ISSN PRINT 2319 1775 Online 2320 7876

*Research paper*  UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

# EVALUATION OF THE PREBIOTIC POTENTIAL OF *MANGIFERA INDICA* LEAF EXTRACT

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**Abstract**
In the present investigation, it was attempted to establish the prebiotic potential of Mangifera mucilage and also perform the proximate analysis of the extracted mucilage. Mangifera mucilage was extracted from the leaves in yield of 5.1% by weight of dry leaf powder. The mucilage was also evaluated for its DPPH radical scavenging potential and its resistance to hydrolysis by gastric juice (acidic hydrolysis) or by α-amylase (enzymatic hydrolysis). The prebiotic potential of the mucilage was studied by assessing its effect on the growth of various lactobacillus strains. The mucilage was found to contain 16.25% moisture, 4.71% ash, and 71.2% carbohydrates. Proteins and fats were totally absent in the mucilage. The total sugar in the Mangifera mucilage sample was determined by Fehling's reagent method and was found to be 61 µg. The $IC_{50}$ value of the mucilage in inhibiting DPPH radical was found to be 43.63 µg/mL. Artificial gastric juice (pH 1) was used to hydrolyze the Mangifera mucilage as well as inulin. The acidic hydrolysis of inulin was found to be 7.14% while that of the Mangifera mucilage was found to be 16.91%. The enzymatic (α-amylase) hydrolysis of inulin was found to be 12.34% while that of the extracted Mangifera mucilage was found to be 18.36%. The prebiotic promoted the growth of Lactobacillus strains in varying degree. The highest growth was obtained for *L. fermentum* (1.71 ± 0.03) followed by *L. rhamnosus* (1.45 ± 0.02 $Log_{10}$CFU/mL) and the least for *L. acidophilus* (1.29 ± 0.03 $Log_{10}$CFU/mL).

**Keywords**
Mangifera indica, mucilage, prebiotic, lactobacillus, 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. Various types of microorganisms, known as gut microbiota, are inhabitants of the human gastrointestinal tract. The human gastrointestinal tract has been reported to be resident for $10^{10}$–$10^{12}$ live microorganisms per gram in the human colon. 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 [1].

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Various types of microorganisms, known as gut microbiota, are inhabitants of the human gastrointestinal tract [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 majority of these microorganisms, which are mostly anaerobes, live in the large intestine.

Although some endogenous factors, such as mucin secretions, can affect the microbial balance, human diet is the chief source of energy for their growth. Particularly, non-digestible carbohydrates can highly modify the composition and function of gut microbiota [4]. Beneficial intestinal microbes ferment these non-digestible dietary substances called prebiotics and obtain their survival energy from degrading indigestible binds of prebiotics [5]. As a result of this, prebiotics can selectively influence gut microbiota. On the other hand, the gut microbiota affects intestinal functions, such as metabolism and integrity of the intestine. Moreover, they can suppress pathogens in healthy individuals through induction of some immunomodulatory molecules with antagonistic effects against pathogens by lactic acid that is produced by Bifidobacterium and Lactobacillus genera [6].

Various compounds have been tested to determine their function as prebiotics. Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and trans-galacto-oligosaccharides (TOS) are the most common prebiotics. Fermentation of prebiotics by gut microbiota produces short-chain fatty acids (SCFAs), including lactic acid, butyric acid, and propionic acid. These products can have multiple effects on the body. Peptidoglycan is another prebiotics fermentation product that can stimulate the innate immune system against pathogenic microorganisms. The structure of prebiotics and the bacterial composition of gut determine the fermentation products [7]. The effects of prebiotics on human health are mediated through their degradation products by microorganisms.

Aqueous extracts from leaves of plants have been found to contain carbohydrates and flavones in them as secondary metabolites and produce mucopolysaccharides 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.

In the present work, we have attempted to examine the prebiotic potential of aqueous extract from the leaves of *Mangifera indica* by studying its effect on the growth of Lactobacillus along with its ability to resist the hydrolysis by gastric juice and α-amylase.

ISSN PRINT 2319 1775 Online 2320 7876

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## Material and Methods

### Collection of Plant Material

The leaves of *Mangifera indica* were collected from the plants found in locality of Gwalior city, Madhya Pradesh.

### Preparation of the Plant Material for Extraction

The leaves of the plant were washed with distilled water and dried in shade (preventing from direct sunlight). The dried leaf has been powdered using slow speed blender and is kept in closed airtight container.

### Extraction of leaf mucilage [8-9]

The *Mangifera indica* leaves (100 g) were soaked for 12 hour 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.

### Phytochemical Screening of Mucilage

The mucilage obtained was tested for the presence or absence of phytoconstituents using different qualitative tests [10].

