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Development of indigenous plant-based prebiotic Nutraceuticals: A Functional and Biological Approach

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Abstract

The study aimed to identify and evaluate indigenous plant materials with high prebiotic potential and to formulate functional plant-based prebiotic supplements. The ability of twenty-nine fruits, vegetables, and other plant-derived materials to stimulate the growth of probiotic microorganisms was tested *in vitro* using Lactobacillus and Streptococcus species. Their prebiotic efficacy relative to fructooligosaccharides (FOS) as the standard was determined through *in vitro* analyses. Many materials exhibited significant growth promotion of >60% FOS activity and hence are highly prebiotic. There was no significant effect from the drying of materials on their prebiotic performance, which thus verifies their stability. The best plant sources were used for the formulation of three formulations, F1, F2, and F3, which were thereafter assessed *in vivo* using Wistar rats for six weeks. All formulations improved gut microbiota, increasing Lactobacillus counts and reducing *E. coli* populations. Total cholesterol, triglycerides, and blood glucose showed significant reduction, while HDL and antioxidant capacity as TEAC were increased as shown by biochemical analyses. This proves that the indigenous plant-based prebiotic formulations are effective, safe, and sustainable alternatives to synthetic prebiotics and hence have a good potential for applications in nutraceuticals and functional foods for gut and overall health improvement.

Keywords: Indigenous plant, prebiotic, Nutraceuticals, Lactobacillus, *Escherichia* coli, *in vitro* and *in vivo* evaluation etc.

Introduction

Over the during the years, researchers become more interested in studying probiotics because they are good for people's health in many ways and because people want them. It has been shown that many probiotic organisms found in human gut and mouth can help protect against infections caused by food-borne pathogens. In general, it is well known that probiotics protect against food-borne pathogens by working in different ways. Immunomodulation, enhancement of gut barrier function, competing exclusion of binding sites and dietary sources, and release of antibiotic compounds are some of the ways it works. One of the problems with probiotic organisms is that they might not be safe for people whose immune systems aren't working well, or who are very sick or in the hospital. In addition to having an effect on the host, probiotics can also work with germs that live with it.

Recently, bacteria that live on plants and have positive benefits were named "Plant Probiotics". There are bacteria called plant probiotics that are good for plants' health when given in certain amounts. They are also part of the "Plant Growth Promoting Rhizobacteria" (PGPR) bacterial community, which may colonize plant roots, enhance plant nutrition, accelerate plant development in several ways (both directly and indirectly), and increase crop quality.

And yet, everyone knows that choosing the right and most effective PGPR is necessary to help plants. So, we need to understand how plants, bugs, and external factors all work together in complex ways. Things of organic compounds, the concentration of inorganic minerals, and the types of plants fluids, can help bacteria grow and settle down. So, the microbe groups that live around plants rely on how the environment grows. To improve plant quality, it is important to know how microbial groups colonise plant roots and how biofertilizers work and interact with them. To keep people and the environment healthy, it is also important to make sure that the germs used in biofertilization methods don't cause disease.

Along with the current food standards, one of the new ones is focused on eating lots of nutritious veggies and fruits.

These chemicals can stop cancer from happening and stop a number of illnesses and health problems. Because of this, a large body of research has examined the advantages of bioactive chemicals on human health. These kinds of things should also be eaten in large amounts for a good diet. One example is berries, which are very good for you because they have a lot of vitamins, minerals, and antioxidants. Berries are also easy to find and can be eaten in many different ways, such as fresh or frozen, and they are used in foods like jam and yoghurt. On the other hand, gardening crops give people a wide range of important vitamins and minerals, such as potassium. Eating a variety of fruits and veggies is linked to a healthy diet generally.

Literature Review

Khan, Fasiha *et al*. (2022) [1]. The nutraceuticals business has recently developed as a promising sector due to the several physiological roles that non-dairy sources the benefits that prebiotics and probiotics provide to the treatment and prevention of metabolic disorders. Prebiotics can be simply and affordably extracted from non-dairy sources. Fruit peels and skins, along with other food processing waste, are "Generally Recognized as Safe" are safe for human consumption and may be used as a source of prebiotics. The gut microbiota is greatly affected by prebiotics derived from non-dairy sources, which in turn decreases the number of harmful bacteria. Isolating next-generation probiotics from sources other than dairy is potentially a possibility. These alternatives to dairy have a lot of promise, as they may provide new types of probiotics. These non-dairy strains are therapeutically useful and have strong probiotic qualities. The review will go into detail on the several alternatives to dairy products that contain beneficial bacteria, how they are characterized, and the substantial physiological potential of these substances.

Mishra, Vibhuti *et al*. (2020) [2]. Dietary supplements with several uses, probiotics have attracted a lot of attention. One of its most well-known and prevalent uses is as a preventative measure against a variety of gastrointestinal diseases and conditions, including IBS, diarrhea, ulcerative colitis, and many more. Researchers have recently shown there is a lot of curiosity in exploring the many additional aspects of probiotic functions. Live microbes have several potential health benefits, including but not limited to: enhancing mental health, protecting against neurodegenerative diseases, alleviating arthritic symptoms, and, most importantly, acting as anticancer agents. Another important role of probiotics is to eliminate dangerous environmental pollutants, including as heavy metals like lead and cadmium. Research on probiotics' roles in the body has shown that they offer great promise as treatments for different types of illnesses. Their potential as antiviral drugs have been suggested by a few recent investigations. Research into probiotics, which may be utilized by healthy people in view of the present COVID-19 pandemic situation, this is essential as an immune system booster as might be a highly beneficial area to examine.

Champagne, Claude *et al*. (2018) [3]. Probiotic bacteria are becoming more popular in a variety of food and supplement items. Conventional wisdom held that assuring cell viability was sufficient to guarantee their efficacy. Nevertheless, probioactives, which are bioactive metabolites unique to probiotics, are being discovered at an alarming rate. So, making sure the items include the probioactives will help them work better for your health. One may argue that by modifying fermentation techniques to create fermented meals or supplements with high concentrations of probioactives, the efficacy of probiotics can be enhanced. Additionally, probiotics must show that they have several uses in food, such as preventing spoiling.

Fredua-Agyeman, Mansa *et al*. (2016) [4]. The popularity and growing use of probiotics have led to a proliferation of these items on the market, but ensuring their quality has remained an ongoing challenge. In light of the claims made by their manufacturers, this research set out to evaluate the composition of many probiotics sold in the UK. The research used seven goods. Isolation, identification, and counting of the bacteria content was carried out using selective media. The findings showed that all of the items tested included probiotic bacteria, however only three of the seven (43% of the total) had the concentration of cultures that were advertised. The probiotic bacteria listed on the labels were not completely present in any of the multispecies products. There were instances of species being mistakenly identified. The findings corroborated those of earlier research demonstrating that commercial probiotics continue to have quality difficulties. The growing popularity of probiotics has prompted calls for strict regulations to regulate the industry's product quality, since the effectiveness of these microorganisms depends on their concentration and is strain dependent.

