10% moringa leaf powder concentration had optimum inhibitory effect on *E. coli* and served as a boost for probiotic *Bifidobacteria*. The powder form of the moringa leaf mixes well with food and quickly starts to break down as the food chyme undergoes an enzymatic process in the mouth.

In addition, the noticeable hyperactivity signs in the albino rats and the bidirectional neurohumoral communication system between the brain and the gut suggest the use of moringa leaf powder as a potential modulator of the gut flora connected with the correlating effect on the vagus nerves which later sends the information about the intestines to the brain.

Further studies can be done on how the moringa leaf supplement induces changes in the gut and the systematic effects it has on the brain. The correlation between the microbiota and human emotions could be checked in further studies putting into consideration possible feedback loops in the gut-brain axis.

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