International Journal of Biomedical Investigation

journal homepage: https://ijbi.edwiserinternational.com

Original Article

Lepidium Sativum Mucilage: From Characterization to Prebiotic Assessment

*Archana** *and Suresh Choudhary*

Lords University, Alwar-Bhiwadi- Delhi Highway, Chikani, Alwar, Rajasthan, India

ARTICLE INFO

Received 15 January 2023
Revised 01 February 2023
Available Online 06 Feb 2023

*Corresponding author: Archana, Research Scholar, Lords University, Alwar-Bhiwadi- Delhi Highway, Chikani, Alwar, Rajasthan-301028, India.

ABSTRACT

Cress or garden cress (Lepidium sativum) has been widely reported for its numerous pharmacological activities. The cress seeds possess numbers of nutraceutical values from rubefacient, galactogogue, laxative to diuretic properties. In the current study mucilage is extracted from the seeds of Lepidium sativum by different methods and further purified exploiting the method of Munir et al. with slight modification. Chemical characterization confirmed the presence of carbohydrate, mucilage and polysaccharide. Proximate analysis confirmed the presence of crude fats, proteins, fibers and carbohydrates. FTIR analysis of Lepidium sativum mucilage shows presence of O-H, C-H and C=O functional group. We further investigated 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. Further stability studies confirmed the stability of L. sativum mucilage over a period of six months making it a potential prebiotic nutrient supplement and pharmaceutical excipient.

Keywords: *Lepidium sativum; Mucilage; Prebiotic; Lactobacillus*

Introduction

This shift towards herbal medication is attributed to their safety profile and the fact that the world's major medical systems like allopathy, homeopathy, ayurveda are directly or indirectly dependent on plant products [1]. Herbal medications are being extensively researched and promoted in various health care programmes in India. Scientist from across the globe are attracted towards the potential medicinal values of different herbs and are working to blend herbal medications with novel formulation technologies to overcome the difficulty associated in developing formulations based on herbal medications [2]. Different animals, plants, bacterial, algae fungi can also biosynthetically develop polysaccharides which are generally regarded as safe for possible human consumption. Among these polysaccharides obtained from plants have a wide range of application and thus have a major application in food industry [3]. Mucilage are complex polysaccharides composed of carbohydrates having highly branched and complex structure of different monomer units like L-rhamnose, D-xylose, L-arabinose, D-galactose and galacturonic acid. Tannins, alkaloids, glycoproteins and steroids are also chief components of mucilage. "Mucilage are sometimes referred to as gums however, both mucilage and gum are mostly related to hemicelluloses in composition, except the sugars produced by hemicelluloses such as xylose, glucose, and mannose

instead of sugars produced by the gums such as galactose and arabinose". Cress or garden cress (Lepidium sativum) has been widely reported for its numerous pharmacological activities. It is also known as mustard, pepperwort, pepper grass and are genetically related to watercress and mustard and have similar tangy, peppery flavour and aroma [4]. The cress seeds possess numbers of nutraceutical values from rubefacient, galactogogue, laxative to diuretic properties. The seed is rich in minerals including calcium, potassium, magnesium, phosphorus and iron. Raw cress also contains protein and other vitamins (i.e. vitamin K, vitamin C and vitamin A) and recommended when additional nutritional required e.g. in pregnancy [5]. 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 [6]. 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 [7].

Prebiotics are naturally present in various fruits, and vegetables [8,9]. As earlier reported, dietary polyphenols and there by-products from microbial degradation can stimulate particular bacterial populations present in the human gut [10].

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 [11]. The seeds have been reported to contain proteins, carbohydrates, lipids, phenolics, tannins, flavonoids and fibers [12]. 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 [13].

In the present work, we have attempted to characterize L. sativum extract and 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. 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.

Characterization of mucilage

Chemical characterization of Lepidium sativum mucilage

Different chemical tests like Molisch's test, iodine test was performed to confirm the presence of mucilage in extracted material.

Molisch's test

To the test solution few drops of alcoholic alpha napthol was added followed by addition of few drops of concentrated sulphuric acid through the side of test tube, a purple to violet color change was observed.

With ruthenium red

To the test solution a few drops of ruthenium red solution was added. A change in colour to pink was observed.