Iqbal, Zeeshan *et al*. (2014) [5]. "Probiotics" "live microorganisms which, when given to the host in sufficient quantities, confer a health benefit on the host" according to a joint FAO/WHO expert consultation study. Lactic acid bacteria (LAB) and bifidobacteria are the two most common kinds of probiotic bacteria. Some other organisms may also act as probiotics; this includes specific yeasts and bacilli. Probiotic supplements are becoming more popular these days. Since the new century, there has been a meteoric rise in the amount of research on probiotics. Possible probiotic health advantages in treating several common ailments have recently attracted a lot of scientific attention. We looked at the ways probiotics help with a few different illnesses here.

Research Methodology

Materials

It was Hi Media laboratories that supplied the materials and chemicals utilized in this project. Pepsin, pancreatin, bile salts, and MRS broth (both ready-made and its ingredients) were among them. Components. It was Qualigens that supplied the furfural. The digested residue was treated with absolute ethanol. Sigma supplied the benzoyl DL-arginine paranitroanilide hydrochloride (BAPNA), and SRL supplied the soluble starch, a-amylase (Diastase from a fungal source), trypsin, cholesterol, bile salts (deoxycholic acid, cholic acid, lithocholic acid) and other relevant substances. We obtained the cholesterol kit from Ranbaxy. Two commercial consortia A and B, together with fructooligosaccharide (Nutraflora FOS), were obtained as an over-the-counter formulation in the USA, and the eleven standard probiotics listed in Table 1 were sourced from NCIM in Pune, India.

We obtained three 500 ml glass jars from a glass fabricator.

Each vessel had three neck inlets and one outlet. The chemostat model was developed using a pH electrode and a peristaltic pump manufactured in Pune by Innovative Analytical Ltd. We employed *E. coli*, *L. acidophilus*, and *L. plantarum* as our test organisms. M5/G10 and fructooligosaccharides (from Nutraflora, USA) were used as experimental substances.

The research used chemicals and components similar to Dulbecco's Modified Eagles. Everything you need for your experiment was sourced from HiMedia: medium, foetal bovine serum (FBS), antimycotic antibiotic reagent, and MTT (5-diphenyltetrazolium bromide)-[4, 5-dimethylthiazol-2-yl]-2, 5-iPt. Glucose, versene, trypsin, and L-Glutamine were purchased from Sigma. We purchased the double-chambered slides from BD Falcon.

Qualigens supplied the chemicals, materials, and diatomaceous earth (DE), charcoal, aniline, and acetonitrile (HPLC grade) used for partial characterisation. Sigma supplied the TLC plates, Dowex SOWXs resins, and naphthoresorcinol. The plates used were Silica Gel G from Whatman and had a 250|im thick layer measuring 20cm x 20cm. The company HiMedia supplied the diphenylamine. An onion served as a reference material for the investigation, along with promising plant materials such as undigested and digested M6, M7, and M8. For comparison, we also employed a commercially available fructooligosaccharides supplement (FOS, Nutraflora USA) and a standard fructoligosaccharide (FOS from Chicory, Sigma).

A dosage response curve was generated by individually monitoring the effects of six nutritional and two antinutritional variables on the development of *E. coli*, two bifidobacterium isolates, one commercialized consortium, and two standard probiotic cultures. The experiments were carried out three times.

Sigma supplied the lysozyme and Equipped with ABTS (2, 2' azinobis 3-ethylbenzothiazoline 6-sulphonic acid diammonium salt) and Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E replacement. We only used analytical grade reagents. We bought biochemical kits from Accurex Biomedical Pvt. Ltd. in India to measure glucose, total cholesterol, HDL cholesterol, and triglycerides. The cyan meth reagent hemoglobin kit was acquired from Ranbaxy Fine Chemical Limited. American company Integrated DNA Technologies supplied the FISH test probes. The micronutrients used in the AIN 93 diet came from Qualigens, whereas the ingredients were sourced from the local market (see appendix).

Table 1: Probiotics selected for study

Organisms	NCIM No.
Lactobacillus acidophilus	2902
Lactobacillus delbrueckii subsp delbrueckii	2025
Lactobacillus helveticus	2733
Lactobacillus plantarum	2084
Lactobacillus casei var. rhamnosus	2364
Lactobacillus acidophilus	2660
Lactobacillus casei	2651
Lactobacillus bulgaricus	2056
Lactobacillus fermentum	2165
Streptococcus thermophilus	2904
Streptococcus lactis	2114
Alliance A	Acidophilus probiotic, Nutrition Now, Inc., USA
Collaboration B	Douglas, Nutri-west, and total probiotics

Method Collection and Processing of Materials

Taxonomists from the University of Pune's Botany Department purchased indigenous plant samples from the Pune market and verified their authenticity. Materials found in abundance in the Indian market that have no connection to their biological origin are called indigenous plant materials. Hydrocolloidal and mucilaginous characteristics were considered while choosing plant materials (appendix). Veggies, fruits, and other plant parts (e.g., roots, gums, blossoms, etc.), and so on) were categorized. To eliminate any dirt or grime, the items were rinsed with water and then blotted on filter paper.

Processing of plant materials

After being finely chopped and oven-dried for two or three days, the materials were crushed into a fine powder and kept at -20 °C. To verify that drying is complete, all materials had their moisture contents monitored simultaneously until a constant weight was achieved. Bile salt binding, water holding capacity, a-amylase inhibition, and trypsin inhibitory potential were among the functional activities assessed utilizing dry powdered material.

***In vitro* Gl digestion treatment for enrichment of prebiotic content:** We used a modified version of Agte *et al*. (1995) [6] simulated GI digesting technique. Following this, the mixture was incubated in a shaker at 37 °C for 2 hours with 2 milliliters of pepsin and 5 percent dried powder. Following peptic digestion, a bile-pancreatin solution with a pH of 7.6 10 milliliters of the digested substance had been added to it. Next, the mixture was left to incubate for 2 hours. After filtering, the residue was collected. Was dried. The free sugar was removed from these powders by treating them with 100% ethanol and rinsing them continuously in a soxhlet extractor overnight. Glucose levels were measured in the samples using dinitro salicylic acid (DNSA) after each treatment step. Once the sample was determined to be sugar-free, the ethanol treatment was maintained. After drying, the residue was put to use in determining its promise for prebiotics.

