



SYNBIOTIC EFFECT OF JUICE MADE FROM GERMINATED ALFALFA SEEDS AND ALOE VERA ON IN-VITRO ACTIVITY OF PROBIOTIC LACTOBACILLI

Sports Science

Shilpa	Sports Nutritionist, Department of Sports Medicine, PGIMS, Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak, Haryana-124001.
Dr. Sushmita Kushwaha*	Assistant Professor, Department of Sports Medicine, PGIMS, Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak, Haryana-124001. *Corresponding Author
Divya Bhalla	Counselor, VLCC
Mandeep	Sports Psychologist, Department of Sports Medicine, PGIMS, Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak, Haryana-124001.
Dr Rajesh Rohilla	Head of Department, Department of Sports Medicine, PGIMS, Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak, Haryana-124001.

ABSTRACT

INTRODUCTION: Probiotics are micro-organism which upon ingestion in certain numbers exerts health benefit in gut beyond inherent general nutrition.

AIM: To study the synbiotic effect of juice made from germinated Alfalfa seeds and Aloe Vera on in-vitro activity of probiotic Lactobacilli.

METHOD: 5%, 25% and 50% concentration of samples prepared using 15g, 30g, and 45g of alfalfa seed and Aloe vera juice, incorporated into the growth media of two strains of lactobacilli (*L. acidophilus* and *L. plantarum*.)

RESULT: Samples with higher concentration showed lesser acidity. Sample with 5% concentration showed maximum growth of *L. acidophilus* and *L. plantarum* as evident from the lesser generation time and higher acidity. No statistically significant change in acidity or growth is seen in samples with different concentration of Alfalfa.

CONCLUSION: At a particular concentration, Aloe vera could possibly be used as a combinational therapy. Additionally, Alfalfa seeds has no major role on growth of probiotic bacteria.

KEYWORDS

Probiotic, Alfalfa, Aloe vera.

INTRODUCTION

Probiotics are live microorganism which upon ingestion in certain number expert health benefit beyond inherent general nutrition. The micro organisms must be present in high number, more than 10^8 - 10^9 probiotic microorganism in daily ingested dose. (1) Human digestive system contain million of bacteria which is important for digestion and immune function. The gut micro flora faces daily challenges that can cause an imbalance between the so-called; 'healthy' and pathogenic bacteria e.g. poor diet, use of antibiotics, stress and food poisoning. By eating foods with added probiotics, healthy bacteria that survive, transit through the gut and arrive in the large intestine is able to colonise there, thereby favorably altering the microbial balance.

Probiotics plays an important role in immunological, digestion and respiratory function and could have a significant effect in alleviating infections disease in children. They are known to improve lactose digestion in lactose mal digesters. (2) Probiotics especially lactobacillus have been shown to prevent infantile and antibiotics associated disorder. (3) Probiotics also have a role in management of patients of inflammatory bowel disease, constipation and urogenital tract infection. (4) There are 2 types of probiotics dairy based and non dairy based. Non dairy sources of production of probiotics includes fruits, vegetables, cereals, soya and Aloe vera.

Aloe vera due to its composition, is an excellent source to promote the growth of probiotic agents. (5) Most important properties of Aloe vera includes its anti-inflammatory, antibiotic, regenerative, antidiabetic and anti-cholesterolemic effects. (6) On the other hand Alfalfa seeds are good source of protein, along with 200 nutrients and phytonutrients. They benefits in reduction of bad cholesterol, promoting liver health, reduction of blood sugar level, relieving muscles and joints pain, alleviating menopausal symptoms. (7)

Both the Aloe vera and Alfalfa is highly nutritious, easily digestible

and economical. So we made a effort through present investigation to observe the activities of Lactobacilli in Aloe vera and Alfalfa juice that could possibly be exploited as a potential combinational remedy.

MATERIAL & METHODS

This was a prospective study, carried out in the department of Nutrition & Dietetics, faculty of applied science, Manav Rachna International University, Faridabad. Strains of Lactobacillus (*L. Acidophilus* and *L. Plantarum*) were procured from IMTECH Chandigarh. Aloe vera juice and Alfalfa seeds procured from the local vendors. Alfalfa seeds were germinated for 3 days at a temperature 37degree Celsius. 3 different samples with different concentration of Alfalfa seeds and Aloe vera juice were prepared. Sample A, B & C contained 15gm, 30gm & 45gm of alfalfa seeds respectively in 100ml of Aloe Vera juice in each. Strain of *L. acidophilus* and *L. plantarum* were activated in 100ml MRS broth separately.

After activation of strains, different concentration of Aloe vera juice i.e. 5%, 25% and 50% with different amount of Alfalfa seeds 15g, 30g and 45g were inoculated with different concentration of MRS both such that the total volume was kept 100ml in each sample. After inoculation samples were incubated in BOD incubator at 37degree Celsius for 48 hours. Chemical & microbial assessment of the samples were done by measuring the acidity & by measuring specific growth grate for early culture at different hours.

Statistic Analysis

Variables were measured at 0, 24 and 48 hours. Specific growth rate for each culture was calculated using the equation: $u = (\ln D_2 - \ln D_1) / (t_2 - t_1)$, where d_2 and d_1 are cell densities at time t_2 and t_1 respectively. Variables were compared using ANOVA test within the group. A p value of <0.05 is considered statistically significant.

