Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(3): 300-306
Received: 13-02-2021
Accepted: 25-03-2021

Selvamuthukumaran M
Biochemistry and Nutrition Division, Defence Food Research Lab, Mysore, Karnataka, India

Corresponding Author:
Selvamuthukumaran M
Biochemistry and Nutrition Division, Defence Food Research Lab, Mysore, Karnataka, India

Evaluation of *in vitro* and *in vivo* prebiotic activity of seabuckthorn products on strains: *Lactobacillus acidophilus* and *Bifidobacterium bifidum*

Selvamuthukumaran M

Abstract
The various seabuckthorn products were developed and tested for its prebiotic effect under *in vitro* and *in vivo* experimental conditions using probiotics *viz*., *Lactobacillus acidophilus* and *Bifidobacterium bifidum.* For assessing efficacy of prebiotic activity by *in vitro*, seabuckthorn products were incorporated at different concentrations *viz*., 0%, 12.5% and 25% using reconstituted skimmed milk as a culture media. For evaluating prebiotic activity by *in vivo*, rats were fed with various seabuckthorn products for a period of one month. The analysis shows that products found to possess prebiotic component of dietary fibres. The *in vitro* result shows that the products exhibited more prebiotic activity at incorporation level of 25%, when compared to commercial prebiotic product honey. The *in vivo* results shows that probiotic counts were significantly higher in colon and feces for pulp fed group, when compared to control group thereby exerting prebiotic activity.

Keywords: Prebiotics, dietary fibre, jam, juice powder, colon

Introduction
The prebiotics are non-digestible food components that may have several health benefits in addition to stimulation of the probiotic bacteria, which includes improved digestion, better gut health, lower stress response, higher immunity, lowers inflammation, obesity and cardiovascular disease etc. (Collins and Gibson, 1999; Tam *et al*., 2020) [4, 24].
Prebiotics hinder the host by promoting the several beneficial bacteria's which are resident of colon (Chowdury *et al*., 2015) [3]. There are four mechanisms, which are involved in hindering the host by prebiotics, the first one is the maintenance of gut epithelial barrier, second one is competitive exclusion of pathogenic organisms, third one is secretion of antimicrobial products and the last one is regulation of mucosal immune system (Collins and Gibson, 1999) [4].
The prebiotics will alter the composition of microflora, which are present in the intestine (Gibson *et al*., 1995; Gibson and Roberfroid, 1995; Kimura *et al*., 1997; Kruse *et al*., 1997) [6-7, 12-13] and therefore the bacterial activities, which are positive to the host, were enhanced and negative to the host health were diminished and they also have specific site for gut action (Parodi *et al*., 1999) [17].
Seabuckthorn (*Hippophae rhamnoides*), a spiny shrub growing widely around the temperate zone of Europe and Asia (Lu, 1992) [15]. The plant yield orange coloured berries, which found to contain rich source of dietary fibres. The fibres found to possess significant health effects and they may enhance the motility of gut (Markowiak and Slizewska, 2017) [16]. The fibres, which are non-digestible in the small intestine, they will enter the colon and here they are utilized by the several gut microflora as a fermentation substrate (Collins and Gibson, 1999) [4].
The research studies depicts that the fibre components plays a major role in enhancing the growth of several probiotic bacterial species, which are already resident of colon (Yadav *et al*., 2017) [28]. The fibre kind of prebiotics can enhance the growth of lactobacilli and bifidobacteria by providing food for the probiotics; it also helps to balance the pathogenic bacteria and toxins produced by them in the digestive tract, thereby stimulating the growth and activity of beneficial microbes in the gut (Chowdury *et al*., 2015; Yao *et al*., 2020) [3, 29].
Therefore the present study was conducted to assess, whether seabuckthorn derived fibre components can exert prebiotic activity by stimulating the growth of selective beneficial bacteria's *viz*., *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, under *in vitro* and *in vivo* experimental conditions.

Materials and Methods

Raw materials

Seabuckthorn berries were collected from Field Research Laboratory located at Leh, Himachal Pradesh, India. The other materials *viz.* sucrose, honey, citric acid, sodium benzoate and sodium bicarbonate were procured locally, while carrageenan, maltodextrin DE 10 and tricalcium phosphate were purchased from Himedia Laboratories Limited (Mumbai, India). All other chemical reagents were procured from Sigma Aldrich Chemicals Limited, Mumbai, India.

Development of seabuckthorn products

Processing of seabuckthorn berries

The berries were cleaned to remove foreign particles, washed and crushed in mixer grinder in order to obtain pulp and it was passed through stainless steel sieve of size 35 mesh. The pulp was pasteurized at 80°C for 35 min and used for prebiotic studies and also for further product development.