Assessment of *in vitro* prebiotic potential

A study was conducted in order to assess the possibility of using several materials, including fresh, undigested dried, digested dried, and the impact of drying. Twenty milligrammes of the corresponding material residue served as the only carbohydrate supply, supplanting glucose from the MRS medium. As a reference, fructooligosaccharide was used. 100 \i\ of individually produced cultures of 0.4 O.D. were added to MRS media containing the corresponding samples or FOS. Then, for 24 and 48 hours, the mixture was kept at 37°C in somewhat anaerobic conditions. First, the growth curves of the organisms were used to determine the link between O.D. and cell count. At 0, a spectrophotometer (Spectronic 21, Bausch & Lomb, UK) was used to measure the optical density at 600 nm at 24-, and 48-hours post-inoculation in order to monitor growth. In order to compare the development of specific organisms in MRS containing samples to that in MRS containing just FOS, a positive control was used. All of the probiotics listed in Table 1 had their growth response assessed three times for each material.

Sample Collection

The rats were starved overnight, put to sleep in an anaesthetic after the 6-week feeding trial, blood samples were collected by heart puncture from subjects in an ether-saturated chamber. Rats were eliminated when dislocating their cervical spines. The cecum was promptly sutured upon dissection to prevent the passage of cecal contents into the colon as a result of the relaxed gastrointestinal muscles following death. Analyses of glucose, hemoglobin, TEAC, plasma MDA, total cholesterol, HDL, and triglycerides were performed on the same day that the serum and plasma samples were collected.

Enumeration of Lactobacilli in Fecal, Cecal and Colon Contents by Fluorescent in Situ Hybridization (Fish)

We used a modified FISH technique developed by Snart *et al*. (2006) [7] to count the lactobacilli. Homogenized (1/10) in phosphate-buffered saline, faecal samples from 0, 2, 4, and 6* weeks were vortexed for 3 minutes. Using a fluorescent microplate reader, we assessed the cells' fluorescence with a 530/25 excitation and 590/35 emission wavelength after they had undergone one last centrifugation in 150 ail of PBS.

Statistical Analysis

The study involved triplicate observations and statistical analysis using Microsoft Office Excel 2007. Data was represented by averages and their respective standard deviations. Significant results were determined by using statistical tests such as ANOVA and crucial difference, with p values less than 0.05. The bacterial population in the feces and colon is known to vary greatly across rats; hence the counts were represented as log CFU/g dry weight. Student t-tests, paired t-tests, critical differences, percentage coefficients of variation, and one and two-way ANOVA were used to examine the data. Every experiment was carried out three times. Mean + standard deviation was the data representation for every group.

Data Analysis and Results

Screening of Indigenous Plant materials for prebiotic potential and their other functional activities

Live-fed probiotic bacteria in the intestines may benefit from indigestible prebiotic carbohydrates. Additionally, they may directly affect the large intestine microbiota. These components may make foods nutraceutical since they offer physiological benefits beyond nutrition. Several foods may be prebiotic. Functional oligosaccharides are found in asparagus, garlic, onion, banana, rye, barley, yacon, wheat, tomato, bamboo shoots, legumes, milk, honey, sugarcane juice, soyabean, and mustard. Produce's inulin and fructo-oligosaccharides (FOS) may be prebiotics. Prebiotic oligosaccharides are made from plant cell wall polysaccharides such arabinan (from sugar beets), arabinoxylan (from wheat flour), polygalacturonan (from soy), and rhamnogalacturonan (from apples). New prebiotic oligosaccharides from jackfruit seed and red meat pitaya. Two novel oligosaccharides, p-D-fructopyranosyl-2-A A fermented drink created from fifty fruits and vegetables included 3-D-glucopyranosyl-(l-3)-D-glucopyranose and p-D-fructopyranosyl-2. Consuming enough Chinese jujube fruit water-soluble carbohydrate concentration (WSCC) may benefit the digestive tract.

Gum acacia is a prebiotic fibre containing low-molecular-weight oligosaccharides. They impact probiotics and the host's health due to their physico-chemical properties. They may stimulate further favourable effects this way. Bile acid and cholesterol binding reduce blood and LDL cholesterol, improve insulin and glucose responses, and speed up water absorption in the digestive tract. These traits may help cancer, infections, constipation, and inflammatory colonic problems. You may simply increase your prebiotic intake by eating more fruits and vegetables. Focus on Indian fruits and vegetables to create a cheaper prebiotic functional dietary supplement. We conducted this study to discover which native plant materials have the most promising prebiotic potential, using probiotic growth as our selection criteria, and to assess their extra functional activities. Prebiotics, short-chain carbohydrates, improve the microbiome by modifying its composition or metabolism. Thus, 29 plant materials were selected, simulated for GI digestion, and tested for prebiotic and other functional activities.

Assessment of prebiotic potential

One possible indicator of prebiotic potential is the ability to grow probiotics using just experimental plant sources for carbohydrate. As a conventional prebiotic source, FOS was used. The achieved growth was reported as the average percentage of growth relative to FOS. To determine how repeatable the FOS findings were, a one-way ANOVA was conducted. The study's consistent technique was evident from the lack of statistically distinguishing features across the various FOS datasets (N=10) sets, F=0.86, p=0.6). In order to measure the variability in each set of FOS, the coefficient of variation was computed using the mean and standard deviations of all sets throughout all tests. The confidence interval for prospective percentage increase was determined by seeing a 21% CV in all FOS sets. In addition, as its mean and standard deviation are 0.623 and 0.13 were

determined for the growth response to standard FOS for all probiotics. Thus, taking into account the mean ± 2S.D. as the confidence interval range for % FOS, a growth response criterion more than 60%, i.e. in the confidence interval (56%-143%), was deemed as a potential prebiotic for screening plant materials, in comparison to FOS.

Analysis of fruits for prebiotic potential

The assessed prebiotic potential of fruits ranged from 25 to 119%. In comparison to FOS, 13 fruits demonstrated significant changes (F=2.8, p<0.001) according to one way ANOVA. Fig 1 shows that out of thirteen fruits, eleven (M8, M7, M24, Ml, several of the molecules (M28, M5, M2, M9, M3, M25, and M4) showed promise as prebiotics of sixty percent or more. However, not all probiotics were successful with all fruits; specifically, only M13 and M14 showed no development. In addition, as compared to FOS, materials M8 and M7 exhibited a greater growth response to all of the organisms.

