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***Research paper***

# *Evaluating the Prebiotic Potential of Lepidium Sativum Seed Mucilage as a Nutraceutical Ingredient*

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ABSTRACT

*The current study investigates the prebiotic potential of Lepidium sativum L. seeds mucilage on different lactobacilli strains. The quantification of the total reducing sugar in the mucilage was attained by Ultraviolet-Visible Spectrophotometric method. The mucilage exhibited efficient resistance against α-amylase and artificial gastric juice hydrolysis compared to standard prebiotic inulin. The mucilage also exhibited efficient activity necessary for the augmentation of almost all strains of lactobacilli. In vitro studies also exhibited that lactobacilli amount was at par to standard prebiotics ($p<0.05$) in the medium supplemented with the mucilage. The study led to conclude that the seed mucilage of Lepidium sativum exhibits efficient prebiotic activity and thereby can be used as a potential functional nutraceutical supplement.*

***Keywords:*** *Prebiotic; Mucilage; Lactobacillus; Gastric juice; Amylase; Hydrolysis*

## *Introduction*

The human gastrointestinal tract (GIT) is a kinetic micro-ecosystem that enables normal physiological functions of host organism unless harmful and potentially pathogenic bacteria dominate it [1].

The human gastrointestinal tract has been reported to be resident for 1010-1012 live microorganisms per gram in the human colon [2]. These residential microbes in the stomach, small, and large intestine are crucial for human health. Human diet, particularly the non-digestible carbohydrates is the chief source of energy for the growth of these microbes [3]. The non-digestible carbohydrates, called as the prebiotics, are fermented by intestinal microbes to obtain their survival energy [4,5]. The prebiotics selectively enhance the survival and growth of the restricted number of gut microbes thus establishes a balanced colonial microflora [6]. By definition, a prebiotic is a "substrate that is selectively utilized by host microorganisms conferring a health benefit" [7]. In recent years, the effects of probiotics and prebiotics on human health are of great interest to both consumers and food manufacturers. Many efforts have been made to develop novel functional foods or preparations containing probiotics and prebiotics. The combinations of probiotics and prebiotics in nutritional supplements in a form of synergism are called synbiotics. It is stated that systematic supplementation of the diet with probiotics, prebiotics or synbiotics may ensure maintaining a proper equilibrium of the microflora in the GIT [8]. A rapid growth is witnessed in the number of products that claim to affect the functions and composition of the microbiota at different body sites to benefit human health [9].

Different genera of microbes particularly, bifidobacteria, lactobacilli, and yeast are being used as a potential source of probiotics which helps to maintain the gut environment and improve the immune system [10]. Among all, lactobacilli and bifidobacteria are the main members of the human intestine ranging about 25 % of the total number of gut microbiota. Therefore, lactobacillus and bifidobacterium genera are being the most significant probiotic strains for human use [11].

Prebiotics are naturally present in various fruits, and vegetables [12,13]. As earlier reported, dietary polyphenols and there by-products from microbial degradation can stimulate particular bacterial populations present in the human gut [14].

Lepidium sativum is a medicinal plant and can be used as an essential drug to improve mother and child health as an abundant source of calcium and phosphorus. The seeds of the plant are reportedly used as diuretic, tonic, demulcent, carminative, galatogogue, emmenagogue, to cure throat diseases, uterine tumour, nasal polyps and breast cancer [15]. The seeds have been reported to contain proteins, carbohydrates, lipids, phenolics, tannins, flavonoids and fibers [16]. Mucilages are mucopolysaccharides produced in early diverging non-vascular plant groups. They are composed of total, acidic or neutral polysaccharides or heteropolysaccharides. Mucilage is well recognized as a prebiotic functional food that can positively affect human intestinal microbiota, leading to the modulation of bowel habits concurrent with the reduction of several ailments, i.e., intestinal tumors. The potential of mucilage as a prebiotic is attributed to its polysaccharide nature, where the high content of soluble heteropolysaccharides, the main progenitor of short chain fatty acids (SCFAs), in mucilage helps to promote the growth of beneficial gut probiotic bacteria [17].

In the present work, we have attempted to examine the prebiotic potential of mucilage extracted from the seeds of Lepidium sativum by studying its effect on the growth of Lactobacillus along with its ability to resist the hydrolysis by gastric juice and α-amylase.

## *Material and Methods*

The seeds of Lepidum sativum were purchased from Vaidya Balmukand and Sons, Ayurvedic and General store, Solan (H.P.), India and Identification and confirmation were done by Department of Botany Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.) India where voucher specimens were deposited with the Herbarium no. Bot/2713. The purchased seeds of the plant were air-dried. The dried seeds sample was crushed to small piece using Mortar and Pestle and grinded using electrical sample miller.