Seabuckthorn jam

The seabuckthorn jam (Kanwal *et al*., 2017) [11] was prepared by adjusting the pulp pH with sodium bicarbonate to 3.30. Then the pulp was boiled for 10 min followed by addition of sugar (80g/ 100g pulp) and carrageenan (3.4%), boiled till TSS of 69° brix is achieved. The product was packed in sterilized glass bottles and utilized for prebiotic studies.

Seabuckthorn squash

The seabuckthorn squash (Girdharilal *et al*., 2013) [9] was developed by blending citric acid incorporated sugar syrup with pulp to get final TSS of 40° brix. The sugar syrup was prepared by adding sugar (24.7g) and water (30.12 ml) followed by addition of citric acid (0.45g). Syrup was passed through strainer and the strained syrup was mixed with seabuckthorn pulp i.e. 28 ml. The product was packed in glass bottles and utilized for prebiotic studies.

Seabuckthorn fruit juice powder

The fruit juice powder (Abadio *et al*., 2004; Chegini and Ghobadian, 2005) [1-2] was developed by addition of maltodextrin i.e. 24% to the pulp taken. The slurry was incorporated with anticaking agent of Tricalcium Phosphate i.e. 2%. The blended slurry was dried in a spray dryer procured from (JISL Instruments Private Limited, Mumbai, India). The slurry was spray dried by using 165°C as an inlet air temperature and 70°C as an outlet air temperature with feed rate and air pressure of 27 rpm and 2.3 kg/cm^2, respectively. The vacuum pressure maintained during drying was 55 Hg. The product was packed in aluminium foil pouches and utilized for prebiotic studies.

Determination of prebiotic component

The amount of soluble dietary fibre (SDF), insoluble dietary fibre (IDF) and total dietary fibre (TDF) in different seabuckthorn products had been determined as per the gravimetric enzymatic method (Prosky *et al*., 1988) [20].

Starter culture propagation

The bacterial cultures *viz. L. acidophilus* and *B. bifidum* were collected from National Dairy Research Institute, Karnal, India. Skimmed milk was used as a culture medium for propagation of cultures. They were incubated at 37˚C for 12-13 h and stored at 5˚C.

In Vitro prebiotic activity of seabuckthorn products

Growth and enumeration of L. acidophilus and B. bifidum

The spray dried skimmed milk powder was used as the growth medium for bacteria *viz*., *L.acidophilus* and *B. bifidum* in order to assess prebiotic activity. The reconstituted skimmed milk (12%) was prepared, pasteurized at 70˚C for 15 min and cooled. The various seabuckthorn products *viz*., pulp, jam, squash, fruit juice powder and honey (commercially available prebiotic product) were adjusted to pH 6.0 by adding sodium bicarbonate, incorporated into 100 ml conical flask containing skimmed milk at two different levels i.e. 12.5 and 25.0%. The lactic acid bacterial culture *viz*., *L. acidophilus* and *B. bifidum* were added at the level of 2.5% to each flask and incubation was carried out at 37˚C until constant pH of 4.0 was reached (Sanz *et al*., 2005) [21]. The samples were transferred immediately to refrigerator for cooling and enumerated. The following analysis was carried out in triplicate for each sample. Total *L. acidophilus* count was obtained by inoculating petridishes with decimal dilutions in Tomato Juice Agar (TJA) and incubating at 37˚C for 24 to 48 h. For Total *B. bifidum* counts, the *Bifidobacterium bifidum* Agar (BBA) was used in place of TJA (Prajapati *et al*., 1987) [18].

In Vivo prebiotic activity of seabuckthorn products

Animals and diet

Permission was obtained from Institute Animal Ethics Committee (IAEC) for conducting the experiments. The compliance had been ensured with all regulatory requirements as per the ethical guidelines of Indian National Science Academy (INSA), New Delhi, India (Institute Animal Ethics Committee, 2010) [10]. The 48 male albino rats of 12 weeks old were selected for the study. The rats were chosen randomly i.e. six/group and totally they were divided into six groups *viz.* pulp, jam, squash, juice powder, honey and control. The control group rats were fed with the casein control diet i.e. 20g as per the diet prepared from various ingredients *viz*. casein (240g), corn starch (660g), groundnut oil & mineral mixtures (40g each) and vitamin & shark liver oil (10g each)/ kg of diet formulations, while the experimental rat groups were fed with various seabuckthorn products of 10 g along with casein control diet i.e. 10 g. The animals were caged and kept as per the laboratory animal guidelines. The rat food intake was recorded on daily basis.