Iodine test

To the test solution a few drop of Lugol's solution was added which produces blue/purple colour indicating the presence of polysaccharide.

Proximate analysis

"Proximate analysis (protein, moisture, ash, fats and fiber contents) of untreated gum was performed according to the protocol as reported in Association of Official Analytical Chemists (AOAC) and by Galla and Dubasi" [14].

Physicochemical characterization of Lepidium sativum mucilage

Loss on drying

"An appropriate quantity of mucilage was weighed and dried at 105°C for 2 hours. After 2 hours it is again weighed to calculate the weight loss on drying, percentage loss of moisture on drying was calculated" [15]. Weight loss on drying was determined by formula:

Weight loss = Initial weight - Final weight

Percentage loss of moisture on drying (LOD) was calculated using the formula:

LOD (%) = (Weight of water in sample/Weight of dry sample) ×100

The difference in weight indicates the amount of moisture present in the material.

Particle size

The particle size of the dried-powder mucilage was determined by the microscopic method. In this method microscope was adjusted for maximum light. Further, calibration of eyepiece was done by using micrometre scale. A small of mucilage suspension was sprinkled on a clean slide and minimum of 300-500 particles was counted to determine average particle size.

pH of solution

The pH was measured in a pH meter by preparing 0.5% solution prepared with distilled water.

Charring

"A few milligrams of dried mucilage were placed in a melting-point apparatus. The temperature was taken and recorded when the material started to char" [15].

Swelling ratio

"The study was carried out using a 100 ml stoppered graduated cylinder. The initial bulk volume of 1 gm of dried mucilage was recorded. Further, water was added in sufficient quantity to yield 100 ml of a uniform dispersion. The sediment volume of the swollen mass was measured after 24 hour, stored at room temperature. The swelling ratio was calculated by taking the ratio of the swollen volume to the initial bulk volume" [15].

Flow property

"The flow properties and compressibility of the dried mucilage, including bulk and tapped density, Carr's index, the Hausner's ratio, and the Angle of repose was calculated as discussed below" [15].

Angle of repose

"Good flow properties are critical for the development of any pharmaceutical powder formulation. It is essential that an accurate assessment of flow properties be made as early in development process as possible so that an optimum formulation can be identified. Interparticle forces or forces between particle as well as flow characteristics evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface of pile of sample and horizontal plane" [16].

The fixed funnel and free-standing cone methods employ a funnel that is secured with its tip at given height, H, which was kept 2 cm, above graph paper that is placed on a flat horizontal surface. With r, being the radius of base of conical pile.

$$\tan\theta = h/r$$

Bulk and tapped density

"Bulk density can be defined as the mass of particles of the material divided by the total volume they occupy. The total volume includes particle volume, inter-particle void volume and internal pore volume. Bulk density is not an intrinsic property of a material; it can change depending on how the material is handled. Bulk density of powder is dependent upon particle size distribution, particle shape and tendency of particle to adhere to one another".

"A Pre-weighted, pre-sieved quantity of dried mucilage was poured into a graduated cylinder, and the volume was recorded. The cylinder was tapped until the powder-bed volume reached a minimum value and the tapped volume was recorded. The bulk and tapped densities were calculated by using tapped or bulk density or Apparent Volume Test Instrument - Type PT-TD200. It is measured in gm/ml".

Bulk density = Mass/Bulk volume

Tapped density = Mass/Tapped volume

Hausner's ratio= Tapped density/ Bulk density

Compressibility index

"It is also known as Carr's index. In which pre weighted, pre-sieved quantity of dried mucilage was poured into a graduated cylinder, and the volume recorded. The cylinder was tapped until the powder-bed volume reached a minimum value and the tapped volume was recorded. Lower the compressibility value indicate better flow" [17]. Carr's Index was calculated using the formula:

Compressibility index = Tapped density-Bulk density/ Tapped density

% Compressibility index = Compressibility index×100

Viscosity

"Rheological studies of dried mucilage were carried out using varying concentrations (0.1–0.5% w/v) prepared in distilled water. The viscosities were measured using a Brookfield viscometer" [17].

