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Combining Different Types of Prebiotic Plant Isolates Toward, Enhancing the Growth of Probiotic Organisms

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Abstract The growth stimulatory effect produced by combining different sources of prebiotics i.e; Fiber isolates from *Musa* sp pseudostem, polyphenol extracts from *Sesbania grandiflora* flower petal and non-digestible polysaccharide extracts from *Artocarpus heterophyllus* seed were assessed against probiotic organisms, *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* BB-12 *in vitro* in liquid cultures. Different combinations were formulated by integrating the three sources of prebiotics at two different levels i.e; fibre (0.2% and 2%), polyphenol extracts (0.2% and 0.6%) and non-digestible polysaccharide extracts (0.2% and 1.2%) to obtain eight treatments. The formulation which consisted 2% fibre, 0.2% polyphenol and 0.2% non-digestible polysaccharide was able to promote significant biomass increment in *Lactobacillus acidophilus*, while the treatment consisting 2% fibre, 0.6% polyphenol and 0.2% non-digestible polysaccharide demonstrated highest proliferation for *Bifidobacterium animalis* subsp. *lactis* BB-12 *in vitro* which were statistically different ($p<0.05$) than other formulations.

Keywords: *L. acidophilus, B. animalis subsp. lactis BB-12, S. grandiflora, Musa sp, A. heterophyllus*

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1. Introduction

The increasing need and trend for functional food in the current society is causing a remarkable change in food pattern. The awareness which initiated interest between diet and health has increased the production of various neutraceutical and functional food ingredients that support health beyond supplying daily nutritional requirement [1]. To achieve a healthier gut system there are several type of probiotic as well as prebiotic supplements that are administrated by humans to increase the proliferation of beneficial gut microbes. This study focuses on integrating different source of prebiotics at suitable quantities to optimize the colonization of *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp *lactis* BB-12 *in vitro*.

L. acidophilus and *B. animalis* subsp *lactis*-BB-12 have been used in food industry for many years in the process of converting carbohydrates into lactic acid which is responsible to the sour taste in most of the fermented dairy food products such as yoghurt and kefir and due to the large array of beneficial physiological benefits they offer [2,3]. This lowers the pH to an extent where the growth of pathogenic and spoilage microorganisms are prevented there by assuring the prevention of various diseases transmissible through food such as gastrointestinal infections. In the meantime the probiotic resident microbiota in the host exerts a microbial barrier against microbial pathogen which is considered a valuable physiological function [4,5]. They are also capable of strengthening the immune system to overcome allergy conditions [6,7].

Under natural conditions a protective gut micro flora develops and there is no need for a probiotic or prebiotic supplement but consumption of large amount of processed food and sterile food prevent the access of natural colonization of certain strains [8]. Beyond that, antibiotic therapy, excessive alcoholic consumption, stress, exposure to toxic component, other diseases are capable of changing the natural equilibrium of intestinal flora which is not considered favorable to host. But the proper prebiotic intake is capable to re-establish the altered gut flora since they are potential heeling ingredients [9]. Processing conditions are also capable of altering the effect that is imposed by prebiotics, there by impairing the benefits imbibed by probiotic strains in order to bring out desirable physiological changes [10].

Fermentable carbohydrates (complex carbohydrates), polyphenols and oligosaccharides, which are not digested or poorly digested in the small intestine are passed to the lower gut where they become available for some colonic bacteria, but are not utilized by majority of the micro flora present in the colon [11]. The criteria as classifying a food

as prebiotics are; It should resist digestion or absorption in the upper gastrointestinal tract, when it enters the colon it should be selectively metabolized by a limited number of beneficial bacteria and capable to change the colonic micro flora to a healthier bacterial flora rendering physiologic effect that is beneficial for host [9,12].