Growth and enumeration of *L. acidophilus* and *B. bifidum*

The diets were given continuously for a period of one month for each groups and at the end of the study, the animals were sacrificed under anesthesia (Nembutal 50 mg/kg body weight). The colons were excised from each animal and 1g sample was homogenized with 9 ml normal saline for 5 min. Serial dilutions were prepared with peptone water solution in triplicate for each sample of colon (Prestamo *et al*., 2003) [19]. Faeces were collected from each group and they were subjected for microbiological evaluation as like colon. Total *L. acidophilus* count was obtained by inoculating petri dishes with decimal dilutions in TJA agar and incubating at 37˚C for 24 to 48 h. Total *B. bifidum* counts were obtained by inoculating petri dishes with decimal dilutes in BBA agar and incubating at 37˚C for 24 to 48 h (Prajapati *et al*., 1987) [18].

Statistical analysis

Triplicate analysis was carried out for all the experiments. Data's were computed in Microsoft Excel 2003 (Microsoft

Corporation, Washington, USA) for carrying out ANOVA (Analysis of variance).

Results and Discussion

Analysis of prebiotic component

The analysis of prebiotic component i.e. fibre content of various seabuckthorn products were shown in Table 1. The result shows that the unprocessed product i.e. pulp found to contain more amounts of soluble, insoluble and total dietary fibres when compared to the processed products. Among the various processed products, the jam exhibited more fibres followed by juice powder and squash, this may be because of more amount of incorporation of pulp during development of jam when compared to fruit juice powder and squash, which might have enhanced the concentration of these fibres to a greater extent for this processed product i.e. jam[23].

Table 1: Prebiotic content* of different seabuckthorn products

S. No	Name of prebiotic component	Seabuckthorn products			
		Pulp	Jam	Squash	Juice powder
1.	Soluble dietary fibre (%)	2.14 ± 0.34	1.09 ± 0.12	0.77 ± 0.10	0.95 ± 0.11
2.	Insoluble dietary fibre (%)	5.60 ± 0.66	2.40 ± 0.37	1.33 ± 0.20	2.12 ± 0.24
3.	Total dietary fibre (%)	7.74 ± 0.71	3.49 ± 0.42	2.10 ± 0.25	3.07 ± 0.30

*Mean ± SD of triplicate analysis

Evaluation of prebiotic activity of various seabuckthorn products (*in vitro*)

Effect of seabuckthorn products on L. acidophilus count

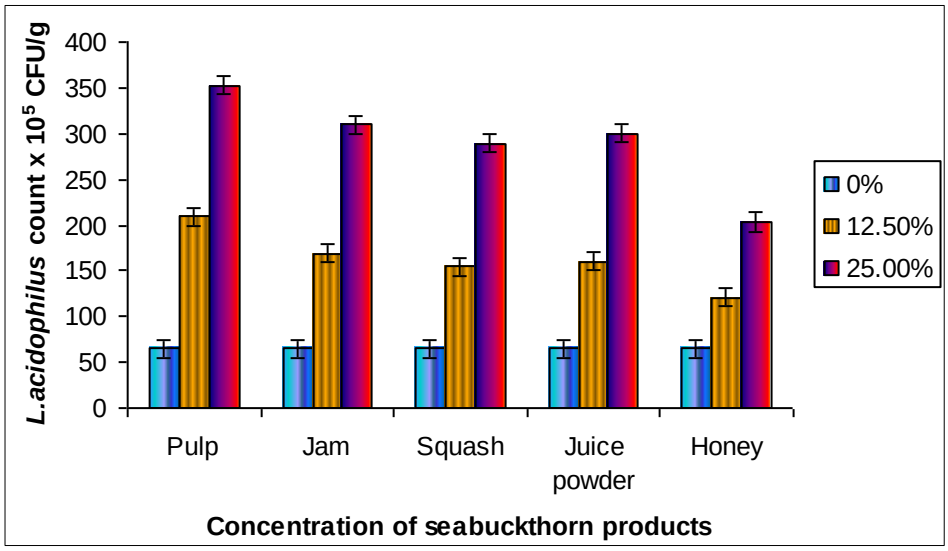
The effect of addition of seabuckthorn products to growth media on count of *L. acidophilus* was given in Fig.1A. The results show that the proliferation was found to be more for 25% level compared to 12.5% and control. Among the various seabuckthorn products incorporated one, the count of *L. acidophilus* was found to be high in pulp followed by jam, juice powder, squash and honey. The pulp showed an increase in *L. acidophilus* count of 3.2, 5.4 fold; jam of 2.6, 4.7 fold; squash of 2.3, 4.4; juice powder of 2.4, 4.6 and honey of 1.8, 3.1 fold at 12.5 and 25.0% concentrations respectively. The increase was found to be significant at the level of $p<0.05$.