Fourier transforms infrared (FTIR) spectral studies

"Fourier transform infrared (FTIR) spectral data were taken on a Shimadzu (model FTIR-8300) instrument to find out chemical stability of the excipients. FTIR spectra of pure drug, mucilage and mixture were obtained. All the samples were crushed with potassium bromide to get pellets at 1 ton/cm2. Spectral scanning was done in the range between 4000-400 cm-1" [17].

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 [18].

Estimation of reducing sugar

The reducing sugar content in the mucilage was determined using Nelson-Somogyi method [19]. 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 [20]. 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 [21]. 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 [22,23]. Basal growth medium was used as the negative control while basal growth medium supplemented with 2% glucose was used as the positive control.

Stability studies of prebiotic/mucilage as per ICH guideline

The stability studies of prebiotic/mucilage were conducted as per ICH guideline Q1A for determining change in pH, viscosity and the prebiotic ability of the mucilage. Change in pH, viscosity and mucilage as source of carbon with inulin was studied for various time intervals (0-month, 1st month, 3rd month and 6th month) to access the difference in their ability to support the growth of multiple probiotic strains, including Lactobacillus fermentum MTCC 903, Lactobacillus rhamnosus MTCC 1423, and Lactobacillus acidophilus MTCC 10307 cultured on MRS broth. The broths were incubated anaerobically for 48 hours at a temperature of 37±2 °C. The sample (0.1 ml) was taken out of this broth solution, further cell count was acquired using a hemocytometer. Three copies of each sample extract were used in the investigation.

Results and Discussion

Chemical characterization of *Lepidium sativum* mucilage

The test for Molisch's reagent and for ruthenium red was positive for extracted material, which confirmed the presence of mucilage obtained from *Lepidium sativum* (Table 1).

Table 1: Organoleptic characteristics of extracted mucilage

Test	Observed	Result
Molisch's test	Purple to Violet colour	Carbohydrate present
Ruthenium test	Pink colour	Mucilage Present
Iodine test	Blue/Purple colour	Polysaccharides present

Proximate analysis

Proximate analysis of *Lepidium sativum* mucilage was processed for biochemical analysis and measures crude fats (2.37%), proteins (3.07%), fibers (4.71%) and carbohydrates (77.72 %) and total calculated energy was 287.62 kcal/gm (Figure 1).

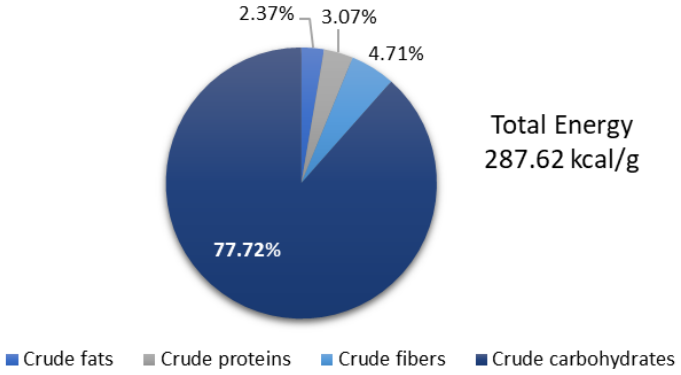


Figure 1: Proximate analysis of *Lepidium sativum* mucilage.

Physicochemical characterization of *Lepidium sativum* mucilage

Loss on drying

The percentage loss of moisture on drying was found to be 4.8% which was available to interact with other material.

Particle size

The average particle size of the dried-powder mucilage was found to be between 500-1000 µm by the microscopic method.

pH of solution

The pH of the 0.5% solution of *Lepidium sativum* mucilage prepared in distilled water was found to be 7.6.

Charring

A charring temperature of *Lepidium sativum* mucilage was found to be 227-229ºC.

Swelling ratio

The swelling ratio of *Lepidium sativum* mucilage determined by measuring the ratio of the hydrated volume to tap volume of dry mucilage in cylinder. The swelling ration was found to be 2.5. There was a significant change in swelling after 24 hours which indicated that the mucilage had good swelling properties.

Flow properties

The flow properties of dried mucilage obtained L. sativum are shown in Table 3. Carr's index, Hausner's ratio and angle of repose were selected as flow indicating parameters. They reflect the particle size, surface characters and moisture content of the mucilage (Table 2).

Table 2: Flow properties of extracted mucilage.