Fiber isolates of *Musa* sp (Banana) pseudostem, polyphenolic extracts of *Sesbania grandiflora* flower petal, and non-digestible polysaccharide extracts of *Artocarpus heterophyllus* (Jackfruit) seed are the major three prebiotic ingredients that were considered in this study. *S. grandiflora* is a widely available plant in family Fabaceae. The flowers of *S. grandiflora* have been reported to have anti-microbial activity against certain pathogenic microbial species [13]. Also recent studies show that the polyphenolic extract of *S. grandiflora* has significant growth promoting effect on *L. acidophilus [14]*. Dietary polyphenols are natural compounds which are chemically large heterogeneous group of compounds. Polyphenols can be classified into flavonoids and nonflavonoids based on their chemical structure and complexity. It has been found that only 5–10% of the total polyphenol intake is absorbed in the small intestine which can be considered low molecular weight polyphenols. The remaining polyphenols (90–95% of total polyphenol intake) may accumulate in the large intestine and be available to colon microbes since they consist oligomeric or polymeric form [15].

The other prebiotic source considered in this research is *Musa* sp pseudostem and jackfruit seed. Banana pseudo stems are rich sources of ligno-cellulosic fiber, which can promote beneficial health effects, but at the present considered as waste product of banana cultivation and not properly utilized [16].

Previous studies shows, jackfruit seed which is used in this study as a prebiotic ingredient, consist considerable amount of extractable indigestible polysaccharide and capable of selectively stimulating the growth of three probiotics, *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Bifidobacterium bifidum* [17]. All three phytonutrient isolates, selected in this study obey the prerequisites to be considered as prebiotics. Designing an integrated prebiotic combination which has far more potential to cause significant biomass increase of *L. acidophilus* and *B. animalis* subsp. *lactis* BB-12 was the core intention of this study. Since inducing proliferation of these organisms can harvest peak performance of probiotics. This sounds the importance of permanent implantation of probiotics in colon.

2. Materials and Methods

2.1. Extraction of Plant Prebiotics

2.1.1. Fiber from *Musa* sp

Fibers were extracted from the pseudostem of *Musa* sp which was purchased from local market in Sri Lanka. The suitable stalk of the plant was cut to a length of 150 cm and its outer sheath was removed, since fibers are located at the outer sheath. Then fibers was extracted from this section using roller drums [18]. Finally the fibers were completely cleaned in water to remove the waste materials and then dried at 60°C for three days to remove excess moisture, then powdered and stored at -18°C for further use.

2.1.2. Polyphenol Extract of *S. grandiflora* Flower Petals

The flower samples was collected from local market Sri Lanka. Then the petals were separated, sun dried and powdered and stored at -18°C for further use. Exactly eight grams of finely dried powder was weighted, and extracted with 80:20 of methanol:water at room temperature and filtered. The extraction was done until the filtrate was colorless. The extracts were pooled, concentrated in the rotary evaporator and freeze dried, then after stored in capped dark bottles at -18°C for further use [19].

2.1.3. Non-digestible Polysaccharide of *Artocarpus heterophyllus* (Jackfruit Seed)

Jackfruit seeds purchased from Sri Lankan local market, were washed, sliced, dried, milled and sieved for size of 2mm and stored at -18°C for further use. Sixty grams of jack fruit seed powder was extracted with 50 %(v/v) ethanol at 60°C shaken at 200 rpm in oil bath for 15 minutes where the liquid: solid ratio was maintained at 8. After extraction the solvent was evaporated using rotary evaporator and stored in dark cap bottle at -18°C for further use [20].

2.2. Bacterial Cultures

Lactobacillus acidophilus LA-5 (Chr. Hansen), *Bifidobacterium animalis* subsp. *lactis* BB-12(Chr. Hansen), purchased in lyophilized form, were used as probiotic test bacteria in this experiment. *Lactobacillus acidophilus* culture was activated in MRS broth (Oxoid) at 37°C under anaerobic conditions for 24 h [21].

Bifidobacterium animalis subsp. lactis BB-12 was activated in MRS broth (Oxoid) supplemented with 0.05% (w/v) L-cysteine HCl, under anaerobic conditions at 37°C for 48 h [22].

2.3. Experimental Design

Fiber (F), polyphenol extract (PE) and non-digestible polysaccharides (NDP) extracts were integrated as below in order to obtain eleven different treatment combinations.