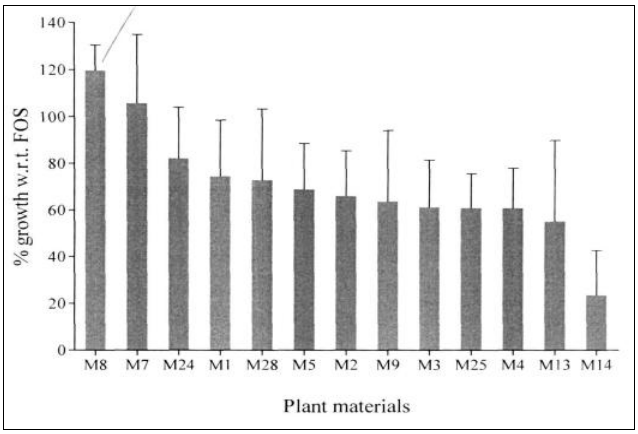

Fig 1: A comparison of the effects of probiotics on fruit development (mean + standard deviation)

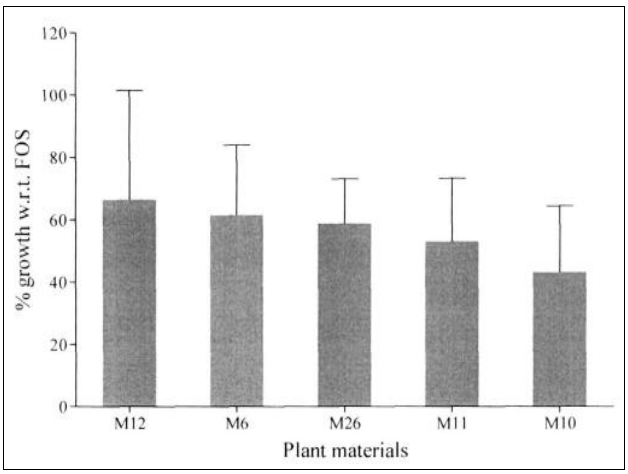

Fig 2: Relative growth response of vegetables to probiotics (mean + S.D. Average of growth given by materials for 13 organisms)

Analysis of vegetables for prebiotic potential

The growth capacity of veggies differed significantly from FOS, according to a one-way ANOVA (F=8.8, p<0.0001). The prebiotic potential range for five different vegetables was shown to be 43-66M12 and M6 were the only ones that showed growth when compared to FOS. Rates more than 60% [Figure 2]. In contrast, M26 and Mil had a growth response of 53-58%. Examination of alternative substances for the possibility of prebiotics.

Materials with a prebiotic potential of 60% or higher included M29, M16, M23, M27, MIB, and M17. [Figure 3], whereas materials M27, MIB, and M29 exhibited growth responses ranging from 55-78%. However, with regard to FOS, the results for M31, M15, M19, and M22 fell within the 55-60% range. These 'other materials' have a very different growth response than FOS, according to one way ANOVA (F=4.9, p<0.0001).

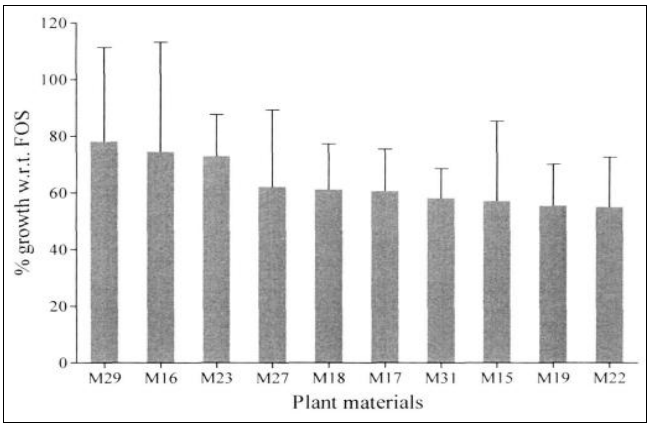

Fig 3: Relative growth response of other materials for probiotics [mean + S.D. Average of growth given by materials for 13 organisms)

When the same data was analyzed for the development of specific probiotics, it was seen that all the materials exhibited growth stimulation for thirteen different probiotics, with responses ranging from forty percent to one hundred and ninety-nine percent. Nevertheless, out of the 34 experimental materials tested, consortia Promising growth over 60% was shown by A, L. casei [L8] and 5, thermophilus (LIO), S. lactis [Lll], *L. plantarum* (L4), L. acidophilus (LI), and L. rhamnosus [L5] (Figure 4). Despite the lack of a statistically significant difference (F=1.9, p=0.2) in a one-way analysis of variance, these six species show potential as probiotics for further research among them.

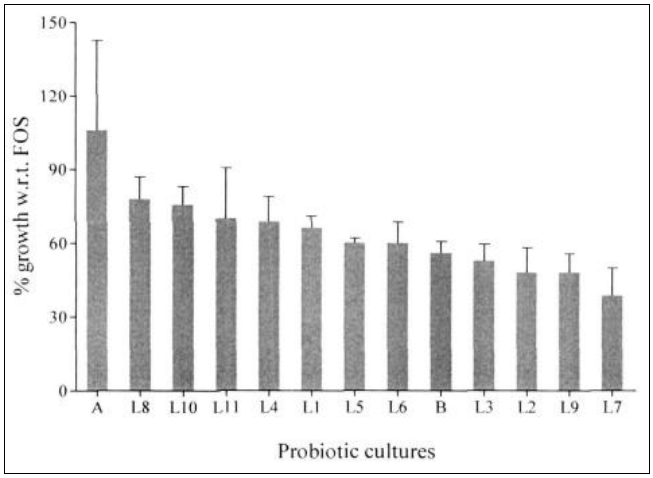

Fig 4: Growth of probiotics to plant materials with respect to FOS (mean ± S.D. Average of growth given by 29 materials)

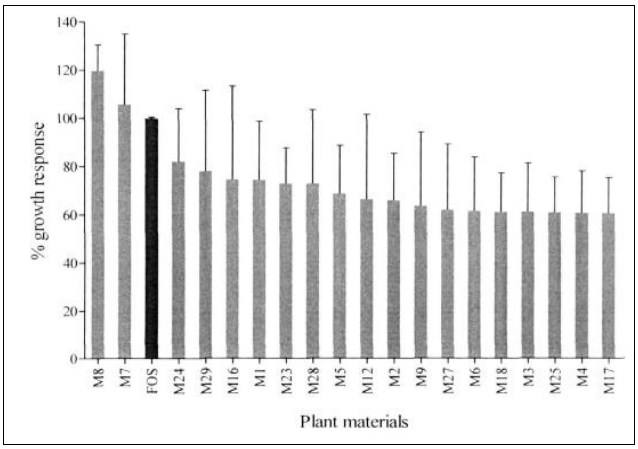

Fig 5: Growth response of materials relative to FOS (mean + S.D. Average of growth for 13 organisms)

Effect of drying on prebiotic potential

Using the same technique, 12 equal-weight samples of fresh and dried materials were used for the prebiotic test. The prebiotic potential of Ml (Figure 6) was higher than that of fresh material, but that of M2 (Figure 7), M3 (Figure 8), and M5 (Figure 9) was lower.