Flow Properties	Value
Carr's index	10.3±0.3
Hausner's ratio	1.17±0.02
Angle of repose	29.4±0.3

Bulk and tapped density

Difference in bulk and tapped density depicts mucilage particles to be surface active with better packing rearrangement. Tapped density (0.73 gm/ml) was higher than bulk density (0.54 gm/ml) suggested that tapping causes interparticle attraction and interlocking. Hausner's ratio is the ration of tapped density to bulk density having the value of 1.35.

Compressibility index

Compressibility index is the ration of difference of tapped and bulk density to tapped density. Compressibility index was found to be 0.26 and percent compressibility index was 26.0%.

Viscosity

On varying concentrations (0.1–0.5% w/v) of mucilage, viscosity also varied and on increasing mucilage concentration viscosity increases as reported in Table 3.

Table 3: Viscosity of mucilage

Concentration of mucilage	Viscosity in cps
0.1%	211.67
0.2%	234.13
0.3%	270.64
0.4%	308.81
0.5%	325.43

Fourier transforms infrared (FTIR) spectral studies

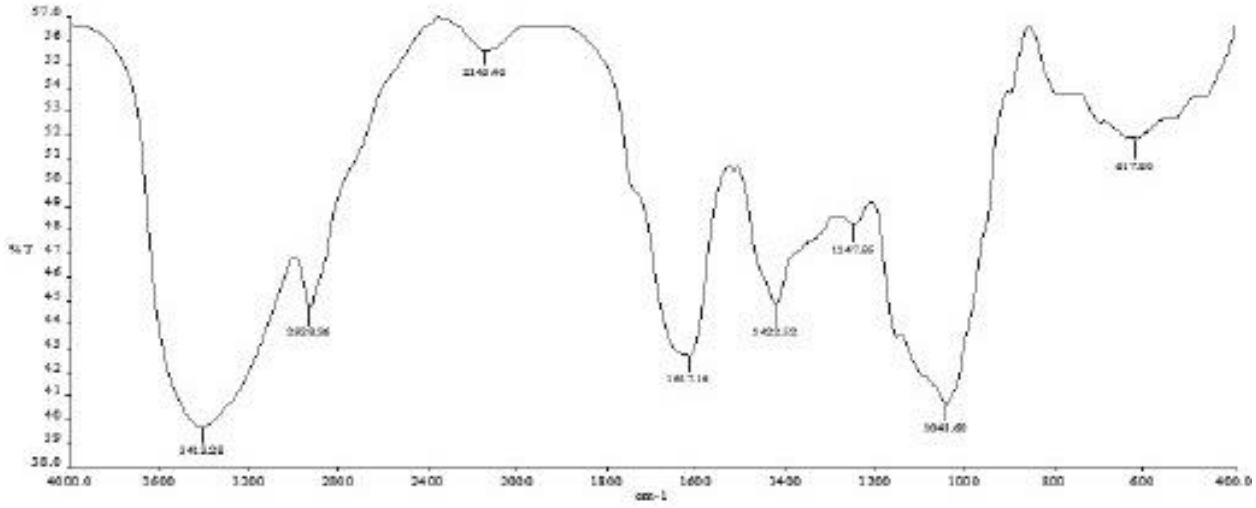


Figure 2: FTIR study for the *Lepidium sativum* mucilage.

The possible functional group present in *Lepidium sativum* mucilage is shown in Table 4 and Figure 2.

Table 4: Functional group present in FTIR spectra

S. No.	Frequency	Band
1	3412	O-H stretching
2	2828	C-H stretching
3	1617	C=O stretching

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 5 shows the total sugar content and the reducing sugar content of the standard prebiotic and the mucilage.

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 5: 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

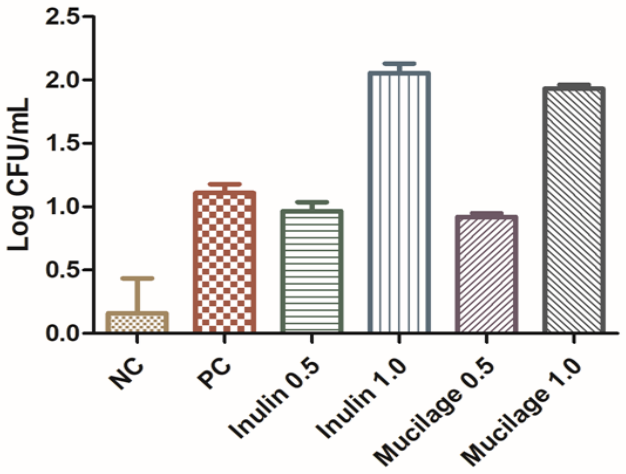


Figure 3a: Effect of various test solution on probiotic growth against L. acidophilus.