The effect of addition of seabuckthorn products to growth media on count of *L. acidophilus* showed an increase pattern, which might be due to presence of fermentable dietary fibres like polysaccharides *viz*. dextrin, gums, glycogen and starch in seabuckthorn products (Tam *et al*., 2020) [24] that might have exerted a prebiotic effect by stimulating the growth of this beneficial bacterium in the skimmed milk media (Collins and Gibson, 1999) [4]. Since the proliferation of *L. acidophilus* count was noticed more for skimmed milk media incorporated with all seabuckthorn products by *in vitro*, therefore, based on these observation one can potentially say that the products will exert postulated beneficial effects after regular consumption by stimulating the growth and activity of specific lactic acid bacteria i.e. *L. acidophilus* in the gut (Ziemer and Gibson, 1998) [31].

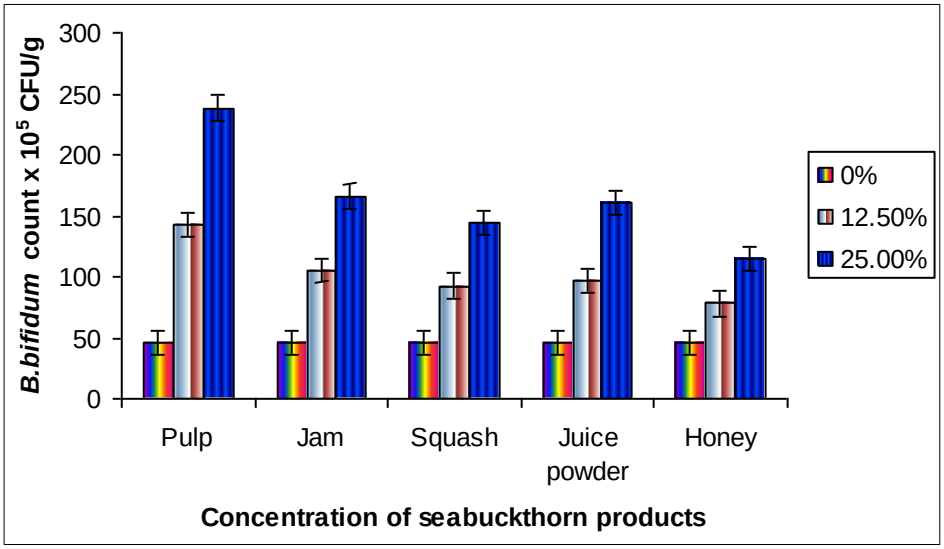
Effect of seabuckthorn products on *B. bifidum* count

The effect of addition of seabuckthorn products to skimmed milk media on the total count of *B. bifidum* was given in Fig.1B. The seabuckthorn pulp pronounced higher increase in *B. bifidum* count of 5.2 fold followed by jam of 3.6 fold, juice powder of 3.5 fold, squash of 3.1 fold and honey of 2.5 fold when compared to control at higher concentration levels i.e. at 25%. Whereas at 12.5% concentration levels, the pulp showed an increase of 3.1 fold; jam, squash, juice powder and honey of 2.3, 2.0, 2.1 and 1.7 fold of *B. bifidum*, respectively. The increase in the total count of *B. bifidum* was found to be significant at level of ($p<0.05$).

The effect of addition of seabuckthorn products to skimmed milk media on the total count of *B.bifidum* also showed similar pattern of increase as like *L. acidophilus*, this might be due to presence of more amount of prebiotic component *viz*., fermentable dietary fibre in higher level incorporated seabuckthorn products (Dongowski, 1996) [5]. As quoted earlier in previous research findings, this product also exerts a similar prebiotic effect by stimulating the growth/activity of *B. bifidum* in the gut after regular consumption (Van Loo *et al*., 1999) [27]. The findings from other research work on in vitro prebiotic studies, which had shown that gut microbiota may be successfully modified by using various prebiotic substrates *viz*. starch (Ze *et al*., 2012) [30], potato peel and bamboo shoots (Thakur *et al*., 2018) [26].



(A)



(B)

Fig 1: Effect of addition of seabuckthorn products on *L. acidophilus* (A) and *B.bifidum* (B) count at pH 4.0

Evaluation of prebiotic activity of seabuckthorn products (*in vivo*)

Food intake

The Fig.2 shows the recorded food intake of various rat groups. The rats consumed seabuckthorn products well with insignificant food intake difference when compared to control group.

