Original Research Article

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

ABSTRACT

Aim: This study was carried out to determine the effects of dried *Moringa oleifera* leaves on *Bifidobacteria* and *Escherichia coli* in the gut of albino rats.

Location: The rats were habituated under laboratory conditions at the animal house of the Department of Zoology, Faculty of Science, University of Ibadan, for two weeks in other to adapt to the environmental conditions during the experiment.

Duration of study: The rats were exposed to the *M. oleifera* feed for four weeks.

Design of study: There were five groups in all. The 5 to 6 weeks old rats were fed with *M. oleifera* powder supplement except for the control groups.

Method: No supplement of *M. oleifera* feed was administered to group A while group B received streptomycin antibiotics. Groups C, D and E received dried leaf supplement of *M. oleifera* (DMO) 1.25 g/kg body weight (2.5 %), 2.5 g/kg body weight (5 %) and 5.0 g/kg body weight (10 %) respectively.

Results: *E.coli* counts increased from $2.3*10^4$ to $2.6*10^4$ colony-forming units per gram (cfu/g) in group E, from $2.2*10^4$ to 3.0*10 cfu/g in group B; but reduced from $4.1*10^4$ to $3.7*10^4$ cfu/g in group D and from $5.4*10^4$ to $3.9*10^4$ cfu/g in group C between day 20 and day 28. As from day 8, the isolates from the non-control groups were resistant to the *M. oleifera* extract except *E. coli* isolates in both 5 % and 10 % *M. oleifera* groups on day 8 with 6 mm zone of inhibition each. The rate of *Bifidobacteria* viable counts increase in group E was expressed as $P = 0.05$ at the beginning of the experiment, unlike *E.coli* counts where there was a decrease.

Conclusion: The *M. oleifera* leaf alters the microbiota in the gut, a situation which sends impulses to the brain. Thus, the *M. oleifera* leaf powder is a potential prebiotic for probiotics like *Bifidobacteria*, and as well as 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, *M. oleifera* is 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 anaemia, is treated using *M. oleifera*. Other diseases like arthritis and rheumatism are also treated using *M. oleifera* [19, 20]. Myriads of infections, ailments and diseases are also treated by the use of *M. oleifera*. These ailments and diseases include constipation, epilepsy, stomach pain, stomach and intestinal ulcers, asthma, cancer, intestinal spasms, headache, heart problems, high blood pressure, diabetes, diarrhoea, kidney stones, fluid retention, thyroid disorders. Furthermore, bacterial, fungal, viral, and parasitic infections are not excluded [12, 13].

M. oleifera contains various nutrients and important classes of food such as proteins, vitamins, and minerals. A very good characteristic of *M. oleifera* is its antioxidant ability because it protects cells from damage. Interestingly, all parts of the *M. oleifera* are edible. Over the years, humans of been consuming all parts of the *M. oleifera* tree. Various chemicals namely alkaloids, proanthocyanidins, cinnamates, flavonoids and anthocyanins have been reported to be found in the *M. oleifera* tree [8, 14]. Thus, the effect of *M. 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. *M. oleifera* is potentially active against free radicals [7].

A laboratory albino rat is a rat of the species *Rattus norvigicus* (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 types of rats for research.

Bifidobacterium is a genus of Gram-negative, non-motile, often branched Anaerobacter. 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* species are one of

the common probiotics and major genera of bacteria that constitute a good fraction of 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 diarrhoea in humans. An example is the O157: H7 strain. 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 *M. oleifera* leaf has since become an important food supplement worldwide because it possesses anti-inflammatory and antioxidant properties [2]. The *M. oleifera* leaf powder's beneficial and bactericidal effects 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. Previous works have been done to show the effect of *M. oleifera* leaves extract at different concentrations on the growth of studied probiotic bacteria which showed that the growth of all studied probiotic bacteria was affected by the *M. oleifera* leaf extract [3]. Furthermore, Abeer, *et al*. [3] reported that increasing the concentration of *M. oleifera* leaves extract from 0 to 8 % led to increase in the probiotic bacterial growth at 37 °C for 24 hours of incubation time. The aim of this study, therefore, is to examine and analyze the effect of *M. oleifera* leaf powder on the population of *Bifidobacteria* spp. and *Escherichia coli* in the gut of Wistar Albino rats.