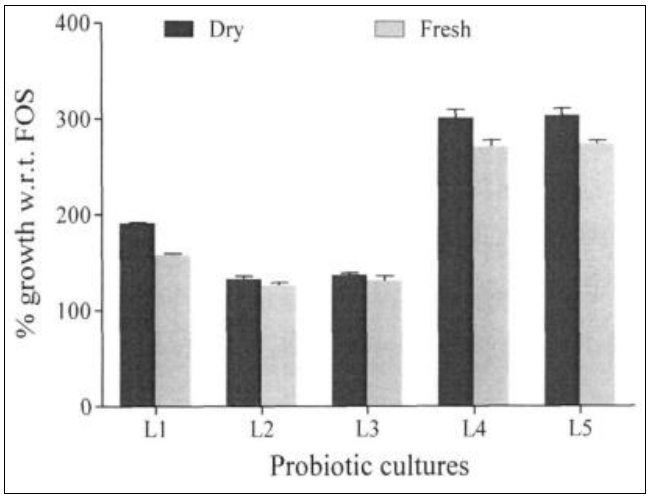

Fig 6: Prebiotic potential of dry Ml vs. fresh Ml (mean ± S.D.)

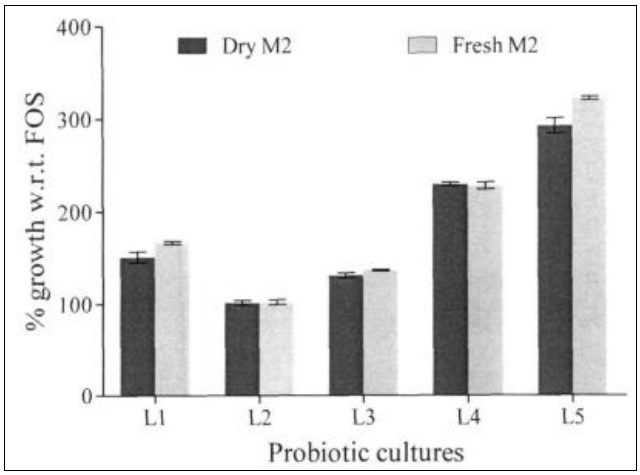

Fig 7: Prebiotic potential of dry M2 vs. fresh M2 (mean + S.D.)

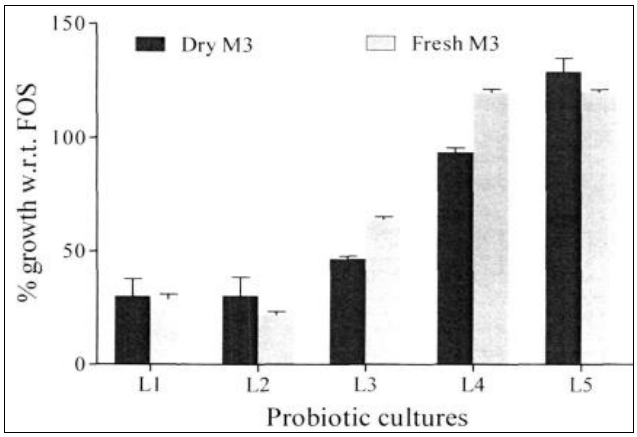

Fig 8: Prebiotic potential of dry M3 vs. fresh M3 (mean + S.D.)

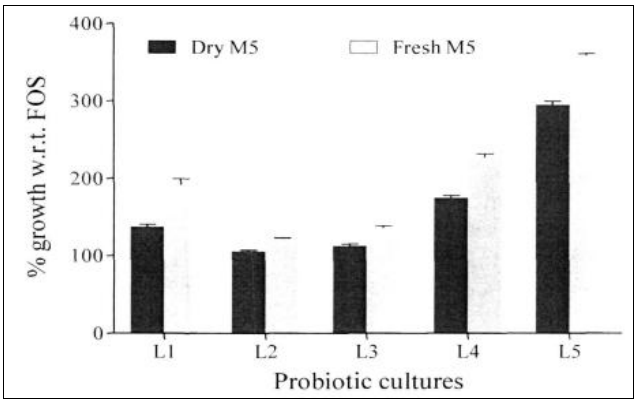

Fig 9: Prebiotic potential of dry M5 vs. fresh M5 [mean + S.D.)

M6, M9, MIO, Mil, and M12 were among the materials whose prebiotic potential increased when dry, but M8 and M13 had the opposite effect when dry compared to when fresh. The prebiotic potential of fresh and dried materials was not significantly different, according to paired t test analysis that assumed unequal variances. Taken together, the data did not point to a drying effect that significantly altered prebiotic potential.

Assessment of prebiotic formulations using *in vivo* rat experiment: Weight increase and feed intake are affected. There were no adverse effects, such as diarrhoea, and all of the animals that were given the experimental food remained healthy during the whole trial. Furthermore, it seemed like the rats acted normally all during the experiment. By substituting 6% formulations and 3% FOS for sucrose, all groups' meals were made isocaloric, with energy levels that were comparable as Kcal/kg of diet (Table 2). All six weeks of feeding, the rats ate the same quantity of food (Table 2).

Table 2: Food intake and Feed efficiency

Animal groups	Food Intake in 6 weeks (Mean ± SD)	Energy value of diet (Kcal/kg)
C	120.9±0.1	3766.0
S	120.3±0.2	3690.8
F1	119.5±0.4	3752.2
F2	120.8±0.1	3749.1
F3	120.8±0.2	3745.0

Weights across the 6-week period for all groups were significantly different from their individual weights at 0 week, according to the two-way ANOVA between animal groups and weeks (F=36.6, p<0.0001). Additionally, there were significant variations in the weights across the animal groups (F=2.4, p<0.05). Nevertheless, the non-significant results of the interaction term between weights and weeks (F=0.18, p=1.0}) suggest that the experiment did not reveal any discernible pattern in the relationship between the two variables. Based on the available data, it seems that the FOS and formulation Fl groups saw less weight gain compared to the control group. Because they were mature animals, it's possible that the drop in body fat percentage contributed to the overall weight loss. In addition, the FOS and formulation groups did not vary significantly from the control category according to mass when evaluated using a one-way analysis of variance (F=1.6, P=0.2) (Figure 10). Indirectly, these findings are corroborated by the findings of Nakamura *et al*. (2011) [10], who postulate that FOS has an impact on reducing the absorption of body fat.