RESULTS

Table 1: Acidity (%lactic acid) of Lactobacilli fermented MRS with different concentration of Alfalfa seeds

Time	Concentration of juices			f- value	p- value
	5%	25%	50%		
Acidity of <i>L. Acidophilus</i> fermented MRS using 15g Alfalfa seeds.					
0 hour	0.04+/-0.001	0.02+/-0.03	0.01+/-0.01	1107.22	0.01
24 hour	0.121+/-0.006"	0.51+/-0.057	0.51+/-0.057	474.50	0.01
48 hour	0.254+/-0.010	0.16+/-0.10	0.11+/-0.07	188.03	0.02

Acidity of <i>L.Acidophilus</i> fermented MRS using 30g alfalfa					
0 hour	0.03+/-0.01	0.10+/-0.01	0.05+/-0.10	1213.168	0.01
24 hour	0.12+/-0.064	0.10+/-0.01	0.81+/-0.01	1.564	0.284
48 hour	0.26+/-0.002	0.11+/-0.05	0.12+/-0.01	163.666	0.02
Acidity of <i>L.Acidophilus</i> fermented MRS using 45g alfalfa					
0 hour	0.04+/-0.001	0.06+/-0.059	0.04+/-0.01	637.083	0.02
24 hour	0.11+/-0.068	0.10+/-0.01	0.07+/-0.010	561.341	0.01
48 hour	0.24+/-0.010	0.21+/-0.010	0.17+/-0.01	420.231	0.02
Acidity of <i>L.plantarum</i> fermented MRS using 15g alfalfa					
0 hour	0.09+/-0.01	0.057+/-0.010	0.02+/-0.105	230.019	0.02
24 hour	0.17+/-0.001	0.13+/-0.015	0.118+/-0.01	60.647	0.01
48 hour	0.19+/-0.010	0.16+/-0.010	0.13+/-0.010	157.207	0.01
Acidity of <i>L.plantarum</i> fermented MRS using 30g alfalfa					
0 hour	0.12+/-0.01	0.01+/-0.071	0.15+/-0.01	6482.470	0.01
24 hour	0.16+/-0.010	0.12+/-0.01	0.06+/-0.01	147.502	0.01
48 hour	0.17+/-0.01	0.15+/-0.01	0.13+/-0.010	5425.000	0.02
Acidity of <i>L.plantarum</i> fermented MRS using 45g alfalfa					
0 hour	0.18+/-0.001	0.09+/-0.01bc	0.37+/-0.01	1.00	0.422
24 hour	0.16+/-0.01	0.12+/-0.038	0.06+/-0.06	4384.926	0.01
48 hours	0.17+/-0.01	0.14+/-0.01	0.11+/-0.01	6152.336	0.02

Table 1 shows that the acidity in MRS fermented with *L.acidophilus* and *L.plantarum* using 15g, 30g and 45g of alfalfa seeds. It shows that the acidity decreases with increase in concentration of juices. Whereas with increase in incubation time acidity of sample with varying concentration (5%, 25% and 50%) increases. This gives statistically significant results between concentration of juices and time of incubation.

The generation time for *L.acidophilus* using different concentration of Aloe vera juice, 5%, 25% and 50% was 40, 90 and 180 minutes respectively for sample A, 42, 88 and 192 minutes for sample B and it was 39, 92 and 200 minutes for sample C. Whereas generation time for *L.plantarum* using different concentration of Aloe vera juice, 5%, 25% and 50% was 55, 98 and 200 minutes respectively for sample A, 52, 96 and 198 minutes for sample B and it was 52, 98 and 202 minutes for sample C.

DISCUSSION

This research highlights on the symbiotic effects of juice made from germinated Alfalfa seeds and Aloe vera on in-vitro activity of Probiotic Lactobacilli. Probiotics present in the market are mainly milk based. But due to vegetarianism, milk cholesterol content and lactose intolerance, some people are not able to consume it. Previous study by Vasudha et al. highlights the use of probiotic beverages from non dairy sources. (8) Tamminen et al., Kyung et al. and Pakbin et al. have used carrot juice, red beets and peach juices respectively, as an alternative carrier for probiotics and found them to be a promising carrier for probiotic Lactobacilli. (9,10,11)

Nagpal et al. in his study innovated the concept of merging both probiotics and Aloe vera as a prospective combinational therapy for its anti-cholesterol and anti-diabetic effect. They found that at a particular concentration Aloe vera could promote the growth of probiotic Lactobacilli. (12) Dixit et al. in their study evaluated the effect of Alfalfa seeds on chicks and found that feeding Alfalfa seeds extract to chick resulted in significant reduction of total cholesterol, phospholipid, triglyceride and LDL cholesterol. (13)

Because of great health benefits of Aloe vera as well as Alfalfa seeds, these are used as the substrate to evaluate the activity of probiotic lactobacilli. An effort was made through present investigation in order to assess the activities of Lactobacilli in Aloe vera and alfalfa juice that could possibly be exploited as a potential combinational remedy.

Present study showed that by increasing concentration of Aloe vera in the samples, their acidity decreases. It is due to increase in the production of some antimicrobial metabolites (short chain organic acid) that could possibly have inhibited the bacteria. But there is no major change in the growth of Lactobacillus by adding different concentration of Alfalfa seeds. It could be because the nutrients uptake by bacteria was more from Aloe vera compared to alfalfa seeds. This favours the utility of Aloe vera as a potential carrier for Probiotic growth compared to Alfalfa seeds. These ingredients are easily available in the local market and low in cost so people from all strata can easily consume it as a part of their daily intake and improve gut microflora.

CONCLUSION

It is seen that there is no major change on growth of probiotic bacteria by addition of different amount of Alfalfa seeds. It is therefore concluded that at a particular concentration i.e. 5% Aloe vera juice, *L.acidophilus* and *L.plantarum* are more viable and thus Aloe vera could possibly be used as a combinational therapy. However, more in-vitro as well as in-vivo studies such as strain specific effect, effect of purified components etc. followed by animal trails are pre-requisites before authenticating and endorsing this kind of combinational therapy and their health claims.

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