MATERIALS AND METHODS

M. oleifera **leaf powder preparation**:
Approximately 500 g of fresh tender leaves of *M. oleifera* were harvested from Orita Challenge suburb of the city of Ibadan in Oyo State, Nigeria. The leaves were authenticated at the Botany Department of the Faculty of Agriculture, University of Ibadan. The *M. oleifera* leaves were washed in water to remove dirt and later washed in 1 % saline solution to remove microbes and washed again with freshwater [4]. Water was allowed to drain for about 15 minutes. The *M. oleifera* leaves were dried in air at 25 to 28 ^{o}C, turned over at intervals with gloves and kept away from sun rays for 7 days. The *M. oleifera* dried leaves were processed into powdery form and kept in well-covered containers to prevent air [9]. One hundred grams of the dry powder was obtained, put in a dry container and stored in a cool dry place.

Animal Grouping:
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 leaf supplement of *M. oleifera* (DMO) 1.25 g/kg body weight/day per rat (2.5 % *M. oleifera* feed)
- Group D – received dried leaf supplement of *M. oleifera* (DMO) 2.5 g/kg body weight/day per rat (5 % *M. oleifera* feed)
- Group E- received dried leaf supplement of *M. oleifera* (DMO) 5.0 g/kg body weight/day per rat (10 % *M. oleifera* feed).

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

Ethical Approval: The Animal Ethics Committee of the Department of Zoology, Faculty of Science, University of Ibadan, gave approval for the purchase of the rats with receipt number 1452592 and housing of the rats in the animal house of the department. A veterinary doctor was also involved in the monitoring and analysis of the rats throughout the period.

Bacteria counts:
The determination of bacteria counts in the faeces was performed. Viable faecal bacteria counts were determined before exposure and determined at 4-day intervals up to the 28th day of exposure. Faeces 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. About 1 ml of the suspensions were serially diluted 10-fold and appropriate dilutions were plated in duplicates on nutrient agar and incubated at 37 ^{o}C for 24 to 48 hours both aerobically and anaerobically. On the 28th day of exposure, the gastrointestinal tract of animals from each group was cut open and samples were taken from the duodenum, ileum, ascending colon and descending colon of the intestines. Swabs of the intestinal parts were taken after they were cut open.

Animal housing and feeding:
The rats (aged between 5 to 7 weeks) were housed five per cage. Cages (24 x 18 x 12 cm) were made of plastic and metal gauze cover. The animals were habituated under laboratory conditions at the animal house of the Department of

Zoology, Faculty of Science, University of Ibadan, for two weeks in other 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 blue (EMB) Agar (HMK ltd), 52 g of MacConkey agar (Biotec ltd) and 28 g of nutrient agar (HMK Ltd) were dissolved in 1l of distilled water, swirled and sterilized by autoclaving for 15 minutes at 121 ^{o}C. The prepared media was allowed to cool to about 45 ^{o}C and 20 ml volumes of the liquid medium were poured aseptically into sterilized Petri dishes and allowed to cool before inoculation with suspected colonies of *E. coli*.

Isolation of *Bifidobacteria*

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

***Bifidobacterium* Medium (BFM 1)** was specially composed with the following ingredients in grams per litre: peptone special (23.0), sodium chloride (5.0), glucose (5.0), L-cysteine hydrochloride (0.3), starch soluble (1.0), agar (15.0), with a final pH of 5.5±0.2 (at 25 °C) [6].

***Bifidobacterium* Medium (BFM 2)** was specially composed of the following ingredients in grams per litre: MRS Agar (25.0), L-cysteine hydrochloride (5.0) [6].

***Bifidobacterium* Medium (BFM 3)** was specially composed with the following ingredients in grams per litre: peptone (5.0), sodium chloride (5.0), lactulose (5.0), L-cysteine hydrochloride (0.5), starch soluble (2.0), tryptone (15.0), meat extract (2.0), yeast extract (7.0), peptone (5.0), riboflavin (0.001), thiamine chloride HCl (0.001), methylene blue (0.016), lithium chloride (2.0), propionic acid (5 ml) (added after sterilization at 55 ^{o}C) at a final pH (at 25 °C) of 5.5 (with 10 N NaOH).