### Proximate Analysis

### Determination of Moisture content [11]

An accurately weighed sample (approximately 1 g) was placed in an aluminum pan and the sample was dried in a previously heated hot air oven at 105°C to a constant weight.

### Determination of Amount of Ash [12]

Accurately weighed sample (approximately 0.1 g) was placed in a ceramic crucible (previously heated and cooled until constant weight was obtained) and was subjected to ashing in a muffle furnace maintained at 550°C until a constant final weight for ash was achieved.

### Determination of Amount of Fat [13]

A known weight of the sample (approximately 10 g/thimble) was defatted in a Soxhlet apparatus using petroleum ether (boiling point range = 40-60°C) as the solvent (product-to-ratio of 1:10 w/v) for 8 hours. Defatted samples were dried overnight (approximately 10-12 h) in a fume hood to remove residual traces of petroleum ether and the samples will be weighed to calculate the lipid content.

Lipid (%) = [initial wt. of full fat product (g) – final weight of defatted product (g)] / [initial weight of full fat product (g)] x100

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**Determination of Amount of Protein [14]**

The micro-Kjeldahl method was used to determine total proteins. Briefly, 0.1 g of the sample was placed in a micro-Kjeldahl flask. A catalyst (mixture of 0.42 g of $CuSO_4$ + 9.0 g of $K_2SO_4$), a few glass beads (to prevent sample bumping), and 15mL of concentrated $H_2SO_4$ (36 N) was added to each sample. Sample digestion was done at 410 °C for 45-75 min. (until a clear green solution was obtained, which ensured complete oxidation of all organic matter). The digest was then diluted with 50 mL of distilled water and the micro-Kjeldahl flask was attached to the distillation unit. After the addition of 45 mL of 15 N NaOH, sample distillation was commenced and released ammonia was collected into boric acid solution containing the indicators methylene blue and methyl red (blue: red :: 1:2). Borate anion (proportional to the amount of nitrogen) was titrated with standardized 0.1 N $H_2SO_4$. A reagent blank was run simultaneously. Sample nitrogen content was calculated using the formula.

% N = [(mL of $H_2SO_4$ for sample – mL of $H_2SO_4$ for blank) x Normality of $H_2SO_4$ x 1.4007] / weight of sample (g)

Protein % = total N (%) x approximate factor for sample.

**Determination of Amount of Carbohydrates**

Total carbohydrates were determined by difference of weights. (Carbohydrates = 100 - % proteins - % fats - %Ash - % moisture).

**Determination of DPPH Radicals Scavenging Activity [15]**

The free radical scavenging activity of the test solution was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH.

Determination of DPPH radicals scavenging activity was performed by the previously reported method. Separately, 1mM solution of DPPH and test solution (50-250 µg/mL) were prepared in ethanol. 1.5ml of the test solution was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared using 3 mL ethanol. The control sample used was 3 mL of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

$$\text{DPPH scavenged (\%)} = \frac{(A_{con} - A_{test})}{A_{con}} \times 100$$

$A_{con}$ - is the absorbance of the control reaction

$A_{test}$ - is the absorbance in the presence of the test solution.

**Prebiotic Activity**

The prebiotic action of the gum obtained was examined for its prebiotic and related actions using previously reported methods.

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**Test organisms**

A whole of three strains of lactobacilli species were examined including *Lactobacillus acidophilus* MTCC 10307, *Lactobacillus rhamnosus* MTCC 1423 and *Lactobacillus fermentum* MTCC 903 and the strains were purchased from Institute of Microbial Technology, Chandigarh in the form of lyophilized culture.

**Assessment of Total Sugar**

The total amount of sugar contained in the Mangifera mucilage was determined using copper reduction method utilizing Lane and Eynon technique that involves titration of Fehling's reagent [16].

**Standardization of the Fehling's Solution**

Weigh 4.75g of sucrose with precision and 50 ml of distilled water was added to it and transfer to a 500 ml volumetric flask. 24 hours should pass after adding 5 cc of concentrated HCl. Make up to volume after neutralising the solution with NaOH while using phenolphthalein as an end point indicator. 25 ml of the mixture should be transferred to a 100 ml volmetric flask and made up to volume (1 ml is equal to 2.5 milligrammes of invert sugar). To a burette, transfer the solution.

5 ml of Fehling A as well as B solutions were taken into an Erlenmeyer flask with a capacity of 250 ml. 10 ml of water, some glass or pumice beads, and combine. Use the burette to release the sugar solution. Heat the solution until it boils. Methylene blue indicator is added three times. Drop by drop, keep adding sugar solution until the blue colour turns brick red at the end ($V_1$).