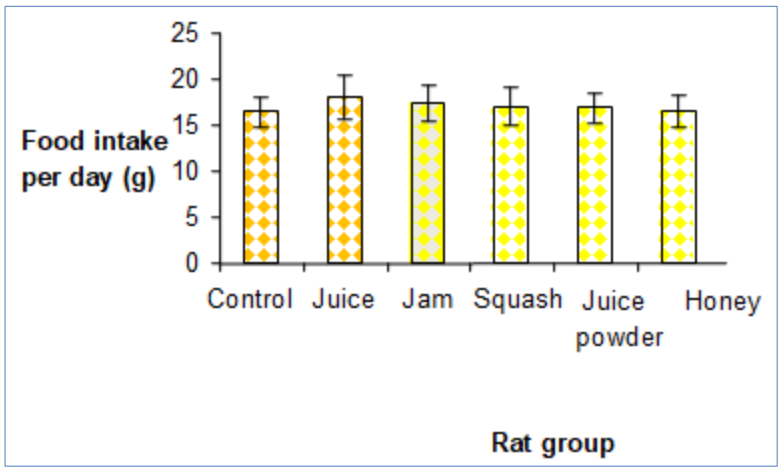


Fig 2: The mean average food intake of various rat groups/day during feeding of various seabuckthorn products

Effect of feeding of seabuckthorn products on *L. acidophilus* and *B. bifidum* count in colon

The effect of feeding of seabuckthorn products on *L. acidophilus* and *B. bifidum* count in rat colon was given in Fig.3 (A and B). The bacterial count was significantly ($p<0.05$) high in all the test groups compared to control. However, the seabuckthorn pulp group exhibited the highest count increase of 18.9 fold followed by jam group of 14.6 fold, juice powder and squash group of 13.4 and 12.4 fold and honey group of 9.5 fold over the *L. acidophilus* counts in control group. The *B. bifidum* count also followed a pattern similar to that of *L. acidophilus* with 17.2, 12.0, 10.9, 9.9 and 7.7 fold increases in the counts with pulp, jam, juice powder, squash and honey over the counts in control samples.

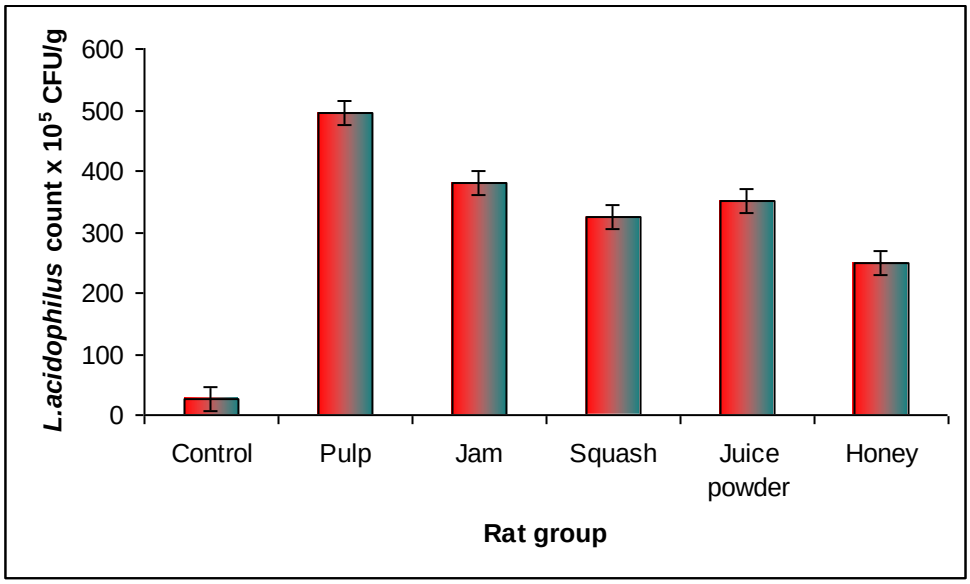
The effect of feeding of seabuckthorn products on *L. acidophilus* and *B.bifidum* count in rat colon showed significant increase, this may be due to presence of prebiotic component i.e. dietary fibres in test products incorporated diet, which might have exerted the prebiotic effect in colon by enhancing the total above bacterial counts *viz*., *L. acidophilus* and *B. bifidum* after continuous feeding (Dongowski, 1996) [5].The increase in intestinal beneficial bacterial counts of lactobacilli and bifidobacteria after continuous feeding of buckwheat for a period of 30 days were reported (Prestamo *et al*., 2003) [19]. The *in vivo* prebiotic effect of almonds were justified by feeding the almonds to rats for a period of one month, which showed significant growth stimulation of both *L. acidophilus* and *B. breve* species in colon and further it also enhanced the metabolic activities in rats (Liu *et al*., 2016) [14]. The similar effect was also achieved by feeding garlic among rats, which had enhanced the growth and activity of strain *L. acidophilus* in colon, which may be ascribed to presence of prebiotic component i.e. fructooligosaccharides (Sunu *et al*., 2019) [23].

Based on the above research findings, it can be observed that the various seabuckthorn products exerted prebiotic effect, which can be obtained from the sources of dietary fibres and it beneficially affected the host by selectively stimulating the growth and activity of bacteria species *viz*., *L. acidophilus* and *B. bifidum* that are already resident in the colon (Gibson and Wang, 1994) [8].