NB: Lactulose is the main carbon source.

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

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

Sub-culturing:

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

Cultural characteristics of organisms

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

Biochemical tests

The tests involved the use of the indole, methyl red, Voges Proskauer and citrate (IMViC) tests as well as catalase, urease and sugar fermentation tests.

The test required to identify *Bifidobacteria* is the fructose-6-phosphate phosphor-ketolase (F6PPK). The F6PPK detection was used for the identification of *Bifidobacteria* were the isolates were grown anaerobically for 42 hours in a 20 ml broth culture at 37 °C. The broth was centrifuged for 3 minutes to harvest the cells at 14 000 × g. The centrifuged broth formed a pellet of harvested cells which was washed twice with a 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 sodium fluoro-iodoacetate and 7 fructose-6-phosphate solutions. The reaction was incubated for 30 minutes at 37 °C and 1.5 ml of hydroxymine chloride (pH 6.5) was added. At room temperature, 1 ml each of 15 % tricarboxylic acid, 4 M hydrochloric acid and iron III chloride hexahydrate was added. The tube was shaken vigorously after the addition of each solution. A reddish-violet colour indicated the presence of fructose -6- phosphate phosphoketolase characteristic of *Bifidobacteria* spp. The result was negative if the colour remained yellow [6].

RESULTS

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

violet [6]. *Bifidobacteria* strains were not affected by the streptomycin but were resistant to it, unlike the *E. coli* strains that were susceptible to streptomycin. Variation in counts of *E. coli* and *Bifidobacteria* in faecal samples in group B shows that *E. coli* was susceptible to streptomycin. Pacheco *et al*. also confirms that there is a low-level resistance of *E. coli* to streptomycin because of the protein-freezing molecules in streptomycin [18]. In group C, D and E, the rats were fed with 2.5 %, 5 %, and 10 % *M. oleifera* rat feed respectively (i.e 1.25 g/kg body weight, 2.5 g/kg body weight and 5.0 g/kg body weight respectively) between days 1 to 28. Table 1 shows the proximate analysis of the *M. oleifera* leaf powder indicating the protein content, ash content and other nutrients. The phytochemical analysis also showed the presence of flavonoids and saponins.

Table 1: Proximate analyses of *M. oleifera* leaf powder.

Parameter	Calculated nutrients values
Dry matter, DM (%)	90.92
Crude protein, CP (% DM)	31.5
Ether extract, EE (% DM)	14.8
Crude fiber, CF (% DM)	37.4
Lysine (% DM)	0.94
Methionine (% DM)	0.42
Ash Content(% DM)	9.0
Calcium, Ca (% DM)	1.05
Phosphorus, P (% DM)	0.69
Vitamin B_1	0.09
Vitamin B_2	0.05
Vitamin B_3	0.8
pH	6.27

Figure 1 shows the growth of *Bifidobacteria* in the non-control groups and the antimicrobial effects of *M. oleifera* on *Bifidobacteria* in the respective groups. Figure 2 shows the growth of *E. coli* in the non-control groups and the antimicrobial effects of *M. oleifera* in the respective groups.