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 3a to 3c and Table 6 represents the effect of prebiotic on growth of different probiotic strains

.

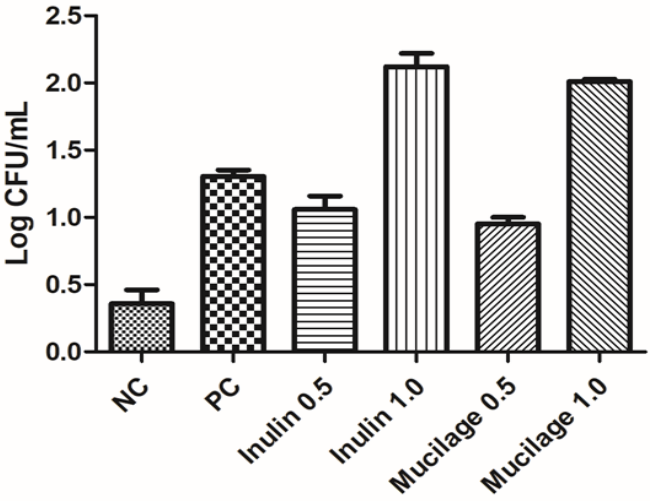


Figure 3b: Effect of various test solution on probiotic growth against L. rhamnosus.

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.

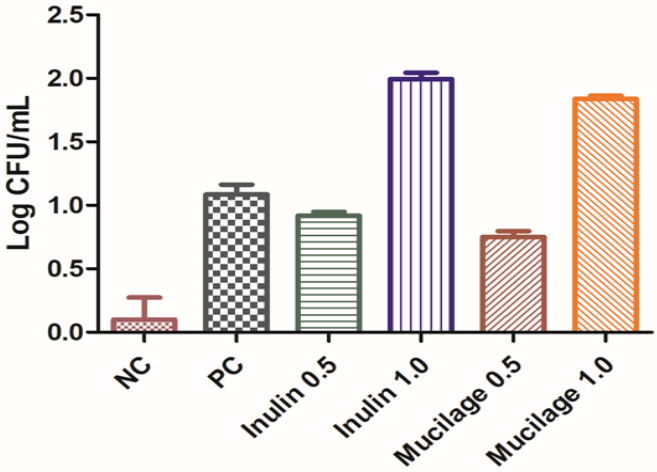


Figure 3c: Effect of various test solution on probiotic growth against L. fermentum.

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 [32] could help them achieve better clinical results.

Table 6: Effect of prebiotic/mucilage on growth Lactobacillus strains

Table 6: Effect of prebiotic/mucilage on growth Lactobacillus strainsS. 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

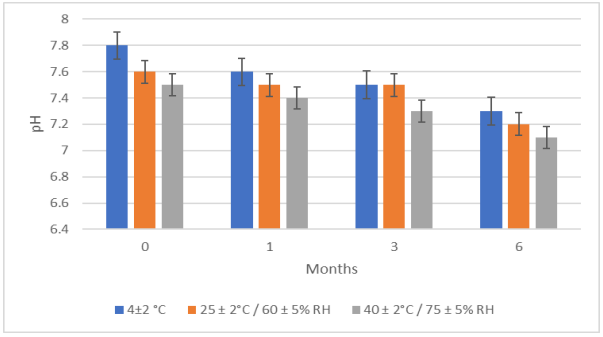
Stability studies of prebiotic/mucilage

The stability study was conducted based on the ICH-guidelines. The mucilage was found to be fairly stable for a period of six months. The pH, viscosity, and the prebiotic ability of the mucilage does not show major variation during the storage at different temperature. The initial and at different time interval results after 0,