The Lepidium sativum seeds (100 g) were soaked for 12 h in distilled water (1litre). Then mucilage was separated by passing through vacuum pump. After that remaining particulate matter separated by passing through muslin cloth. The separated clear material was treated with 15 mL acetone and allowed to stand for 30 min precipitate the mucilage. The mucilage was dried in hot air oven at 60°C for 16 h [18]. Then powder was passed through 80# mesh sieve and weighed to calculate the yield.

### Test organism

A total of three lactobacilli strains were investigated namely Lactobacillus acidophilus MTCC 10307, Lactobacillus rhamnosus MTCC 1423 and Lactobacillus fermentum MTCC 903 were purchased from Institute of Microbial Technology, Chandigarh in the form of lyophilized culture.

### Estimation of total sugar

The total sugar content in the mucilage was determined using copper reduction method utilizing Lane and Eynon procedure involving titration of Fehling's reagent [19].

### Estimation of reducing sugar

The reducing sugar content in the mucilage was determined using Nelson-Somogyi method [20]. Pipette out aliquots of 0.1 or 0.2 ml and 1.0 ml of the mucilage in separate test tubes label. Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard into separate tubes and label. Using distilled water make up the volume to 2 ml in both tubes of sample and standard. Set up a blank in another tube with 2 ml water blank. Add 1.0 ml of alkaline copper tartarate to each tube and place all the tubes in boiling water bath for 10 minutes. Cool the tubes and add 1.0 ml of arsenomolybdic acid to all the tubes. Make up the volume to 10 ml with distilled water in all the tubes. After 10 minutes, read absorbance of the blue colour developed at 620 nm. Plot a graph with µg of sugar against absorbance and calculate the amount of reducing sugar present in the mucilage.

### Determination of gastric juice hydrolysis activity

Acid resistance of various dried plant extracts was carried out along with inulin and FOS as a prebiotic reference. The hydrochloric acid buffer (g/l) was mimicked as an artificial human gastric juice: $NaH_2PO_4$, 14.35; $CaCl_2.2H_2O$, 0.1; KCl, 0.2; NaCl, 8; $Na_2HPO_4.2H_2O$, 8.25; and $MgCl_2.6H_2O$, 0.18. The pH of the buffer was maintained at pH 1 using 5 M HCl [21]. The sample was prepared by dissolving the mucilage as 1% (w/v) in water. Artificial gastric juice (5 ml) was added to the sample solution (5 ml) with further incubation for 6 h at 37±2°C in a water bath. The estimation of total and reducing sugar was done at both 0 and 6 h [22]. The percentage hydrolysis of the mucilage was estimated as the reducing sugar released and the total sugar content of the sample as below:

$$\text{Hydrolysis } (\%) = \frac{\text{Reducing sugar}}{\text{Total sugar} - \text{Initial reducing sugar}} \times 100$$

### Determination of α-amylase hydrolysis activity

For enzymatic hydrolysis α-amylase, 2 units $mL^{-1}$ was prepared in sodium phosphate buffer (20 mM) adjusted to pH 6.9 using 6.7 mM of sodium chloride [23]. The sample was made as 1% (w/v) of mucilage dissolved in the buffer. The sample solution 5 ml was further incubated with 5 ml of enzyme solution at pH 6.9 at 37± 2°C for 6 h. Enzymatic hydrolysis was estimated by the evaluation of total and reducing sugar in the sample. The percentage of hydrolysis was estimated:

$$\text{Hydrolysis } (\%) = \frac{\text{Reducing sugar}}{\text{Total sugar} - \text{Initial reducing sugar}} \times 100$$

### Prebiotic potential of plant leave extracts

Mucilage with inulin as standard was used as the source of carbon to stimulate the augmentation of different probiotic strains. Various probiotics strains i.e., Lactobacillus acidophilus MTCC 10307, Lactobacillus rhamnosus MTCC 1423 and Lactobacillus fermentum MTCC 903 were grown on MRS broth for 24 h at 37 ± 2°C. The prebiotics were tested against the 5 ml of mucilage solution (0.5 and 1%, w⁄v) and sterilized by passing through a membrane filter of 0.45 µm pore size (Millipore). MRS broth (Carbohydrate free) was used as a basal growth medium. The activated bacterial culture (1%) was transferred into basal growth media along with mucilage and standard prebiotic (0.5 & 1% w/v). The broths were incubated at 37 ± 2°C in anaerobic conditions for 48 h. From this broth solution, the sample (0.1 ml) was withdrawn, and further cell count was obtained using the hemocytometer. The study was carried out by three replicates of each sample extracts [24,25]. Basal growth medium was used as the negative control while basal growth medium supplemented with 2% glucose was used as the positive control.