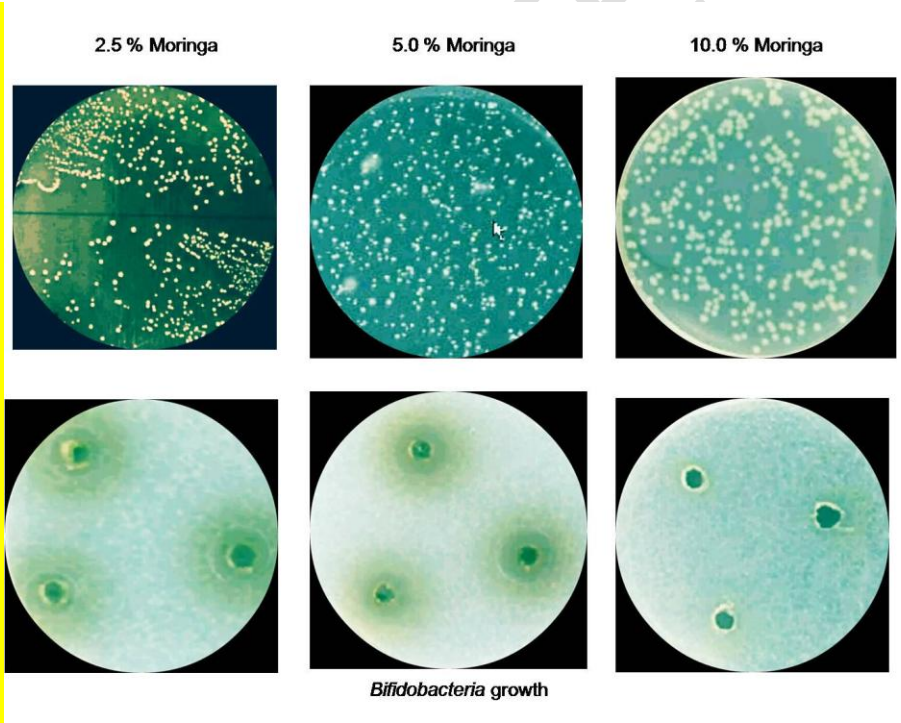

Figure 1: The growth of *Bifidobacteria* in non-control groups and the antimicrobial effects of *M. oleifera* in the respective groups.

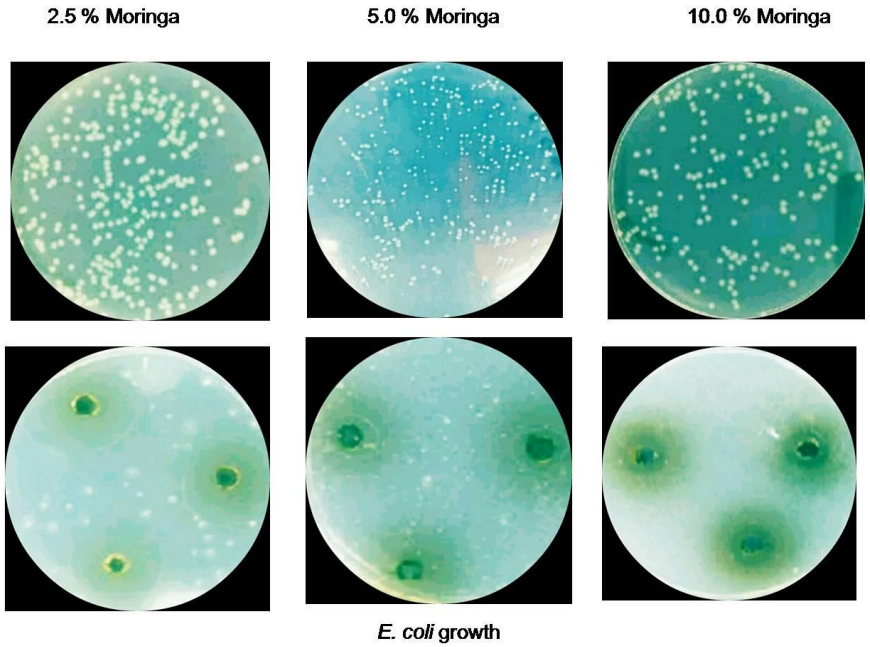

Figure 2: The growth of *E. coli* in non-control groups and the antimicrobial effects of *M. oleifera* in the respective groups.

Table 2 shows the antimicrobial activity of *M. oleifera* and streptomycin on intestinal isolates of *E.coli* and *Bifidobacteria* in Groups A-E, while Table 3 shows that of the faecal isolates.

Table 2: Antimicrobial activity of *M. oleifera* and streptomycin on intestinal isolates of *E.coli* and *Bifidobacteria* in Groups A-E.