Table 1. Prebiotic treatment combinations and composition

Treatment Combinations	Composition of F, PE, NDP (w/v)
A	0.2%, 0.2%, 0.2%
B	2%,0.2%,0.2%
C	0.2%,0.6%,0.2%
D	2%, 0.6%, 0.2%
E	0.2%,0.2%,1.2%
F	2%,0.2%,1.2%
G	0.2%,0.6%,1.2%
H	2%,0.6%,1.2%
I	**0.2%,0%,0%**
J	**0%,0.2%,0%**
K	**0%,0%,0.2%**

*The treatments in bold-face are designed using one prebiotic ingredient
*The treatments in non-bold-face are designed with three prebiotic ingredients combined at different concentrations.

2.3.1. Growth promoting property of integrated and non-integrated prebiotic treatments on *L. acidophilus*

The eleven treatments mentioned in Table 1, were introduced to fresh MRS broth (5 mL) separately and were inoculated with 0.1 mL *L. acidophilus* culture which contains 5.5×10^5 CFU/mL. In the positive control set *L. acidophilus* culture was grown in fresh MRS broth (5 mL) omitting prebiotic ingredients.

The cultures were incubated at 37°C for 24 h under anaerobic conditions. After incubation the samples were diluted in MRS broth and plated on MRS agar using pour plate technique and incubated at 37°C under anaerobic conditions. All steps were carried in triplicates. The effect of eleven treatments on *L. acidophilus* was determined comparing the number of CFU in the presence of different combinations of prebiotic ingredients, against those obtained from controls.

2.3.2. Growth Promoting Property of Integrated and Non-integrated Prebiotic Treatments on *B. animalis* subsp. *lactis* BB-12

The eleven treatments mentioned in Table 1 were introduced to fresh MRS broth enriched with 0.05% cysteine HCL (5 mL) separately and were inoculated with 0.1 mL *B. animalis* subsp. *lactis* BB-12 culture which contains 6.3×10^4 CFU/mL. In the positive control set *B. animalis* subsp. *lactis* BB-12 culture was grown in fresh MRS broth enriched with 0.05% cysteine HCL (5 mL) omitting prebiotic ingredients.

The cultures were incubated at 37°C for 24 h under anaerobic conditions. After incubation the samples were diluted in MRS broth and plated on MRS-cysteine agar using pour plate technique and incubated at 37°C under anaerobic conditions. All steps were carried in triplicates. The effect of eleven treatment on *B. animalis* subsp. *lactis* BB-12 was determined comparing the number of CFU in the presence of different combinations of prebiotic ingredients, against those obtained from controls.

2.3.3. Determination of Total Polyphenolic Content in *S. grandiflora* Flower Extract

S. grandiflora flower extract (2 mg) was initially dissolved in 40 µl of dimethyl sulfoxide (DMSO) and 2 and 1 mg/ml concentrations were made using distilled water. Series of gallic acid concentrations (1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.015625 mg/ml) were used to construct the standard curve (n=6). Total Polyphenolic Content was determined by method described by Singleton. The assay was carried out in a micro plate reader. Total polyphenolic content was expressed as mg gallic acid equivalents per g of *S. grandiflora* flower extracts [23].

2.4. Statistical Analysis

Statistical analysis was carried out in order to determine the significant difference among all treatments towards growth promoting ability, using Minitab- 16.1.1. One-way ANNOVA.

3. Results

3.1. Initial Count of *L. acidophilus* and *B. animalis* subsp. *lactis*

Table 2. Initial bacterial counts of inoculum

Organism	Log CFU/ mL
Lactobacillus acidophilus	5.73±0.0404
Bifidobacterium animalis subsp. *lactis* BB-12	4.79±0.0153

Values are expressed as log means of three trials (mean ±SD, n=3).

3.2. Total Polyphenolic Content in *S. grandiflora* Flower Petals

The polyphenolic extracts of S. grandiflora flower used in this study contain a total polyphenolic content of 28.31± 0.44 mg of GAE/g of dry weight (mean ± SD, n=6).

3.3. Growth Stimulation Caused by Prebiotic Treatments toward *L. acidophilus* and *B. animalis* subsp. *lactis*

The effect of eight integrated prebiotic treatments and three non-integrated prebiotic treatments were assessed in terms of increased CFU/mL on *L. acidophilus* and *B. animalis* subsp. *lactis* compared to control.