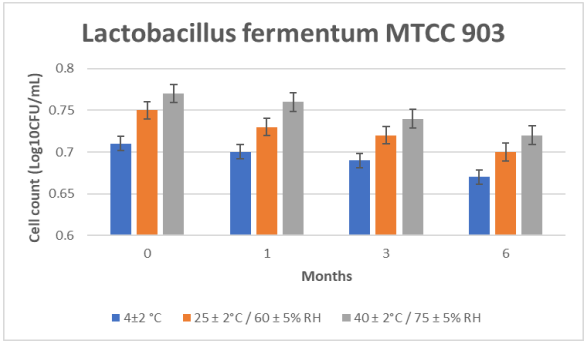
1st, 3rd, and 6th month of the stability study were shown in the below table and figure. The result revealed that there is no significant ($p>0.05$) difference was observed which proved the stability of the mucilage (Table 7 and Figures 4a to 4e.

Table 7: Stability study data of the plant mucilage as per stability guidelines.

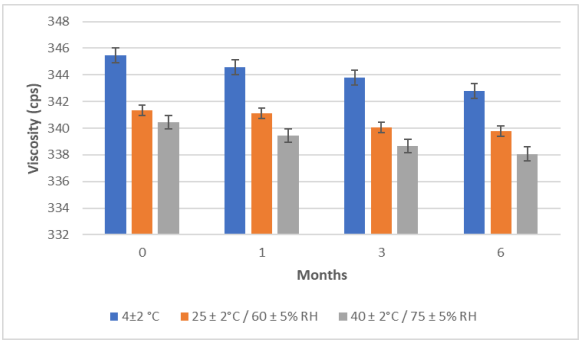
No. of days	Temperature/ Relative humidity	pH	Viscosity	Cell count (Log_{10}CFU/mL)		
				Lactobacillus fermentum MTCC 903	Lactobacillus rhamnosus MTCC 1423	Lactobacillus acidophilus MTCC 10307
0 days	4±2 °C	7.8	345.45	0.71 ± 0.05	0.92 ± 0.05	0.92 ± 0.03
	25 ± 2°C / 60 ± 5% RH	7.6	341.33	0.75 ± 0.05	0.95 ± 0.05	0.95 ± 0.03
	40 ± 2°C / 75 ± 5% RH	7.5	340.43	0.77 ± 0.05	0.96 ± 0.05	0.96 ± 0.03
1 month	4±2 °C	7.6	344.55	0.70 ± 0.05	0.91 ± 0.05	0.91 ± 0.03
	25 ± 2°C / 60 ± 5% RH	7.5	341.12	0.73 ± 0.05	0.94 ± 0.05	0.94 ± 0.03
	40 ± 2°C / 75 ± 5% RH	7.4	339.45	0.76 ± 0.05	0.95 ± 0.05	0.95 ± 0.03
3 month	4±2 °C	7.5	343.79	0.69 ± 0.05	0.90 ± 0.05	0.90 ± 0.03
	25 ± 2°C / 60 ± 5% RH	7.5	340.03	0.72 ± 0.05	0.92 ± 0.05	0.93 ± 0.03
	40 ± 2°C / 75 ± 5% RH	7.3	338.66	0.74 ± 0.05	0.94 ± 0.05	0.94 ± 0.03
6 month	4±2 °C	7.3	342.81	0.67 ± 0.05	0.89 ± 0.05	0.89 ± 0.03
	25 ± 2°C / 60 ± 5% RH	7.2	339.75	0.70 ± 0.05	0.91 ± 0.05	0.92 ± 0.03
	40 ± 2°C / 75 ± 5% RH	7.1	338.07	0.72 ± 0.05	0.93 ± 0.05	0.93 ± 0.03



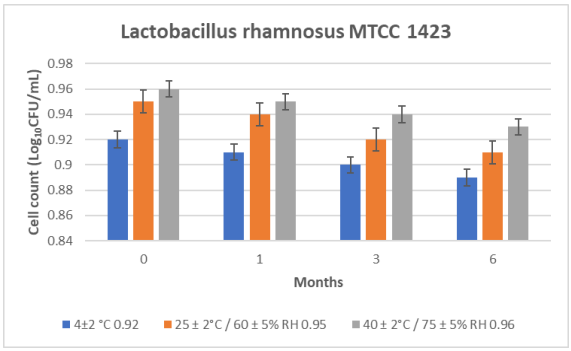
(a)