		ZONES OF INHIBITION (mm)			
		Bifidobacteria		***E. coli***	
Group	Intestinal part	M(50 mg/l)	S(0.64 mg/l)	M(12.5 mg/l)	S(0.16 mg/l)
A	Du	N	N	N	20
	I	N	N	N	16
	Ac	N	N	N	20
	Dc	N	N	N	20
B	Du	N	R	N	10
	I	N	R	N	16
	Ac	N	R	N	8
	Dc	N	R	N	12
C	Du	R	R	R	14
	I	R	R	R	18
	Ac	R	R	R	20
	Dc	R	R	R	18
D	Du	R	R	R	20
	I	R	R	R	16
	Ac	R	R	R	18
	Dc	R	R	R	20
E	Du	R	R	R	16
	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, M-*Moringa*, S-Streptomycin

However, Figures 3 and 4 show 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. The effect of *M. oleifera* leaves extract at different concentrations on the growth of studied probiotic bacteria (i.e *Bifidobacteria* spp.) showed that the growth of all studied probiotic bacteria was affected by the *M. oleifera* leaf extract [3]. Furthermore, Abeer, *et al*. [3] reported that increasing the concentration of *M. oleifera* leaves extract from 0 to 8 % led to increase in the probiotic bacterial growth at 37 °C for 24 hours of incubation time. The statement above is in tandem with the result obtained in this research work where much increase in *Bifidobacteria* counts was observed at 10 % *M. oleifera* concentration (in group E) than all other groups between days 12 and 16.

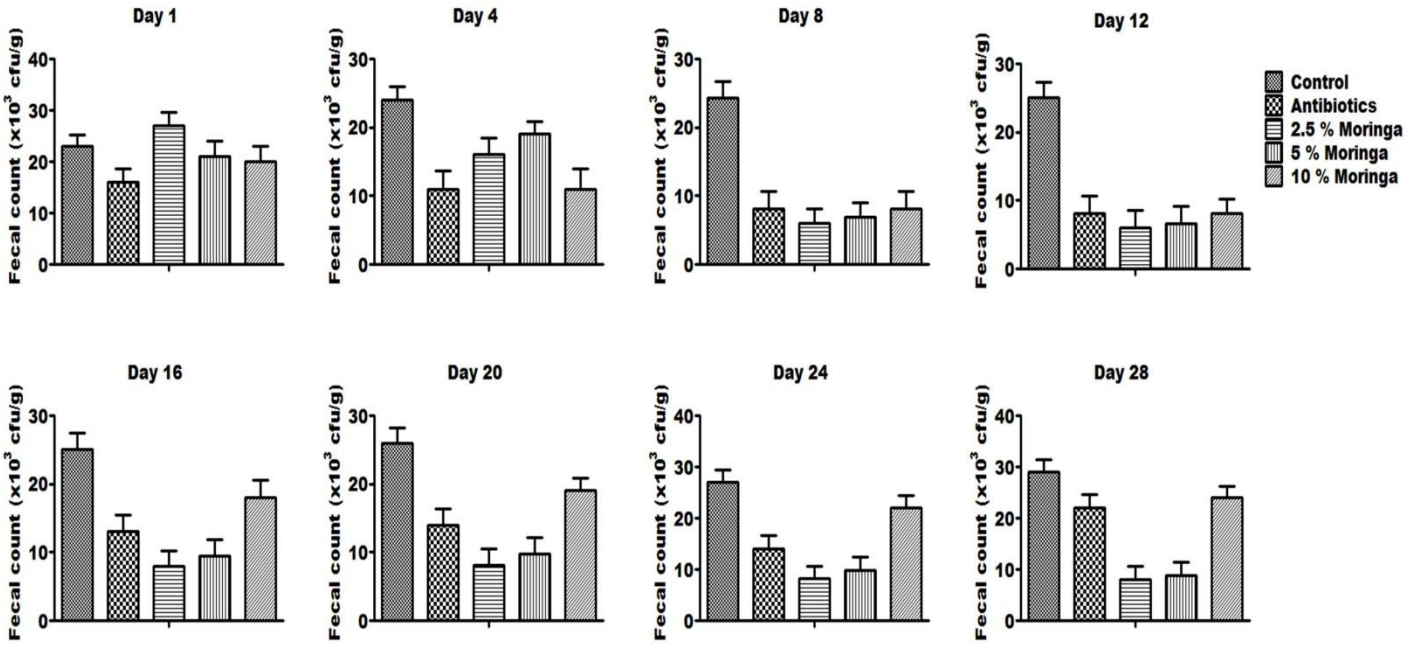

Figure 3: *Bifidobacteria* faecal counts in all groups between days 1 and 28.