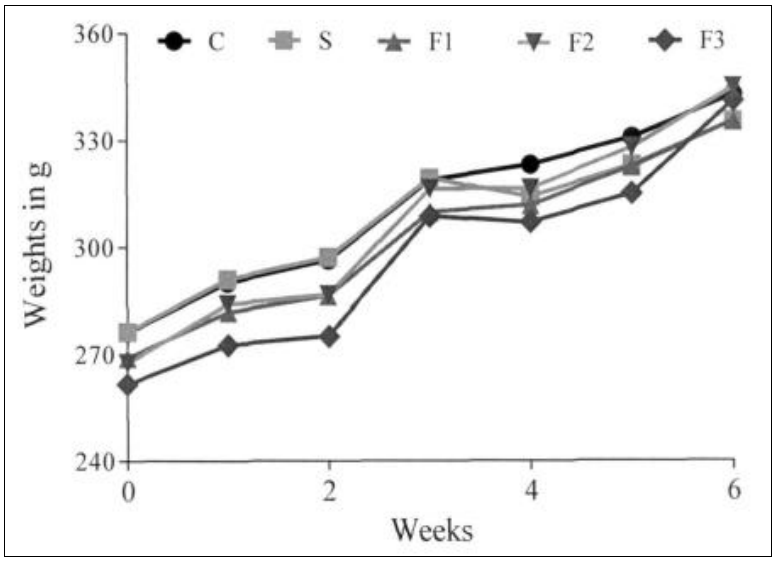

Fig 10: Effect of formulations on weights (g) of rats during 0 to 6 weeks

By dividing weekly weight increase by total food intake, the feed efficiency (FE) value was determined. Over the course of the six weeks, all groups ate almost the same quantity of food. Just as there was no significant interaction between the weeks of the experiment and weight growth, feeding efficiency similarly revealed no significant interaction between the weeks of the experiment and feeding efficiency (F=0.19, p=1.0) (Figure 11). Formulations Fl, F2, and F3 had reduced feeding efficiency than the control and FOS groups from 0 to 6 weeks. One possible explanation is the impact of dietary fiber, which is that large fibers make you feel full faster and may have reduced your appetite as a result. The findings are corroborated by According to research conducted by Wang *et al*. (2009), there was no discernible impact on feed efficiency or body weight when *L. plantarum* was added to a high-cholesterol diet.

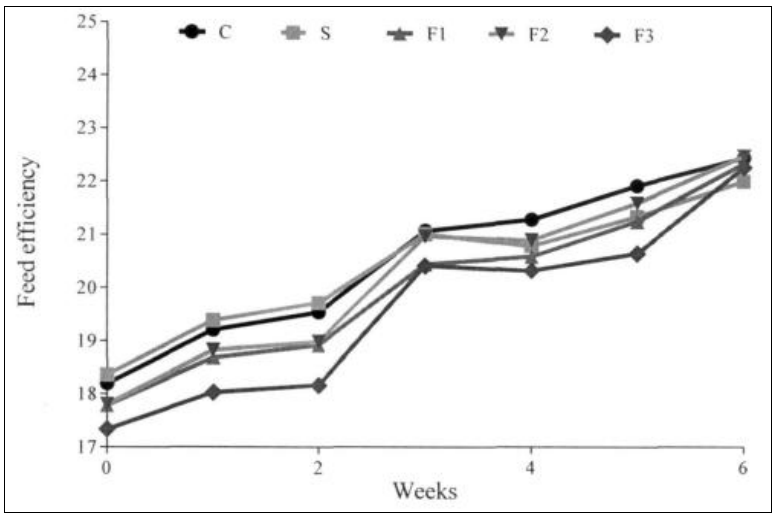

Fig 11: Feed efficiency of rats (feed efficiency=wt gain/food intake per week)

Effect on the fecal weight and moisture content

There was a significant difference shown by the two-way ANOVA in the animal groups (F=2.2, p=0.07) and across weeks the statistical significance was high (F=15.1, p<0.0001). After running the numbers, we found no statistically significant shift in faecal weight (F=1.3, p=0.22)

when comparing all experimental groups, including control and standard. Total faecal weight increased from 0 to 6'a week in the regular FOS, formulation Fl, and F2 groups as contrasted with the normative group (Figure 12). Group F3 had slightly higher total feces weight each day, but the difference was not statistically significant. Additionally, compared to their moisture levels at 0 week, formulations F2 and F3 showed an increase in the percentage of moisture in faeces. There may be a correlation between the water-holding ability of the dietary fibers of the plant materials used in the formulation groups and the increase in faecal weight and faecal water content.

This quality may be effective in the treatment of constipation as it increases the size of faeces and the amount of moisture in faeces. Both the this is supported by data from both *in vitro* and *in vivo* studies, which pertain to the materials' water-holding capacities.