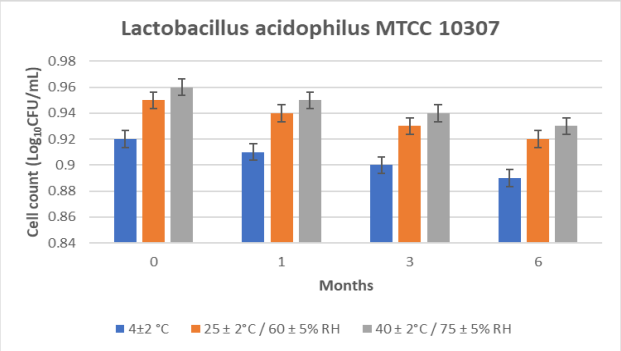
(c)



(b)



(d)



(e)

Figure 4(a-e): Stability study graph of prebiotic/mucilage for change in (a) pH (b) viscosity (c-e) Cell count (Log_{10}CFU/mL) of Lactobacillus fermentum MTCC 903, Lactobacillus rhamnosus MTCC 1423, Lactobacillus acidophilus MTCC 10307 respectively as per stability guidelines.

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.

Funding

The authors did not receive any financial sponsorship for the research.

Conflict of Interest

The author declares no conflict of interest.

References

1. Jahangir MA, Jain P, Verma R, Taleuzzaman M, Ahsan MJ, Chettupalli AK, Muheem A, Mirza MA. Transdermal Nutraceuticals Delivery System for CNS Disease. CNS & Neurological Disorders Drug Targets. 2022 Jan 1
2. Jahangir MA, Khan S, Kala C. Phytonutrients and Technological Development in Formulations. J Pharm Res Sci Technol 2022; 6 (1): 159. doi: 10.31531/jprst.;1000159:39.
3. Chawla P, Kumar N, Bains A, Dhull S.B, Kumar M, Kaushik R and Punia, S. (2020). Gum arabic capped copper nanoparticles: Synthesis, characterization, and applications. Int J Biol Macromol. 146: 232–24
4. Jangra SS, Madan VK. Phytochemical and Nutritional Composition of Different Parts of Garden Cress (Lepidium sativum L.). Int. J. Curr. Microbiol. App. Sci. 2018;7(11):1136-4
5. Dybka-Stępień K, Otlewska A, Góźdź P, Piotrowska M. The Renaissance of Plant Mucilage in Health Promotion and Industrial Applications: A Review. Nutrients. 2021 Oct;13(10):335
6. Reza MA, Hossain MA, Lee SJ, et al. In vitro prebiotic effects and quantitative analysis of Bulnesia sarmienti extract. J Food Drug Analysis 2016; 24(4): 822-830.
7. Azmi AF, Mustafa S, Hashim DM, et al. Prebiotic activity of polysaccharides extracted from Gigantochloa levis (Buluh beting) shoots. Molecules 2012;17: 1635-165
8. Wichienchot S, Thammarutwasik P, Jongjareonrak A, et al. Extraction and analysis of prebiotics from selected plants from southern Thailand. Songklanakarin J Science Technol 2011; 33(5).
9. Lidiyawati A, Widodo E, Sudjarwo E. Potency of green cincau leaves (premna oblogifolia merr) juice as prebiotics and its effect on laying hen performances. J Experimental Life Science 2015; 5: 92-9
10. Lee HC, Jenner AM, Low CS, et al. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. Res in Microbiology 2006; 157: 876-88
11. Al-Snafi AE. Chemical constituents and pharmacological effects of Lepidium sativum-a review. Int J Curr Pharm Res 2019; 11: 1-10.
12. Hussain I, Khattak MU, Riaz Ullah, et al. Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyber Pakhtunkhwa, Pakistan. Afr J Pharm Pharmacol 2011; 5 :746-750.
13. Kassem IAA, Ashaolu TJ, Kamel R, et al. Mucilage as a functional food hydrocolloid: ongoing and potential applications in prebiotics and nutraceuticals. Food Function 2021; 12: 4738-4748.
14. Galla NR and Dubasi GR. (2010). Chemical and functional characterization of Gum karaya (Sterculia urens L.) seed meal. Food Hydrocoll. 24(5): 479-48
15. Bhatia NM, Salunkhe SS, Mali SS, Gadkari SS, Hajare AA, Gaikwad SV, Karade RS. Extraction and characterization of mucilage from Lepidium sativum Linn. seeds. Scholars Research Library der Pharmacia Lettre. 2014;6(1):65-70.
16. Akhand K. Design and characterization of floating microparticles of a cyclooxygenase inhibitor for arthritis related disorders. JDDT [Internet]. 1 [cited 11Apr.2022];3(6):6-
17. A. Bharathi K. Vijaya Sree, D. Chandra Sekhar Naik. Formulation and Evaluation of Roflumilast