However, the antibacterial activity exhibited on some bacterial isolates by *M. oleifera* could be as a result of the presence of flavonoids and tannins, since these phytochemicals are reported to confer antibacterial activity [2, 18]. Figure 3 shows the antibacterial effect of *M. oleifera* on *E. coli* especially on the rats in the 2.5 % *M. oleifera* feed group. The *E. coli* and *Bifidobacteria* counts in faecal samples in all groups between days 1 to 28 are shown in Table 3. Group A without *M. oleifera* feed supplement is the control group. Group B was given with normal rat feed and streptomycin 40 mg/kg body weight.

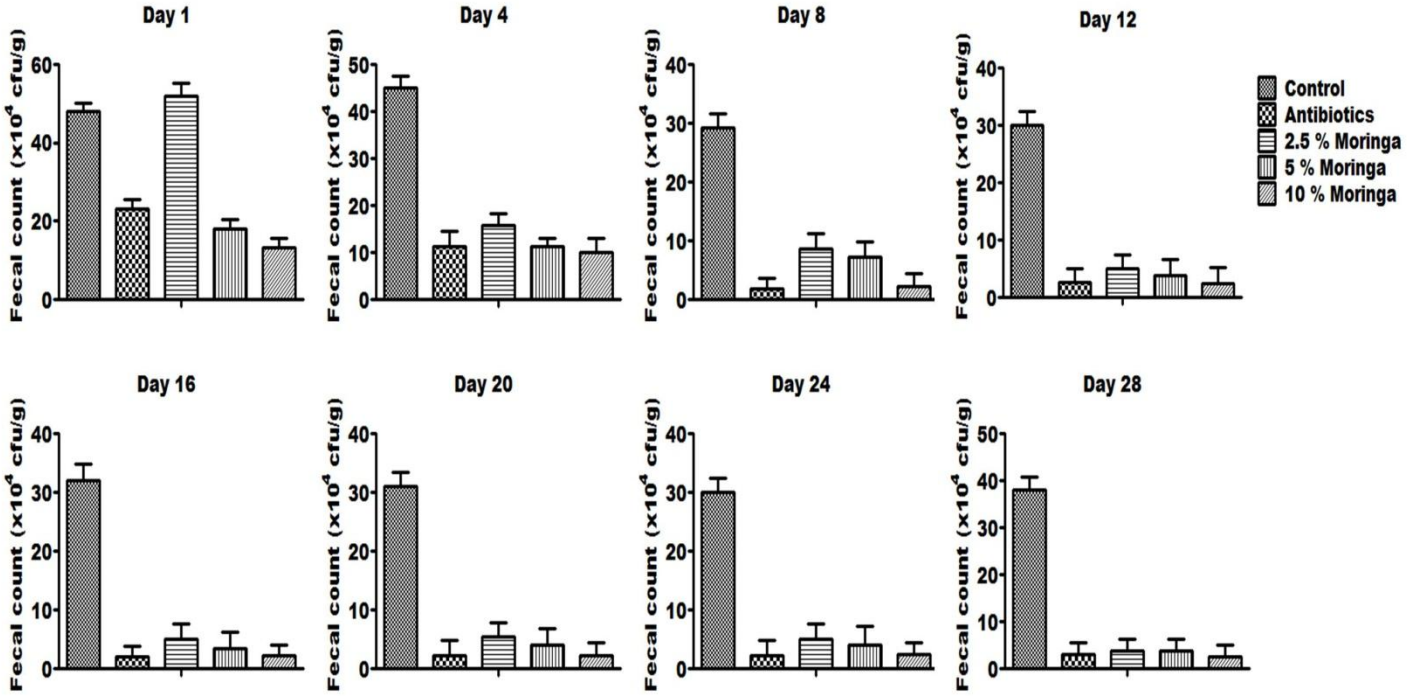

Figure 4: *E. coli* faecal counts in all groups between days 1 and 28.