Table 3. Growth of probiotic species with integrated prebiotic treatments

	Viable counts (log CFU/mL)	
	L. acidophilus	*B. animalis* subsp. *lactis* BB-12
Treatments		
A	$^{b}6.96 \pm 0.0115$	$^{b}6.17 \pm 0.0052$
B	$^{*}7.49 \pm 0.0049$	$^{c}6.08 \pm 0.0361$
C	$^{b}7.00 \pm 0.0058$	$^{d}5.94 \pm 0.0173$
D	$^{b}6.86 \pm 0.0096$	$^{*}6.48 \pm 0.0015$
E	$^{b}7.08 \pm 0.0058$	$^{e}5.86 \pm 0.0006$
F	$^{c}6.74 \pm 0.046$	$^{f}6.05 \pm 0.0101$
G	$^{d}7.18 \pm 0.0125$	$^{e}5.89 \pm 0.0058$
H	$^{d}7.26 \pm 0.0053$	$^{g}6.35 \pm 0.0189$
Control	$^{a}5.78 \pm 0.2223$	$^{a}5.68 \pm 0.0012$

Values are expressed as log means of three trials (mean ±SD, n=3)
Value in the same column with the same superscript letter are not statistically significant (p< 0.05) than each other.
Superscript * indicates the treatment producing maximum growth proliferation.

Table 4. Growth of probiotic species with non-integrated prebiotic treatments

	Viable counts (log CFU/mL)	
	L. acidophilus	*B. animalis* subsp. *lactis* BB-12
Treatment		
I	$^{*}6.51 \pm 0.0674$	$^{m}5.79 \pm 0.0173$
J	$^{m}6.15 \pm 0.0050$	$^{*}5.84 \pm 0.0029$
K	$^{m}6.12 \pm 0.0125$	$^{n}5.76 \pm 0.0051$
Control	$^{x}5.78 \pm 0.2223$	$^{x}5.68 \pm 0.0012$

Values are expressed as log means of three trials (mean ± SD, n=3)
Value in the same column with the same superscript letter are not statistically significant (p< 0.05) than each other.
Superscript * indicates the treatment producing maximum growth proliferation.

4. Discussion

4.1. Prebiotic Potential of Plant Extracts

Functional food is one step ahead in delivering more health promoting effects beyond satisfying basic nutritional requirement. Prebiotics are phytonutrients that falls in to the category of functional food and will amend the gut micro biome in a potential way of delivering tremendous health benefits for the host by increasing the

proliferation and growth of beneficial bacteria who are not pathogenic, especially *Lactobacilli*, and *Bifidobacteria* [1,9].

Table 1, indicates the eight prebiotic treatments, designed by integrating fiber, polyphenol extracts and non-digestible polysaccharide extracts at different levels and three prebiotic treatments designed by including fiber, polyphenol extracts and non-digestible polysaccharide extracts separately. The effect of these eleven treatments, toward increasing the growth of *L. acidophilus* and *B. animalis* subsp. *lactis* BB-12 were assessed in terms of increased CFU/mL in liquid MRS cultures when incubated with the prebiotic treatments under anaerobic conditions.

The results obtained in Table 3 and Table 4, clearly indicates all treatments are significantly ($p< 0.05$) capable to induce growth of *L. acidophilus* and *B. animalis* subsp. *lactis* BB-12 compared to the control. This demonstrate the prebiotic potential of ingredients used in this experiment.

Fibers of *Musa* sp, polyphenol extracts of *S. grandiflora* flower and non-digestible polysaccharide extract of *A. heterophyllus* seed, when tested integrated and separately showed statistically significant increase in biomass of *L. acidophilus* and *B. animalis* subsp. *lactis* BB-12 *in vitro* emphasise, they are qualified functional foods possessing prebiotic potential. Also the phytonutrient isolates are capable to, initiate favorable health benefits in host, because the action they undertake toward increasing the proliferation of probiotics.

