

## Research Article

# Development and Functional Characterization of a Plant-Based Symbiotic Beverage Enriched with *Lactobacillus plantarum*

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**Abstract** : The growing demand for plant-based functional foods has encouraged the development of non-dairy probiotic beverages with added health benefits. This study focuses on the formulation and evaluation of a symbiotic beverage prepared using *Lactobacillus plantarum* and selected herbal ingredients including banana stem, aloe vera, amla, and honey. Fresh extracts were obtained from the plant materials, blended in suitable proportions, and subjected to mild heat treatment. The probiotic culture was incorporated under sterile conditions, followed by fermentation at 37°C for 24 hours. The prepared beverage was analyzed for physicochemical properties, antioxidant potential, anti-inflammatory activity, and phytochemical constituents. The fermented product exhibited an acidic pH of 3.7, which supports probiotic stability and inhibits undesirable microbial growth. With an IC<sub>50</sub> value of 20.38 µg/mL, the antioxidant activity, as measured by the DPPH assay, demonstrated efficient free radical scavenging. Anti-inflammatory activity assessed through RBC membrane stabilization indicated approximately 50% protection. The presence of important bioactive substances such as flavonoids, phenols, alkaloids, and steroids was verified by phytochemical screening. Overall, the developed beverage demonstrates promising functional and therapeutic properties. The study highlights the potential of combining probiotics with plant-based ingredients to produce a health-promoting symbiotic drink suitable for improving digestive health.

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## Introduction

In recent years, increasing awareness of the relationship between diet and health has driven the demand for functional foods and beverages. Incorporating bioactive chemicals, vitamins, minerals, and helpful microbes into beverages has the dual purpose of providing basic nourishment and delivering additional physiological advantages. Among these, probiotic drinks have garnered a lot of interest because of their potential to improve immunity, preserve intestinal health, and lower the risk of gastrointestinal illnesses [1]. The creation of non-dairy probiotic substitutes has been prompted by concerns about lactose intolerance, cholesterol levels, and the increasing inclination towards plant-based diets, despite the fact that traditional probiotic products are primarily dairy-based [2].

Probiotics are live microorganisms that, when ingested in sufficient quantities, confer health benefits on the host. One of the most extensively studied probiotic strains is *Lactobacillus plantarum*, known for its ability to tolerate acidic conditions and adapt to diverse environmental substrates. This organism contributes to gut health by maintaining microbial balance, suppressing pathogenic bacteria, and improving digestion [3]. Incorporation of *Lactobacillus plantarum* into plant-based beverages enhances their functional properties and makes them suitable for regular dietary intake.

In recent years, symbiotic formulations that blend probiotics and prebiotics have become increasingly important. The term "prebiotic" refers to indigestible substances that selectively promote the formation of beneficial bacteria in the gut. Natural ingredients such as banana stem and honey serve as effective prebiotic sources, supporting probiotic survival and activity. The synergistic interaction between probiotics and prebiotics improves gut microbiota composition and enhances overall health benefits [4].

Banana stem (*Musa* spp.) is an underutilized agricultural by-product rich in dietary fiber, potassium, and bioactive compounds. It has traditionally been used for its digestive and detoxifying properties. Its high fiber content supports gut health by promoting beneficial microbial growth, making it a suitable base for functional beverage development [5]. Similarly, *Aloe barbadensis* Miller (aloe vera) is widely recognized for its therapeutic properties. The polysaccharides, vitamins, and phenolic substances found in the gel have anti-inflammatory and antioxidant properties, which help protect and heal the gastrointestinal tract [6].

The Indian gooseberry, or amla (*Emblica officinalis*), is an excellent food choice as it is rich in antioxidants and vitamin C. It excels in improving immunity, lowering oxidative stress, and bolstering general health. However, its high acidity necessitates careful formulation to ensure the survival of probiotic organisms in the final product [7]. Honey, a natural sweetener, provides fermentable sugars that facilitate probiotic growth and fermentation. In addition, it exhibits antimicrobial and antioxidant properties, improving both the safety and sensory quality of the beverage [8].

Probiotic beverages cannot be produced without the fermentation process, which involves the use of bacteria to convert carbohydrates into organic acids. This process enhances shelf life, improves nutrient availability, and contributes to the characteristic taste of the product. Maintaining optimal fermentation conditions, such as temperature (37°C) and duration (24 hours), is essential to achieve maximum probiotic viability. The resulting acidic pH (3.5–4.5) creates a favorable environment for beneficial microorganisms while inhibiting harmful pathogens [9].

Evaluation of functional beverages involves assessing antioxidant and anti-inflammatory activities. The DPPH radical scavenging assay is a typical method for determining antioxidant capacity, as it indicates how effectively a sample can neutralize free radicals. Anti-inflammatory activity can be

determined using the RBC membrane stabilization method, which indicates the capacity of the formulation to protect cell membranes from damage [10]. To further characterize the beverage's medicinal qualities, phytochemical screening is carried out to detect bioactive components such as tannins, alkaloids, flavonoids, and phenols [11].