**Reducing sugars in sample**

1 g of Mangifera mucilage should be weighed and shifted to a 50 mL volumetric bottle. To the phenolphthalein end point, add approximately 100 ml of water and neutralise with NaOH solution. Ten minutes after adding 10 ml of neutral lead acetate solution, shake the mixture. Add modest volumes of potassium oxalate solution until precipitation stops. Make the solution up to volume, thoroughly mix it, and then run it through a Whatman No. 1 sieving circle. The filtrate was transferred to a 50 ml burette, and then follow the steps in 5.8.6.2.2 to titrate. Calculate the percent reducing sugar in the solution using the formula:

$$\%\ reducing\ sugar = \frac{0.0025\ X\ V1\ X\ V2\ X\ 100}{V3\ X\ W}$$

Where W – weight of sample; $V_1$ – Titre volume for standardization; $V_2$- Dilution volume of sample; $V_3$ – Volume of sample solution used

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**Estimation of reducing sugar**

The extent of reducing sugar contained in the Mangifera mucilage was estimated using Nelson—Somogyi method [17]. Measure accurately aliquots of 0.1 or 0.2 ml and 1.0 ml of the Mangifera mucilage in separate labelled test tubes. Transfer 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standards into separate tubes and label. Using distilled water top up the volume to 2 ml in both tubes of sample and reference. Set up a blank in another tube with 2 ml water blank. Place all the tubes in a boiling water bath for 10 minutes after adding 1.0 ml of alkaline copper tartarate to each one. Arsenomolybdic acid is added to all of the tubes once they have been cooled. Fill each tube to a volume of 10 ml with filtered water. After 10 minutes, read the absorbance of the developed blue colour at 620 nm. Plot a graph with µg of sugar against absorbance and calculate the amount of reducing sugar present in the Mangifera mucilage.

**ESTIMATION OF GASTRIC JUICE HYDROLYTIC ACTION**

Research on the ability of different dried plant extracts to withstand acidity was done using inulin and FOS as prebiotic standard materials. The following ingredients were used to simulate artificial human gastric juice in the hydrochloric acid buffer (g/l): $NaH_2PO_4$, 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. Using 5 M HCl, the pH of the buffer was kept at 1 [18]. The sample was made by dissolving 1% (w/v) of Mangifera mucilage in water. The sample solution (5 ml) was combined with artificial gastric juice (5 ml), which was then incubated for an additional 6 hours at 37±2°C in a water bath. At both 0 and 6 hours, total and reducing sugars were estimated. The quantity of reducing sugar released and the sample's overall sugar content were used to estimate the percentage of gum hydrolysis:

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

**Estimation of A-Amylase Hydrolysis Action**

2 units mL-1 of α-amylase were produced for enzymatic hydrolysis in sodium phosphate buffer (20 mM), which was then pH-adjusted to 6.9 using 6.7 mM of sodium chloride [19]. The sample was created by dissolving 1% (w/v) of Mangifera mucilage in the buffer. The sample solution was combined with 5 ml of enzyme solution and incubated for a further 6 hours at pH 6.9 and 37 2 °C. By measuring the quantity of total and reducing sugar in the sample, enzymatic hydrolysis was determined. Estimates of the hydrolysis rate were made:

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

**Prebiotic Potential of *Mangifera indica* Mucilage**

To encourage the augmentation of various probiotic strains, Mangifera mucilage was used as the source of carbon with inulin as the benchmark. Different probiotic strains, including

ISSN PRINT 2319 1775 Online 2320 7876

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*Lactobacillus fermentum* MTCC 903, *Lactobacillus rhamnosus* MTCC 1423, and *Lactobacillus acidophilus* MTCC 10307, were cultured on MRS broth for 24 hours at 37 2°C. The prebiotics were sterilised by passing through a membrane filter with a pore size of 0.45 m before being tested against the 5 ml of Mangifera mucilage solution (0.5 & 1%, w/v) (Millipore). As a base growth media, MRS broth (carbohydrate-free) was employed. Mangifera mucilage and common prebiotics (0.5 & 1% w/v) were added to the basal growth media along with the activated bacterial culture (1%). 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 and a further cell count was acquired using a hemocytometer. Three copies of each sample extract were used in the investigation [20,21]. Basal growth medium served as the positive control, and 2% glucose supplemented basal growth medium served as the negative control.

## Results and Discussion

### Extraction and phytochemical screening of mucilage

The mucilage from *Mangifera indica* was extracted using the reported method and the yield of mucilage was 5.1 % w/w of the dry leaf powder. The mucilage was yellowish in color, tasteless and amorphous. The mucilage was found to contain flavonoid, tannins, phenolics and carbohydrates.

### Proximate Analysis

The content of moisture, fat, ash, protein and carbohydrate in Mangifera mucilage was established using the previously reported methods and the results obtained are presented in Table 1.