4.1.1. Prebiotic Potential of *Musa* sp Fiber Isolates

There are tremendous health benefits that host obtain from consumption of fiber, out of all, the outstanding feature becomes maintaining good gastrointestinal health which this study focus more on. Fibre components are capable to undergo partial or complete fermentation by the gut micro flora in the large bowel (colon). The complex fiber which undergo fermentation produces gases such as hydrogen, methane and carbon dioxide and short chain fatty acids such as acetate in major proportion, propionate and butyrate. The produced short chain fatty acids are capable to decrease the pH in the colon and there by inhibit the growth of pathogenic organisms and also the formation of toxic breakdown products. Also the short chain fatty acids that are generated will be absorbed into the systemic circulation and account as an energy source while inducing beneficial effects on lipid metabolism. The availability of fermentable substrates in the colon results in an increase in the number of bacteria and thereby increase the stool weight which is attributed to the capability of some fiber components to absorb fluid and so increase stool weight [24]. Recent studies carried on *Musa* sp pseudostem has revealed that, the outer sheath consists higher ratios of insoluble fiber where soluble fibers are present in minor ratios [25]. Fibers can be classified as soluble and insoluble fibers, soluble fibers have gel forming property thereby increasing the viscosity in intestinal tract and undergo complete fermentation than insoluble fibers. However, not all soluble fibers are viscous and some insoluble fibers may also be well fermented [26]. Previous studies executed on fermentation of soluble and insoluble fibers by *Lactobacilli* spp and *Bifidobacterium* spp and production of short chain fatty acids, grandly complies with the results obtained in Table 3, and Table 4 [27,28]. The previous studies insist that *Musa* sp fibers can be fermented by *L. acidophilus* and *B. animalis* subsp. *lactis* BB-12 and thereby induce proliferation in liquid culture media.

4.1.2. Prebiotic Potential of Polyphenol Extracts of *S. grandiflora* Flower

The poor bioavailability of native polyphenols is the major reason to consider polyphenols as prebiotic candidates [29]. *S. grandiflora* flower petals consist of numerous antimicrobial properties towards most of the pathogenic microorganism mean while possessing growth stimulatory effect on *L. acidophilus* has been discussed in previous work carried out. HPLC analysis of the flavonoids extracted from the broth which was cultured with polyphenol extracts of *S. grandiflora* flower and *L. acidophilus*, before and after the incubation has showed that there is a reduction of rutin content and increment of biomass of the bacterium in the broth after incubation for 24h [14]. Rutin, absorbs more slowly since it requires the assistance of cecal microbial enzymes to be hydrolyzed. This implies the ability of rutin to resist digestion by digestive juices and be available in the large intestine for microbial digestion [30]. This is a clear evident that insists polyphenol extracts of *S. grandiflora* flower is a potential candidate to cause growth of *L. acidophilus*. Also the previous studies carried out shows that *Bifidobacterium* spp is capable to biotrasformate rutin and fermentation of polyphenols stimulates proliferation of *Bifidobacterium* spp [31]. These previous studies, dictate the capability of polyphenol extracts to cause growth stimulation against *L. acidophilus* and *B. animalis* subsp. *lactis* which are clearly shown in Table 3, and Table 4.

4.1.3. Prebiotic Potential of Non-digestible Polysaccharide Extracts of *A. heterophyllus*

Jackfruit (*A. heterophyllus*) seed are proven in previous studies to possess prebiotic properties and stimulate the growth of beneficial intestinal probiotics. The non-digestible polysaccharide of jackfruit seed which consist degree of polymerization 5, has confirmed their prebiotic capacity and selective fermentation by *Lactobacillus* spp and *Bifidobacterium* spp. Also the fermentation is associated with production of significant amount of butyric acid under anaerobic conditions [32]. This forms a link between the results obtained in current study, declaring *A. heterophyllus* seed extracts which contain non-digestible polysaccharides are capable to increase the growth of *B. animalis* subsp. *lactis* BB-12 and *L. acidophilus* under anaerobic conditions when integrated with other prebiotic ingredients and as separate entities, as shown in Table 3 and Table 4.