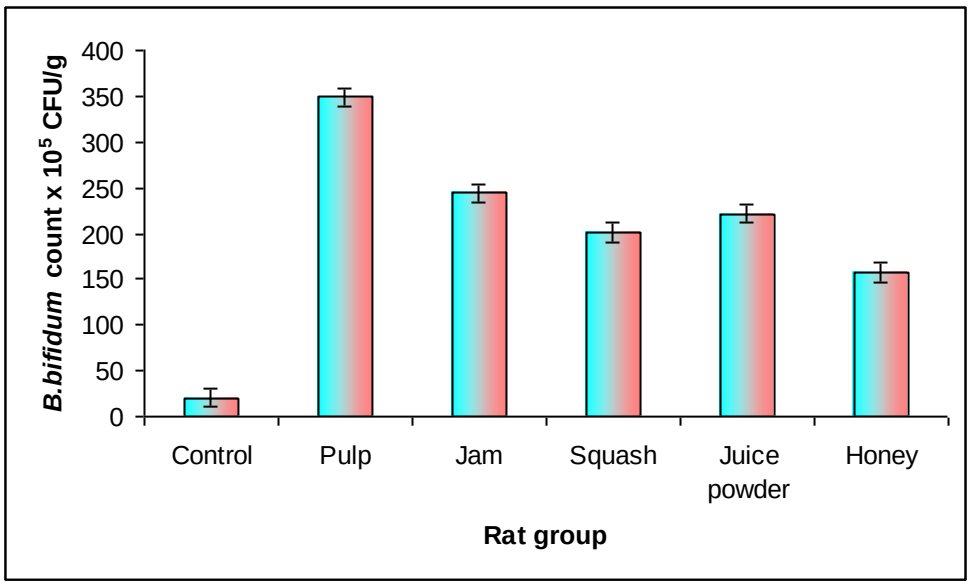
L.acidophilus and B.bifidum count in faeces

The Fig.3 (C and D) shows the *L. acidophilus* and *B. bifidum* count in faeces of rats fed control diet and diets with seabuckthorn products. The pulp fed rat group showed higher *L. acidophilus* count in faeces followed by jam, juice powder, squash, honey and control groups after continuous feeding for 30 days with 15.0, 11.4, 10.4, 9.1 and 6.0 fold increases, respectively. The *B. bifidum* count also showed a pattern similar to that of *L. acidophilus* of 12.6, 8.8, 8.2, 7.1 and 5.1 fold increases in the counts with pulp, jam, juice powder, squash and honey over the counts in control group. The increase in count of *L. acidophilus* and *B. bifidum* was found to be significant at level of $P<0.05$.

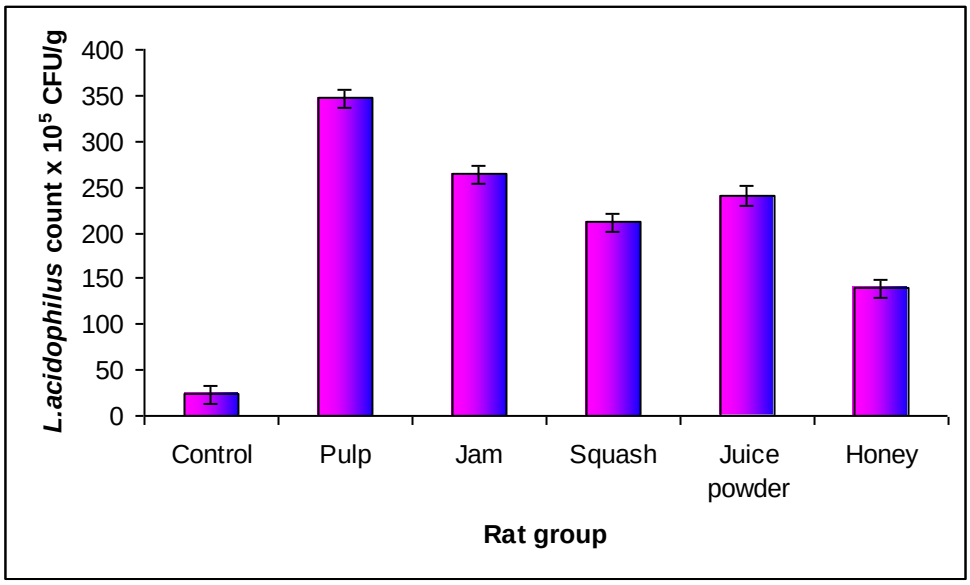
Seabuckthorn products fed group exhibited more bacterial faecal counts of *L. acidophilus* and *B.bifidum* when compared to control group. This may be because of intake of dietary fibre from incorporated diet containing various seabuckthorn products, which might have enhanced the *L. acidophilus* and *B.bifidum* counts (Dongowski, 1996) [5]. The result of metabolic conversion in the body leads to excretion of some bacteria's through faeces, which might have enhanced the faecal population with above said bacteria to a higher extent (Tannock, 2002) [25]. Based on the results, it has been depicted that these products may exert a prebiotic effect, ascribed due to presence of dietary fibre. This fibre may enhance metabolization process in a smooth manner (Simon and Gorbach, 1984) [22]. It also helps in increasing the biomass and faecal/stool bulking and also at the same time by significantly increasing the above bacterial counts in the faecal bulk (Gibson and Wang, 1994) [8]. The increase in faecal beneficial bacterial counts of lactobacilli and bifidobacteria after continuous feeding of buckwheat for a period of 30 days were reported (Prestamo *et al*., 2003) [19].