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

	ZONES OF INHIBITION (mm)																			
	Group A (Control)				Group B (Antibiotics) [Positive control]				Group C (2.5 % Moringa)				Group D (5 % Moringa)				Group E (10 % Moringa)			
	E. coli		Bfd		*E.coli*		Bfd		*E.coli*		Bfd		*E. coli*		Bfd		*E. coli*		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- *M. oleifera*, S-Streptomycin, R-Resistant, N-Negligible(< 6)

Table 4 reveals that at 50 mg/l for *M. oleifera* and 0.64 mg/l for streptomycin, *E. coli* is strongly inhibited while *Bifidobacteria* is slightly inhibited. Figure 5 shows the effect of the *M. oleifera* leaf powder on *Bifidobacteria* and *E. coli* in mean and standard deviation form. Table 5 reveals a significant increase in both *Bifidobacteria* in group E and *E. coli* in group C with high maximum limits of the organisms in the respective groups using the Dunnet's test.

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

	M. oleifera (mg/l)			
	**50*	25	12.5	6.25
E. coli (Zones of inhibition)	24 mm	16 mm	8 mm	0 mm
Bifidobacteria (Zones of inhibition)	6 mm	0 mm	0 mm	0 mm
	Streptomycin (mg/l)			
	*0.64	0.32	0.16	0.08
E. coli (Zones of inhibition)	32 mm	20 mm	12 mm	0 mm
Bifidobacteria (Zones of inhibition)	6 mm	0 mm	0 mm	0 mm

*Concentrations where there is significant inhibition.

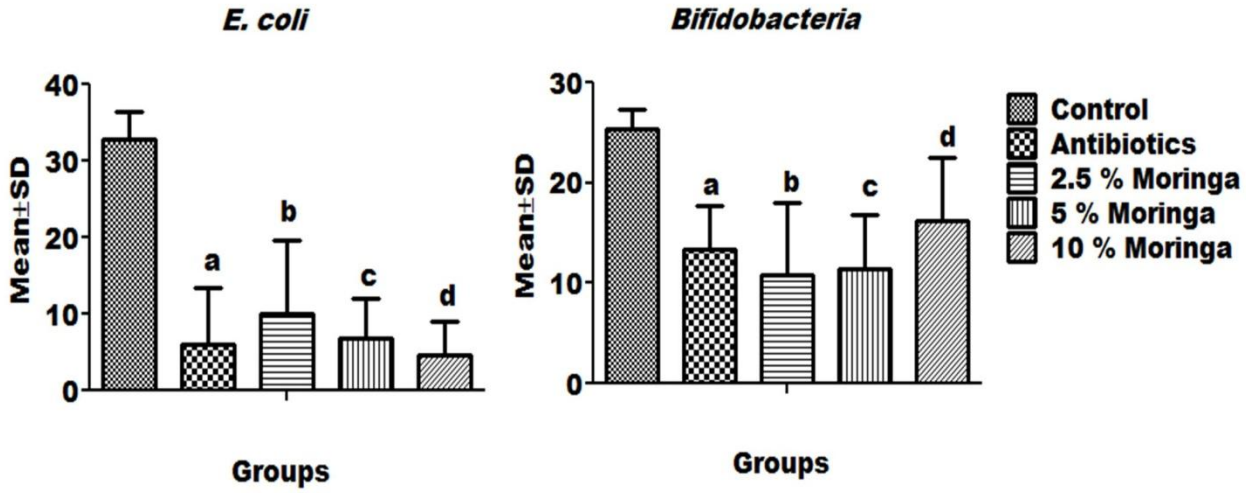

Figure 5: Effect of *M. oleifera* leaf Powder on *Bifidobacteria* and *E. coli* counts in all Groups in "Mean ± Sd" form
a= Statistically significant compared to the control group.
b= Statistically significant compared to the control and antibiotics group.
c= Statistically significant compared to the control, antibiotics and 2.5 % Moringa group.
d= Statistically significant compared to the control, antibiotics, 2.5 % and 5 % Moringa group

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

	Bifidobacteria		***E. coli***	
Group	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

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

DISCUSSION

The *M. oleifera* is a very important source of micro and macronutrients, including dietary fibre. It has respectable antioxidant and anti-inflammatory potency. As a single plant, it contains almost all nutrients from all other herbs combined. It is a potential source of tocopherols, pro-vitamin A, vitamin C, calcium, protein and minerals. The *M. oleifera* leaf is the most nutritious part of the plant containing significant quantities of crude protein, vitamins and minerals. The percentage dry weight of the crude protein of the *M. oleifera* leaf powder used in this study (31.5 % dry matter) is higher than that of other vegetable leaves. Abiodun *et al*. [2] reported that the *M. oleifera* leaf powder has 25.29 % protein dry weight of which no other vegetable has close to such amount.