4.2. The Impact of Integrated Prebiotic Combinations on *L. acidophilus*

The results obtained in Table 3, remarkably dictates, treatment B which consist 2% fiber, 0.2%, polyphenol extracts and 0.2% non-digestible polysaccharide has the highest potential to cause statistically significant ($p<0.05$) growth of *L. acidophilus* than the other seven integrated prebiotic treatments and control in liquid culture media. Treatment H (2% F, 6% PE, 1.2% NDP) and G (0.2% F,

0.6% PE, 1.2% NDP) are capable to induce statistically significant growth of *L. acidophilus* than the control next to treatment B but there is no statistically significant difference among these two groups, which emphasise that the effect imposed by both the treatments are the same toward enhancing the growth of *L. acidophilus*. Treatment E (0.2% F, 0.2% PE, 1.2% NDP), C (0.2% F, 0.6% PE, 0.2% NDP), A (0.2% F, 0.2% PE, 0.2% NDP) and D (2% F, 0.6% PE, 0.2% NDP) stand next in place to cause significant growth than the control on *L. acidophilus* but there is no significant difference among these treatments. The contribution of Treatment F (2% F, 0.2% PE, 1.2% NDP) towards the growth of *L. acidophilus* is significantly effective than the control but, the effect is quite minute compared with other integrated treatment combinations.

4.3. The Impact of Non-integrated Prebiotic Treatments on *L. acidophilus*

The results obtained in Table 4, clearly shows that when fiber isolates of *Musa* sp (treatment I), polyphenol extracts of *S. grandiflora* (treatment J) and Non- digestible polysaccharide extracts of *A. heterophyllus* (treatment K) when tested separately with a concentration of 0.2% (w/v) are able to promote growth of *L. acidophilus* than the control in a statistically significant ($p< 0.05$) manner. Among all three non-integrated treatments treatment "I "which consisted 0.2% fiber extracts showed significant growth promotion than the other treatments [33,34]. Also there were no significant difference between treatment J and K in producing growth stimulation in the case of *L. acidophilus*. It is evident that the growth stimulation caused by the non- integrated prebiotic treatments are higher than the control but lower than the integrated prebiotic treatments, which is clear by Table 3 and 4.

4.4. The Impact of Integrated Prebiotic Treatments on *B. animalis* subsp. *lactis* BB-12

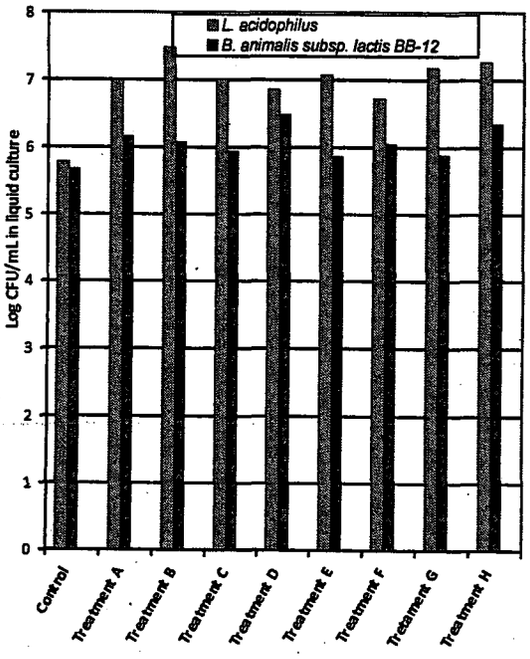

Figure 1. The effect imposed by integrated prebiotic treatments on *L. acidophilus* and *B. animalis* subsp. *lactis* BB-12 in liquid culture media

The results obtained in Table 3, remarkably dictates, treatment D which is constructed using 2% fiber, 0.6% polyphenolic extracts and 0.2% non-digestible extracts showed outstanding performance in promoting significant ($p<0.05$) biomass increment of *B. animalis* subsp. *lactis* BB-12, than rest of the seven integrated treatments and control in liquid culture media. The rest seven treatments are also capable to induce significant biomass increase of *B. animalis* subsp. *lactis* BB-12 than the control in the order of, treatment H (2% F, 0.6% PE, 1.2% NDP) > A (0.2% F, 0.2% PE, 0.2% NDP) > B (2% F, 0.2% PE, 0.2% NDP) > F (2% F, 0.2% PE, 1.2% NDP) > C (0.2% F, 0.6% PE, 0.2% NDP) > G (0.2% F, 0.6% PE, 1.2% NDP) > E(0.2% F, 0.2% PE, 1.2% NDP). But there is no significant difference between treatment G and E in relation to growth promotion.