## *Results and Discussion*

### Mucilage extraction and proximate analysis

The mucilage form seed of Lepidium sativum was obtained as off-white, tasteless amorphous powder, off white in color with an 8% yield by weight. The proximate analysis revealed the presence of crude fats (2.37%), proteins (3.07%), fibers (4.71%) and carbohydrates (77.72 %) with the total calculated energy (287.62 kcal/g). Previously reported literature mentioned 24.18 % proteins, 28.03 % lipids, 32.87% carbohydrates and 6.75% fibers in the seeds of the plant. This reveals higher carbohydrate content in the mucilage that is in agreement with literature.

### Total Sugar and reducing sugar estimation

The presence of high percentage of carbohydrate relates to the ability of the plant to provide energy required to maintain physiological functions of the plant [26]. The total sugar in the mucilage was estimated using Copper reduction method. The reducing sugars play role as reducing agents and may be helpful in several pathological conditions. The reducing sugar content of the samples was analyzed by Nelson-Somogyi method. Table 1 shows the total sugar content and the reducing sugar content of the standard prebiotic and the mucilage.

**Table 1: Quantification of sugar content in samples**

| S. No. | Sample | Total Sugar Content (mg/g) | Reducing Sugar Content (mg/g) |
|---|---|---|---|
| 1 | Inulin | 87.67 ± 4.51 | 21.43 ± 0.80 |
| 2 | Mucilage | 182.33 ± 2.08 | 59.08 ± 3.95 |

*Results are average ± standard deviation; n=3*

### Hydrolytic effect of gastric juice

Artificial gastric juice (pH 1) was used to hydrolyze the mucilage as well as inulin. The acidic hydrolysis of inulin was found to be 7.26% while that of the extracted mucilage was found to be 12.89%. The extracted mucilage was able to resist the acidic hydrolysis. The incubation time of 6h was also in part responsible to hydrolysis of the mucilage as well as inulin allowing for conversion of polysaccharides to mono and di-saccharides. Since the mucilage was able to withstand about 90% hydrolysis, it could be assumed that it might reach the intestine surpassing the hydrolytic effect exhibited by the gastric juice in stomach.

### Hydrolytic effect of α-amylase

Apart from the acidic degradation, enzymatic hydrolysis in the stomach plays a vital role in conversion of the complex polysaccharides to simple carbohydrates. An active food ingredient that is not degraded in the upper gastrointestinal tract might be a good prebiotic candidate. The percent hydrolysis of the mucilage in presence of α-amylase was determined by quantifying the reducing sugar. The enzymatic (α-amylase) hydrolysis of inulin was found to be 11.34% while that of the extracted mucilage was found to be 10.73%. The mucilage was found to be even more

resistant to enzymatic hydrolysis in comparison to the standard prebiotic. Hence, the extracted mucilage presents a great potential to be a source of carbon in the gut microflora and establishing itself as a prebiotic.

**Table 2: Effect of prebiotic/mucilage on growth Lactobacillus strains.**

| S. No. | Prebiotic | Concentration (%) | Cell count ($Log_{10}$CFU/mL) | | |
|---|---|---|---|---|---|
| | | | ***Lactobacillus acidophilus*** | ***Lactobacillus rhamnosus*** | ***Lacotbacillus fermentum*** |
| 1 | Inulin | 0.5 | 0.97 ± 0.07 | 1.06 ± 0.10 | 0.92 ± 0.03 |
| | | 1 | 2.06 ± 0.07 | 2.12 ± 0.10 | 1.99 ± 0.05 |
| 2 | Mucilage | 0.5 | 0.92 ± 0.03 | 0.95 ± 0.05 | 0.75 ± 0.05 |
| | | 1 | 1.93 ± 0.03 | 2.01 ± 0.02 | 1.84 ± 0.03 |
| 3 | Negative Control | - | 0.15 ± 0.27 | 0.36 ± 0.10 | 0.10 ± 0.17 |
| 4 | Positive Control | - | 1.11 ± 0.07 | 1.31 ± 0.05 | 1.09 ± 0.08 |