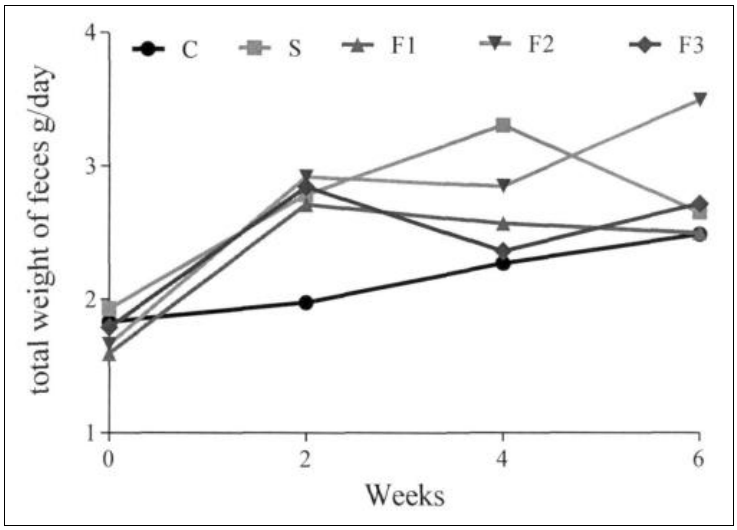

Fig 12: Effect of different formulations on the fecal weight

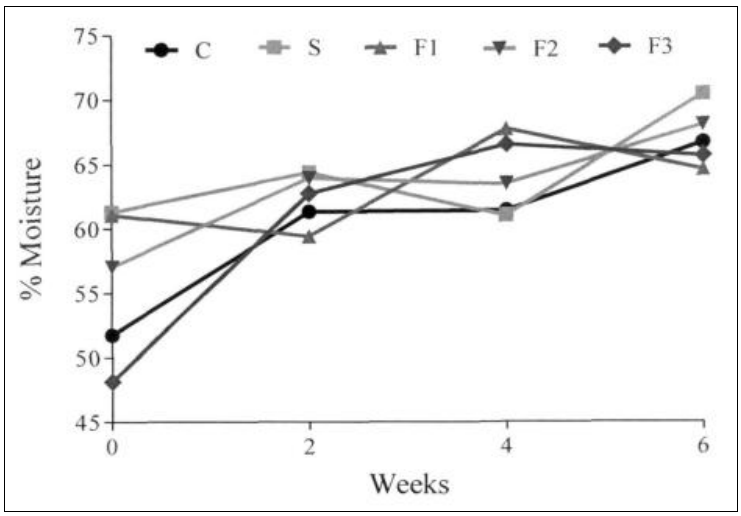

Fig 13: Effect of different formulations on the fecal moisture content

Fecal microflora analysis

The total viable counts of lactobacilli and *E. coli* were determined over the 6-week trial using MRS and MacConkey's medium, respectively, to assess the faecal microflora. Log CFU/g dry weight of faeces was the unit of expression for the findings.

Analysis of lactobacilli

From the beginning to after the trial was over, the viable count of lactobacilli grew considerably (Table 3). Lactobacilli count varied significantly across animal groups ($F=26.5$, $p<0.0001$}) and from 0 to 6 weeks ($F=23.8$, $p<0.0001$), per the results of the two-way analysis of variance. The groups of animals interacted with one another and with the number of weeks in the experiment ($F=3.7$, $p<0.0001$). This finding provided further evidence that the 6-week feeding of formulations led to a more favorable profile of gut flora.

Table 3: Fecal micro flora analysis for lactobacilli and *E. coli*

Group	0 week	2 weeks	4 weeks	6 weeks
C	8.5179±0.72	8.2010±0.54	8.6044±0.44	8.6595±0.37 (NS)
S	8.5004±0.41	7.6297±0.43	8.7594±0.24	9.0896±0.45[a2]
F1	8.9201±0.57	8.1624±0.58	8.2091±0.78	9.0827±0.23[a2]
F2	8.9518±0.26	8.9416±0.61	10.0759±0.22	10.6170±0.14[a2c]
F3	8.7948±0.70	8.0874±0.49	9.7947±0.20	10.1832±0.14[a2c]

Data represented Logio values as mean + S.D.
a: indicates significance level when compared for inter group difference as compared to control group at 6 weeks. (ai p< 0.02, a2 p<0.001)
c: indicates significance level when compared for inter group difference as compared to FOS group at 6 weeks. (‘a p<0.001)
NS: Not significant

The number of lactobacilli in the placebo group did not change at the 0, 2, 4, or 6-week points. There was an increase in the count from 0 to 6 weeks in the standard FOS, Fl, F2, and F3 groups. There was an increase in the count for 6a*1 week compared to 0 week, but a drop at 2" aa week, according to the statistics. Oral administration of the probiotic pill to the rats may have necessitated a two-week period for colonization. This may explain why the FOS, Fl, and F3 groups had lower lactobacillal counts at 2 weeks.
The results of the one-way ANOVA shown that substantial disparities (F=48.6, p<0.0001) in the lactobacilli count between the groups at 6*a week. The control group had a considerably lower count compared to the F2 and F3 groups, while the FOS and Fl groups were very close behind with p<0.02 and p<0.02, respectively, suggesting major differences.

Analysis of *E. coli*: There were significant variations (F=10.8, p<0.0001) in the *E. coli* count during the weekly analysis. Across groups (F=18.4, p<0.0001), and when groups and weeks interacted (F=2.0, p<0.02). These differences were confirmed by two-way ANOVA. Additionally, there was no change in the *E. coli* count for the control and FOS groups at 2'"*, 4aaa, and 6aa weeks. In contrast to the 0-week group, the *E. coli* counts in Fl, F2, and F3 were lower. Fig 14 shows that all three formulation groups-Fl (p<0.001), F2 (p<0.001), and F3 (p<0.001) exhibited a substantial decline in count compared to the control group when analyzed intergroup at 6'*a week using one way ANOVA (F=13.7, p<0.001). In addition, as compared to the control group, FOS shown a non-significant decline. Nonetheless, when compared with conventional FOS, group F2 (p<0.001), F3 (p<0.001), and Fl (p<0.01) exhibited a significant decline.

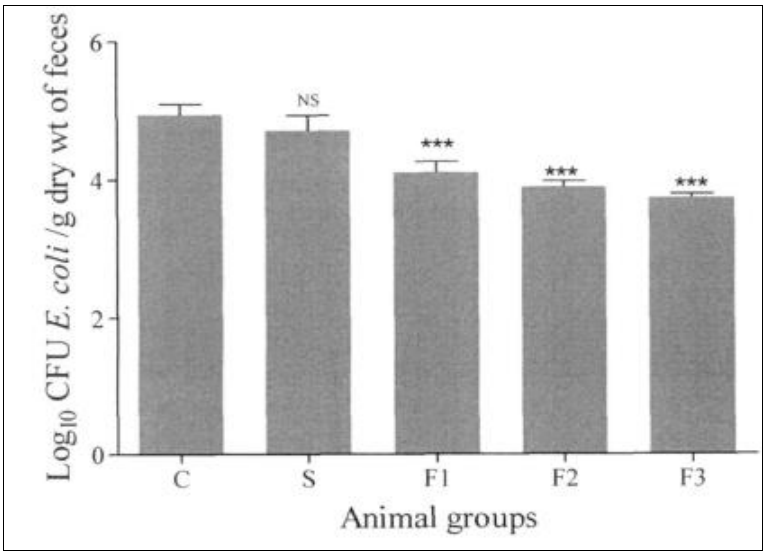

Fig 14: Effect of different formulations on *E. coli* count at 6* week

The findings showed that the formulations were efficient in boosting the lactobacilli count and decreasing the *E. coli* count after 6 weeks of feeding the animals.

Biochemical analysis of blood
On the very same day as the dissection, hemoglobin levels were determined by drawing whole blood. Table 4 shows the results of the analyses of rat plasma and whole blood, which were used to monitor changes in the antioxidant capacity and lipid profile. When comparing Fl and F3 (p<0.001), F2 and FOS (p<0.01), and the control group, biochemical examination of blood revealed a substantial difference (F=7.3, p<0.001) in total cholesterol. While Fl, F2, and FOS all shown a declining trend in TG levels, there was no statistically significant difference between them. The levels of HDL increased in all of the experimental groups, but the one-way ANOVA (F=3.6, p=0.02) and critical difference tests only found a significant rise in F3 (p<0.01) and Fl (p<0.05). Although hemoglobin levels rose with both FOS and formulations, a one-way analysis of variance revealed no statistically significant difference (F=1.3, p=0.31).