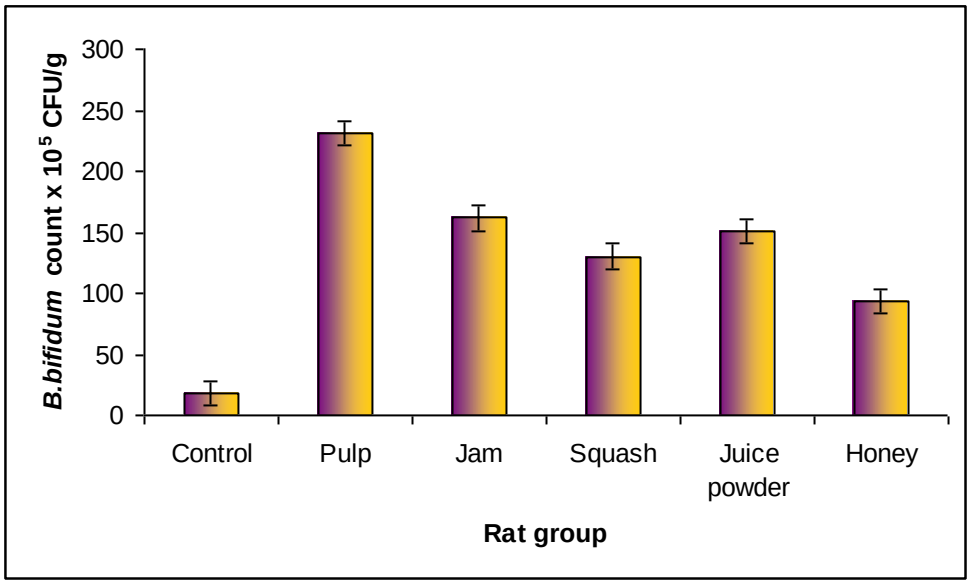
(A)



(B)



(C)



(D)

Fig 3: Effect of feeding of seabuckthorn products on *L acidophilus* and *B.bifidum* count in rat colon (A-B) and faeces (C-D) after 30 days

Conclusions

Based on the findings of the *in vitro* study, it was precluded that this seabuckthorn products had exerted prebiotic activity by incorporation at the level of 25%. The in vivo prebiotic effect may be ascribed due to presence of prebiotic promoter i.e. dietary fibre in various fed seabuckthorn products, which had selectively stimulated the growth of bacterial species *viz*., *L. acidophilus* and *B. bifidum*, which are already resident in rat colon. Therefore regular consumption of prebiotic foods derived from seabuckthorn may exert prebiotic effect by augmenting the beneficial bacterial counts *viz. L. acidophilus* and *B. bifidum*, in the colon, which may further improve the digestion and enhance immunity for the consumers. The *in vivo* study also further depicts that, it also enhanced the metabolization process to a greater extent as a result of increasing the biomass and stool bulk by exerting above activity. Therefore this product can not only alter the gut microflora but it also maintains gut health significantly. The consumers may reap health benefits by including seabuckthorn based prebiotic products as part of their daily diet.

Acknowledgement

Defence Research and Development Organization is greatly acknowledged for providing fund from project DIP-233 for this research work.

Conflict of Interest

The authors don't have any conflict of interests.