Expressed as mean ± standard deviation; n=3

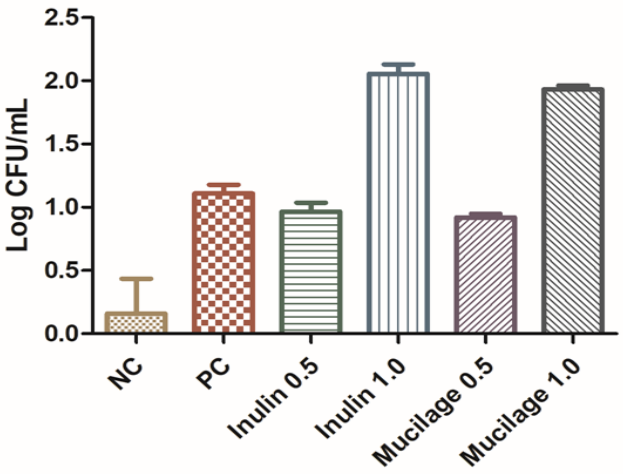


**Figure 1a**: Effect of various test solution on probiotic growth against L. acidophilus.

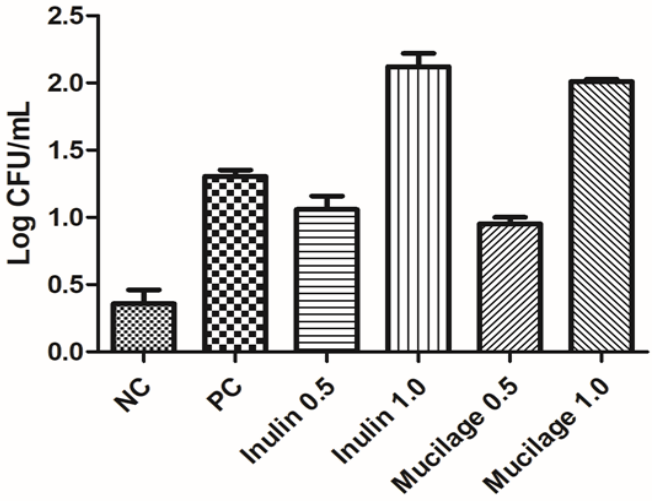


**Figure 1b**: Effect of various test solution on probiotic growth against L. rhamnosus.

**Prebiotic potential of mucilage**

The effect of the prebiotic on the growth of the probiotic strain was studied by counting the number of cells as colony forming units per mL of the prebiotic. The effect of the concentration of the prebiotic on growth of probiotic was also observed. Figures 1a to 1c and Table 2 represents the effect of prebiotic on growth of different probiotic strains.

The data was statistically analyzed using one way ANOVA followed by Dunnetts post-test. The results indicate that at all the concentrations, the mucilage was able to significantly promote the growth of the Lactobacillus strains in comparison to the basal growth medium ($P<0.05$). It was also observed that the prebiotic promoted the growth of Lactobacillus strains in varying degree. The highest growth was obtained for L. rhamnosus followed by L. acidophilus and the least for L. fermentum.

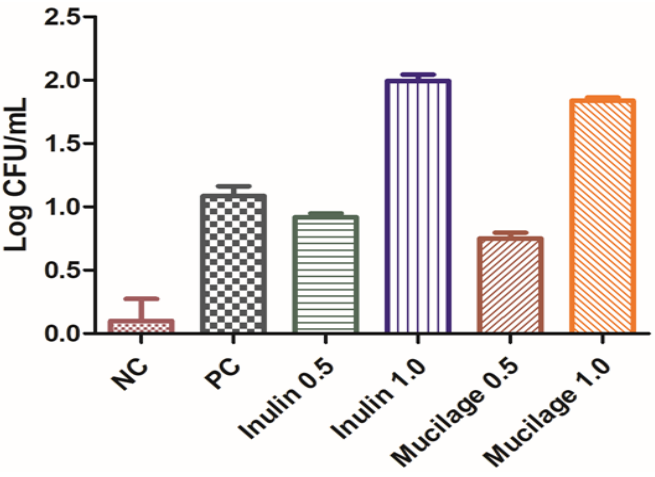


**Figure 1c**: Effect of various test solution on probiotic growth against L. fermentum.

The significantly improved growth of the probiotic strains could be attributed to the presence of sugars in the prebiotic. Higher levels of sugar variably cause a significant growth to probiotic [27]. Hence, the advent of nutraceuticals [28] and various approaches to develop them into potent deliver systems like use of liposomal technology [29,30], nano-emulsions [31,32] nanoparticulate systems [33,34] could help them achieve better clinical results.

## *Conclusion*

In the present study, the mucilage obtained from seeds of Lepidium sativum was extracted and compared with inulin for its prebiotic potential. The mucilage exhibited significant resistance to hydrolytic degradation in acidic pH (gastric juice) as well as by enzyme (α-amylase). The mucilage also improved growth of the probiotic bacterial strains supporting the theory that the mucilage could be of great use as a prebiotic and nutraceutical.

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## *Conflict of Interest*

The author declares no conflict of interest.

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