Fast dissolving Tablets employing Lepidium sativum mucilage using 23 Factorial design. International Journal of PharmTech Research, 2020,13(1): 52-65

18. Determination of reducing sugars, total reducing sugars, sucrose and starch. Available at https://egyankosh.ac.in/bitstream/123456789/12041/1/Experiment-pdf
19. Romadhoni RP, Purbaningtias TE, Muhaimin, Fauzi'ah L. Determination of reduction sugar form banana (Musa acuminat a Balbisiana colla) with different cooking process by uv-visible spectrophotometer. Proceeding. The 2nd International Seminar on Chemical Education. 2017; 403-409.
20. Korakli M, Gänzle MG, Vogel RF. Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by Lactobacillus sanfranciscensis. J Applied Microbiology 2002; 92: 958-96
21. Wichienchot S, Jatupornpipat M, Rastall RA. Oligosaccharides of pitaya (dragon fruit) flesh and their prebiotic properties. Food Chemistry 2010; 120: 850-85
22. Lidiyawati A, Widodo E, Sudjarwo E. Potency of green cincau leaves (premna oblogifolia merr) juice as prebiotics and its effect on laying hen performances. J Experimental Life Science 2015; 5: 92-9
23. Wang Y, Han F, Hu B, et al. In vivo prebiotic properties of alginate oligosaccharides prepared through enzymatic hydrolysis of alginate. Nutrition Res 2006; 26: 597-60
24. Bhatt S, Singh B, Gupta M. Antioxidant and prebiotic potential of Murraya koenigii and Brassica oleracea var. botrytis leaves as food ingredient. J Agriculture Food Res2020; 2: 100069.
25. Jahangir MA, Muheem A, Imam SS, et al. High-Altitude Edible Plants: A Great Resource for Human Health and their Socio-Economic Significance. In Edible Plants in Health and Diseases 2022 (pp. 161-180). Springer, Singapore.
26. Jahangir MA, Anand C, Muheem A, et al. Nano phytomedicine based delivery system for CNS disease. Current Drug Metabolism. 2020; 21: 661-73.
27. Imam SS, Alshehri S, Jafar M, et al. Liposomal Carrier Systems. In Liposomes for Functional Foods and Nutraceuticals 2022 (pp. 145-163). Apple Academic Press.
28. Jahangir MA, Muheem A, Haque MA, et al. Formulation and Challenges in Liposomal Technology in Functional Food and Nutraceuticals. In Liposomes for Functional Foods and Nutraceuticals 2022 (pp. 165-195). Apple Academic Press.
29. Imam SS, Jahangir MA, Gilani SJ, et al. Nanoemulsions as Delivery Vehicle for Nutraceuticals and Improving Food Nutrition Properties. In Nanoemulsions in Food Technology 2021 Oct 17 (pp. 187-204). CRC Press.
30. Ahmad J, Nollet LM, editors. Nanoemulsions in Food Technology: Development, Characterization, and Applications. CRC Press; 2021 Oct 17.
31. Jahangir MA, Khan S, Singh AD, et al. Nanophytomedicine in clinical management: An introductory evidence-based review. Journal of Pharmaceutical Research Science & Technology [ISSN: 2583-3332]. 2022 Feb 7;6(1):26-37.
32. Archana CS, Kalra N. Evaluating the Prebiotic Potential of Lepidium Sativum Seed Mucilage as a Nutraceutical Ingredient. J Pharm Res Sci Technol 2022; 6 (2): 163. doi: 10.31531/jprst. 1000163 e-ISSN: 2583.;3332:91.