References

1. Abadio FDB, Domingues AM, Borges SV, Oliveira VM. Physical properties of powdered pineapple (*Ananas comosus*) juice-effect of maltdextrin concentration and atomization speed. J. Food Eng 2004;64:285-287.
2. Chegini RG, Ghobadian B. Effect of spray drying conditions on physical properties of orange juice powder. Dry Technol 2005;23:657-668.
3. Chowdury S, Das S, Bhattacharjee D, Saha PK, Mukherjee S, Bhattacharyya BK. Prebiotic-clinical relevance. Indian J. Nat. Prod. Resour 2015;6:91-97.
4. Collins MD, Gibson GR. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial

ecology of the gut. Am. J. Clin. Nutr 1999;69:S1052-S1057.
5. Dongowski G. Investigation on polysaccharide, pectin and dletary fiber fractions of buckthornton berries. Z Lebensm Unters Forsch 1996;203:302-308.
6. Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 1995;108:975-982.
7. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr 1995;125:1401-1412.
8. Gibson GR, Wang X. Enrichment of bifidobacteria from human gut contents by oligofructose using continuous culture. FEMS Microbiol. Lett 1994;118:121-128.
9. Girdharilal G, Sidappa S, Tandon GL. Preservation of Fruits and Vegetables. Indian Council of Agricultural Research Publications, New Delhi 2013,124.
10. Institute Animal Ethics Committee (IAEC), Guidelines on the Regulation of Scientific Experiments on Animals. Indian National Science Academy, New Delhi, India 2010,9-21.
11. Kanwal N, Randhawa MA, Iqbal Z. Influence of processing methods and storage on physico-chemical and antioxidant properties of guava jam. Int. Food Res. J 2017;24:2017-2027.
12. Kimura K, McCartney A, McConnell MA, Tannock GW. Analysis of fecal populations of bifidobacteria and lactobacilli and investigation of the immunological responses of their human hosts to the predominant strains. App. Environ. Microbiol 1997;63:3394-3398.
13. Kruse HP, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. Brit. J. Nutr 1997;82:375-382.
14. Liu Z, Wang W, Huang G, Zhang W, Ni L. In vitro and in vivo evaluation of the prebiotic effect of raw and roasted almonds (*Prunus amygdalus*). J. Sci. Food Agric 2016;96:1836-1843.
15. Lu R. Seabuckthorn: A Multipurpose Plant Species for Fragile Mountains. International Centre for Integrated Mountain Development, Kathmandu 1992,1.
16. Markowiak P, Slizewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 2017;9:1021.
17. Parodi PW. The role of intestinal bacteria in the causation and prevention of cancer: modulation by diet and probiotics. Aust. J. Dairy Technol 1999;54:103-121.
18. Prajapati JB, Shah RK, Dave JM. Survival of *Lactobacillus acidophilus* in blended-spray dried acidophilus preparations. Aust. J. Dairy Technol 1987;42:17-21.
19. Prestamo G, Pedrazuela A, Penas E, Lasuncion MA, Arroyo G. Role of buckwheat diets on rats as prebiotic and healthy food. Nutr. Res 2003;23:803-814.
20. Prosky L, Asp NG, Schweizer TF, Devries JW, Furda I. Determination of insoluble, soluble, and total dietary fiber in foods and food products: inter laboratory study. J. AOAC Intl 1988;71:1017-1023.
21. Sanz ML, Polemis N, Morales V, Corzo N, Drakoularakou A, Gibson GR *et al*. In vitro investigation into the potential prebiotic activity of honey oligosaccharides. J. Agric. Food Che 2005;53:2914-2921.
22. Simon GL, Gorbach SL. Intestinal flora in health and disease. Gastroenterology 1984;86:174-193.
23. Sunu P, Sunarti D, Mahfudz LD, Yunianto VD. Prebiotic activity of garlic (*Allium sativum*) extract on *Lactobacillus acidophilus*. Veterinary World 2019;12:2046-2051.
24. Tam CCL, KM, Cheng LW. Prebiotics, probiotics and bacterial infections. In Franco Robles E and Ramirez Emiliano J (eds) Prebiotics and probiotics: Potential benefits in nutrition and health, Intech Open, London, UK 2020.
25. Tannock G. Methodologies for quantification of faecal bacteria: application to prebiotic effects. Br. J. Nutr 2002;87:S199-S201.
26. Thakur K, Xu GY, Zhang JG, Zhang F, Hu F, Wei ZJ. In vitro prebiotic effects of bamboo shoots and potato peel extracts on the proliferation of lactic acid bacteria under simulated gut conditions. Front. Microbiol 2018;9:2114.
27. Van Loo J, Cummings J, Delzenne N, Englyst H, Franck A, Hopkins M *et al*. Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). Brit. J. Nutr 1999;81:121-132.
28. Yadav SA, Gite SS, Nilegaonkar SS, Agte VV. Comparitive functional potential of some plant materials in India. Indian J. Nat. Prod. Resour 2017;8:159-166.
29. Yao M, Xie J, Julian D, McClements, Xiao H, Li L. Progress in microencapsulation of probiotics: a review. Compr. Rev. Food Sci. F 2020;19:857-874.
30. Ze X, Duncan SH, Louis P, Flint HJ. *Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon. ISME J 2012;6:1535-1543.
31. Ziemer CJ, Gibson GR. An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. Int. Dairy J 1998,473-479.