**Table 1 Proximate composition of mucilage**

| S. No. | Parameter | Percentage |
|---|---|---|
| 1 | Moisture | 16.25 |
| 2 | Ash | 4.71 |
| 3 | Fat | 0 |
| 4 | Protein | 0 |
| 5 | Carbohydrate | 71.2 |

### DPPH radicals scavenging activity

DPPH is stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, then losing colour stoichometrically with the number of electrons consumed, which is measured

ISSN PRINT 2319 1775 Online 2320 7876

*Research paper*  UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

spectrophotometricallty at 517 nm. The deep purple color of DPPH decreases if the compound exhibits antioxidant action (Table 2).

**Table 2 DPPH radical scavenging potential of *Mangifera indica* leaf mucilage**

| Concentration (µg/mL) | DPPH Scavenging % | |
|---|---|---|
| | Test Solution | Ascorbic acid |
| 50 | 57.3 ± 0.361 | 94.37 ± 0.702 |
| 100 | 72.09 ± 1.513 | - |
| 150 | 85.13 ± 1.464 | - |
| 200 | 86.07 ± 0.551 | - |
| 250 | 93.41 ± 0.781 | - |

Results are reported as mean ± SD (n=3)

The test solution exhibited a dose depended DPPH scavenging action with a dose of 250µg/mL producing protection against DPPH almost equivalent to the standard drug ascorbic acid (50µg/mL). The $IC_{50}$ value of the Mangifera mucilage against DPPH was found to be 43.63 µg/mL.

**Reducing Sugar**

The total sugar in the Mangifera mucilage sample was determined by Fehling's reagent method and was found to be 61 µg.

**Hydrolytic effect of gastric juice**

Artificial gastric juice (pH 1) was used to hydrolyze the Mangifera mucilage as well as inulin. The acidic hydrolysis of inulin was found to be 7.14% while that of the Mangifera mucilage was found to be 16.91%. The Mangifera mucilage was able to resist the acidic hydrolysis. The incubation time of 6h was also in part responsible to hydrolysis of the Mangifera mucilage as well as inulin allowing for conversion of polysaccharides to mono and di-saccharides. Since the Mangifera mucilage was able to withstand about 85% 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 Mangifera mucilage in presence of α-amylase was determined by

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quantifying the reducing sugar. The enzymatic (α-amylase) hydrolysis of inulin was found to be 12.34% while that of the extracted Mangifera mucilage was found to be 18.36%. The Mangifera mucilage was found to be resistant to enzymatic hydrolysis almost equivalent to the standard prebiotic. Hence, the extracted Mangifera mucilage presents a great potential to be a source of carbon in the gut microflora and establishing itself as a prebiotic.

**Prebiotic potential of Mangifera 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. Table 3 represents the effect of prebiotic on growth of different probiotic strains.

The data was statistically analyzed using one way ANOVA followed by Dunnett's post -test. The results indicate that at all the concentrations, the Mangifera 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. fermentum* followed by *L. rhamnosus* and the least for *L. acidophilus*.

**Table 3. Effect of prebiotic/ Mangifera mucilage on growth Lactobacillus strains**

| S.N | Prebiotic | Concentration (%) | Cell count ($Log_{10}$CFU/mL) | | |
|---|---|---|---|---|---|
| | | | ***Lactobacillus acidophilus*** | ***Lactobacillus rhamnosus*** | ***Lacotbacillus fermentum*** |
| 1 | Inulin | 0.5 | 0.91 ± 0.07 | 1.01 ± 0.10 | 0.86 ± 0.03 |
| | | 1.0 | 1.82 ± 0.07 | 1.96 ± 0.10 | 1.79 ± 0.05 |
| 2 | *Mangifera* Mucilage | 0.5 | 0.66 ± 0.03 | 0.61 ± 0.05 | 0.89 ± 0.05 |
| | | 1.0 | 1.29 ± 0.03 | 1.45 ± 0.02 | 1.71 ± 0.03 |
| 3 | Negative Control | - | 0.16 ± 0.27 | 0.39 ± 0.10 | 0.11 ± 0.17 |
| 4 | Positive Control | - | 1.15 ± 0.07 | 1.34 ± 0.05 | 1.15 ± 0.08 |

Expressed as mean ± standard deviation; n=3

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.

**Conclusion**

In this study, the proximate composition of *Mangifera* mucilage was evaluated and the effect of *Mangifera* mucilage on microbiota of gut was studied using various strains of lactobacillus in vitro. The ability of *Mangifera* mucilage to resist the acidic and enzymatic hydrolysis was also established. The results obtained led to the conclusion that *Mangifera* mucilage exhibits prebiotic effects comparable to inulin.

ISSN PRINT 2319 1775 Online 2320 7876

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