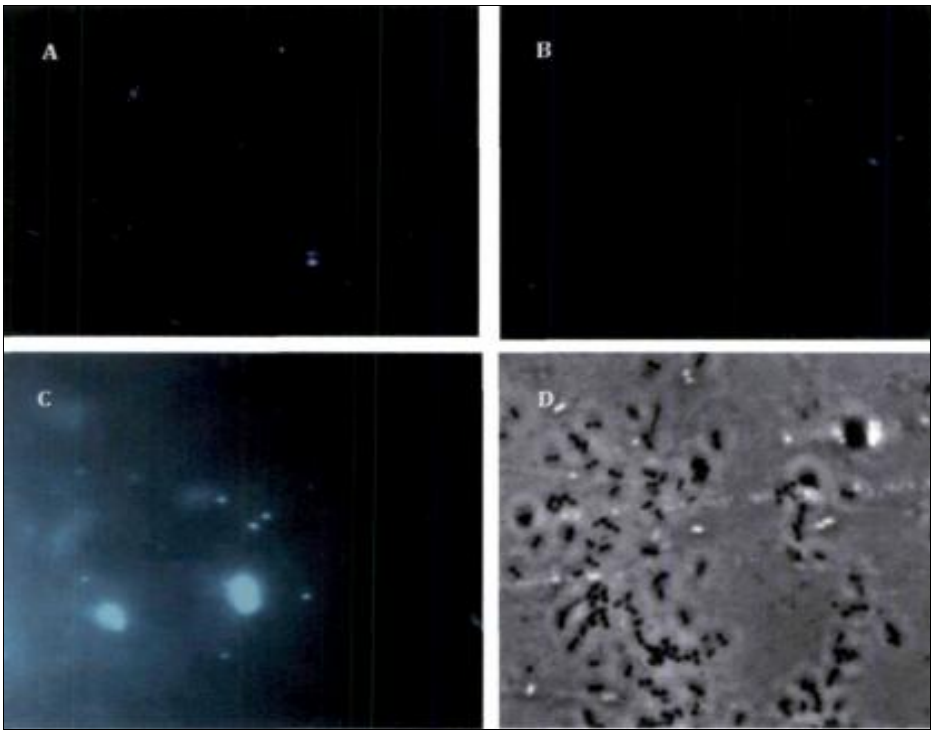

Fig 15: Fish analysis of lactobacilli, bifidobacteria and *E. coli* from feces (Magnification 400X): A) bifidobacteria in feces B) lactobacilli in feces C) fecal *E. coli* D) Phase contrast of lactobacilli

The blood glucose levels of the Fl (p<0.05) and F2 (p<0.001) formulation groups were significantly lower than the control group in the one-way ANOVA and CD analyses (F=9.2, p<0.0001). When compared to the control group, F3 had no statistically significant change in glucose level. There was no statistically significant reduction in blood glucose level between the control group and F3 and FOS. The trolox equivalent antioxidant capacity (TEAC) of the plasma in the Fl and F3 formulations was higher than in the FOS group, however this difference was not statistically significant (F=1.2, p<0.31). There was a significant difference in MDA levels across all formulations (F=2.8, p<0.05), however only F2 demonstrated a significant reduction (p<0.01) in CD analysis compared to the control. Nonetheless, there was no statistically significant reduction in MDA levels in the Fl, F3, and conventional FOS groups as compared to the control group. Indicators of better antioxidant status include increased TEAC and lower MDA.

Table 4: Biochemical parameters of blood sample of rats

Parameters	Control	FOS	Fl	F2	F3
Glucosea	135.8+16.3	122.2+14.5NS	115.5+23.2a3	92.1+14.0='3b3	142.26. 7 NS
Hemoglobin*	13.6+0.6	14.5±1.0Ns	14.4+1.2 NS	14.7+0.9 NS	15.3i2.1Ns
HDLJ	41.3+5.0	44.3±2.1Ns	45.7+4.5a1	41.8±3.1 NS	48.1±2.9a2
Total Cholesterol*	97.1+9.7	81.4+3.8=a2	71.6±13.4=3	79.7+5.6a2	76.4+7.7a3"
Triglycerides*	53.2±15.4	50.6±19.7Ns	51.7+14.1 NS	42.7±6.5 NS	71.0+15.6NS
Plasma MDA*	5.7+2.7	4.3±3.0Ns	3.7+1.8 NS	2.0±0.3a2	4.5+0.4 NS
Plasma TEAC*	31.5±3.5	29.1+2.3 N	33.8±4.0Ns	31.2+3.8 NS	32.5+5.0 NS

Data represented as mean+ S.D. *: mg/dl, *: nm/ml
a: Indicates significance level when compared to control (a' p<0.05, aa p<0.01, =3 p<0.001)
NS: Non-significant

Conclusion

The research was able to confirm that a number of native plant materials have high prebiotic potential that can lead to the growth of probiotic bacteria. Among the twenty-nine materials tested, most of the fruits, vegetables, and other sources of vegetables recorded growth responses greater than 60 percent relative to the standard FOS control, which implies that they can be used in the development of functional prebiotic formulations. The prebiotic efficacy was not significantly affected by drying of the materials, and this showed that they were stable and could be used in practice. Three formulations (F1, F2, and F3) of the most promising sources of plants (M7, M8, and others) were subsequently confirmed by *in vivo* studies done on Wistar rats. These compositions showed a high level of gut micro biota regulation in terms of a high number of Lactobacillus and a low number of *E. coli*, thus improving the health and microbalance of the entire intestinal tract.

Another significant effect of microbial modulation was seen in the biochemical analysis of blood samples which showed significant health benefits of the plant based pre-biotic formulations. The treated groups also had less total cholesterol, triglycerides and blood glucose, and increased HDL levels and better antioxidant status as indicated by

higher TEAC and lower MDA values. The histopathological analysis also proved that the treated groups have improved villi formations in the intestine and bacterial adhesiveness, which indicated improved nutrient absorption and mucosal integrity. All the results confirm the functional efficacy of these natural preparations as safe and sustainable and nutritionally beneficial to synthetic prebiotics. The research therefore provides a solid platform on the establishment of indigenous plant based prebiotic nutraceuticals that can be used to promote the health of the gut of the human and animal body.

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