4.5. The Effect of Non-integrated Prebiotic Treatments on *B. animalis* subsp. *lactis*

The results obtained in Table 4, clearly shows that when fiber isolates of *Musa* sp (treatment I), polyphenol extracts of *S. grandiflora* (treatment J) and Non- digestible polysaccharide extracts of *A. heterophyllus* (treatment K) when tested separately with a concentration of 0.2% (w/v) are able to promote growth of *B. animalis* subsp. *lactis* BB-12 than the control in a statistically significant ($p< 0.05$) manner. Among all three non-integrated treatments, treatment J which consisted 0.2% polyphenol extracts showed significant growth promotion than the other treatments[31]. Comparison made between Table 3 and 4, dictates growth stimulation caused by the non-integrated treatments are low than the growth induced by the integrated treatment combinations but higher than the control.

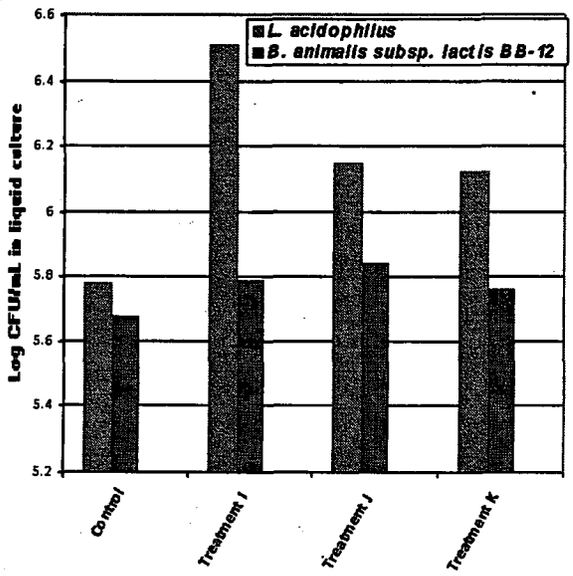

Figure 2. The effect imparted by non-integrated prebiotic treatments on *L. acidophilus* and *B. animalis* subsp. *lactis* BB-12 in liquid culture media

Further studies are required to understand the biotransformation mechanism and biotransformed products of all eight integrated prebiotic treatments when fermented by the test probiotic strains. Also detailed study is required to understand, the biotrasformated product

mixture that is generated after fermentation e.g: short chain fatty acid production of fibers in the presence of polyphenolic extracts. Since, previous researchers have shown that it is not the initial component initiates proliferation of probiotics, but it is the biotransformed product environment that create the growth of probiotics [24]. The study demonstrates the ability of the prebiotic combination to enhance growth *in vitro*, but more work needed to be carried out to understand how these integrated prebiotic treatments work *in vivo* and the potential concentration to proliferate growth *in vivo*.

5. Conclusion

In conclusion we can say that the force, imposed by integrated prebiotic combinations toward causing significant increase of the biomass of *L. acidophilus* and *B. animalis* subsp. *lactis* BB-12 is greater than the force imparted by single prebiotic ingredients. But essentially not all integrated combinations are effective in causing maximum proliferation. Combination containing 2% fiber, 0.2% polyphenol extracts and 0.2% non-digestible polysaccharide extracts is significant in promoting growth of *L. acidophilus*, where 2% fiber, 0.6% polyphenol extracts and 0.2% non-digestible polysaccharide extracts combination is potential to cause remarkable growth of *B. animalis* subsp. *lactis* BB-12 *in vitro* in liquid culture. The effect caused by the integrated prebiotic combinations do not reflect reactions of the bacteria in the intestine, but the *in vitro* growth stimulatory effect on probiotic bacteria might indicate the *in vivo* interaction of the prebiotic combinations and probiotic organisms. This integrated prebiotic combinations can be employed in neutraceutical development, since the merged prebiotic combinations are capable to cause remarkable proliferation of probiotics which can render beneficial physiological health benefits for host.

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