Over the years, people have made use of plants and herbs to cure several illnesses and diseases like fever, wounds and bruises, constipation, weight management, cardiovascular diseases and many others. The effect of *M. oleifera* leaves extract at different concentrations on the growth of studied probiotic bacteria (i.e *Bifidobacteria* spp.) showed that the growth of all studied probiotic bacteria was affected by the *M. oleifera* leaf extract [3]. Furthermore, Abeer, *et al*. [3] reported that increasing the concentration of *M. oleifera* leaves extract from 0 to 8 % led to increase in the probiotic bacterial growth at 37 °C for 24 hours of incubation time. Likewise in this study, the increase in the *M. oleifera* feed across the groups also resulted in corresponding increases in bacteria counts, especially probiotic *Bifidobacteria*. Also, *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 a load of *Bifidobacteria* in the gastrointestinal tract tends to reduce the rate of IBD occurrences [6].

The statement by Abeer, *et al*. [3] is in tandem with the result obtained in this research work where much increase in *Bifidobacteria* counts was observed at 10 % *M. oleifera* concentration (in group E) than all other groups between days 12 and 16. Abeer *et al*. [3] also stated that optimum growth was recorded for all probiotic bacteria at 8 % concentration of the *M. oleifera* leaves extract at 37 °C for 24 hours incubation time. *Lactobacillus jonsonii* and *Bifidobacteria adolescentis* exhibited a higher growth than *L. casei* and *B. lactis* respectively. The presence of essential amino acids in the *M. oleifera* leaves improved the growth of the organisms [3]. However, the antibacterial activity exhibited on some bacterial isolates by *M. oleifera* could be as a result of the presence of flavonoids and tannins, since these phytochemicals are reported to confer antibacterial activity [2, 18]. In this study, there was a considerable reduction in the amount of both *E. coli* and *Bifidobacteria* between day 1 and day 8; thereafter, *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. The study shows the highest reduction of *E. coli* at 2.5 % *M. oleifera* concentration.

Only at 10 % *M. oleifera* 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 % *M. oleifera* leaf powder concentration was not effective enough in inhibiting the growth of *E. coli* and exhibiting the growth of *Bifidobacteria,* while the 10 % *M. oleifera* leaf powder concentration had an 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.

Furthermore, this study reveals the *M. oleifera* leaf powder is being used as a modulatory tool against probable disruption of gut microbiota by certain factors causing an intestinal microbial imbalance which exposes the compromised host to opportunistic infections. All animals in the groups fed with the *M. oleifera* leaf powder supplement were found to be healthier and stronger throughout the days of the experiment. The animals in groups C, D, and E appeared to be more active especially animals in group E with 10 % *M. oleifera* leaf supplement.

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

CONCLUSIONS AND RECOMMENDATIONS

The present study suggests that the *M. oleifera* leaf's mechanism of action in *E. coli* involves antipropulsive and antisecretory effects. *M. oleifera* can be used to create high activated carbons which are able to sequester and remove cyanobacterial microcystin-LR quite effectively. The leaf extract also appears to be capable of suppressing cyanobacterial growth because 20 to 160 mg of *M. oleifera* extract per litre of water is able to suppress the 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 *M. oleifera* plant leaf to restore the gut microbiota after antibiotic treatment. *M. oleifera* leaf supplement is suitable for improving the intestinal microbial balance thus could serve as a modulating tool for the immune system [4, 10]. Therefore, 10 % moringa leaf in the powder form is recommended as a prebiotic for adults per meal per day for a substantial boost in a load of *Bifidobacteria* and other probiotics in the gastrointestinal tract and for an increase in activity as observed in this study.

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