Therefore, the present study aims to develop a plant-based symbiotic beverage incorporating *Lactobacillus plantarum* and herbal extracts of banana stem, aloe vera, amla, and honey. The formulated beverage is evaluated for its physicochemical characteristics, antioxidant activity, anti-inflammatory potential, and phytochemical composition. This approach highlights the potential of combining probiotics with plant-derived ingredients to produce a functional beverage with significant health benefits.

## **Materials and Methods**

### **Collection and Preparation of Raw Materials**

Fresh banana stem (*Musa* spp.), amla (*Embluca officinalis*), and commercially available honey were obtained from a local retail outlet (Thirumalayampalayam) in Tamil Nadu, India. Aloe vera leaves were collected from the institutional herbal garden. All plant materials were selected based on freshness and quality, transported under hygienic conditions, and washed thoroughly with distilled water to remove adhering impurities prior to use (Figure 1).

### **Preparation of Herbal Extracts**

The cleaned banana stem, aloe vera gel, and amla fruits were cut into small pieces and processed separately using a sterile mechanical grinder to obtain fresh extracts. The resulting pulp was filtered through sterile muslin cloth to remove fibrous residues and obtain a clear juice. The extracts were stored under refrigerated conditions until further use [12,13].

### **Preparation of Functional Symbiotic Beverage**

The formulation of the beverage was carried out under aseptic conditions. Measured volumes of individual extracts were mixed in a sterile container in the following proportion: banana stem extract (50 mL), aloe vera extract (20 mL), amla extract (15 mL), and sterile distilled water (10 mL). To lower the microbial load while preserving bioactive compounds, the mixture was mildly heated to 60°C for ten minutes. After cooling to room temperature, honey (5 mL) was added as a natural sweetener and prebiotic agent. A probiotic culture of *Lactobacillus plantarum* (1–2 g capsule powder containing viable cells) was inoculated into the mixture. To promote fermentation, the inoculated formulation was incubated for 24 hours at 37°C. The fermented beverage was stored at 4°C for further analysis following incubation [14,15].

### **Flow Process of Beverage Preparation**

The preparation steps followed a sequential process: Raw materials → Washing → Grinding → Filtration → Mixing → Heat treatment → Cooling →

Honey addition → Probiotic inoculation → Fermentation → Refrigerated storage

#### **Determination of pH**

A calibrated digital pH meter was used to determine the pH of the fermented beverage. The observed pH value (3.7) falls within the optimal acidic range (3.5–4.5) suitable for probiotic viability. This acidic environment improves product stability and shelf life by promoting the survival of beneficial bacteria and preventing the growth of harmful and spoilage microorganisms [16].

#### **Sensory Evaluation**

The sensory characteristics of the developed beverage, including taste, aroma, and color, were evaluated using a 9-point hedonic scale by a semi-trained panel. The evaluation provided insight into consumer acceptability and overall product quality [17].

#### **Anti-inflammatory Activity (RBC Membrane Stabilization Assay)**

The red blood cell (RBC) membrane stabilization method was used to assess the beverage's anti-inflammatory properties. Fresh human blood was collected with EDTA as an anticoagulant and centrifuged to obtain packed RBCs. The cells were washed with normal saline and prepared as a 10% suspension. The reaction mixture containing the RBC suspension and sample extract was incubated at 50°C for 15 minutes and then centrifuged. The absorbance of the supernatant was measured at 560 nm. The percentage of membrane stabilization was calculated using the following formula:

$$\text{Membrane stabilization (\%)} = [(Ac - At) / Ac] \times 100$$

where Ac represents control absorbance and At represents test absorbance. This technique reflects the sample's capacity to prevent cell membrane lysis [18].

#### **DPPH Radical Scavenging Assay: Antioxidant Activity**

The DPPH free radical scavenging method was used to assess the beverage's antioxidant capability. DPPH solution was combined with sample extracts of different concentrations, and the mixture was left in the dark for 20 minutes. The absorbance was measured at 517 nm, and the concentration needed to block 50% of DPPH radicals was reported as the IC<sub>50</sub> value [19].

#### **Phytochemical Screening**

Using standard procedures, a qualitative phytochemical analysis of the beverage was carried out to identify bioactive compounds. The tests included Mayer's test for alkaloids, the alkaline reagent test for flavonoids, the ferric chloride test for tannins, the foam test for saponins, the ferric chloride test for phenols, and the Liebermann–Burchard test for steroids. These analyses confirmed the presence of therapeutic phytoconstituents responsible for biological activities [20,21].

### Isolation of Probiotic Bacteria

The probiotic bacterium *Lactobacillus plantarum* was isolated from the fermented beverage using De Man, Rogosa, and Sharpe (MRS) agar medium. The sample was serially diluted and then spread onto sterile MRS agar plates. The plates were incubated under anaerobic conditions at 37°C for 24–48 hours. Distinct colonies showing typical morphological characteristics were selected and subcultured to obtain pure isolates.. The cultures were maintained on MRS slants at 4°C for further analysis [22,23].

### Results and Discussion

#### Physicochemical Analysis (pH)

The developed functional beverage exhibited an acidic pH of 3.7, indicating a suitable environment for probiotic survival. This pH range (3.5–4.5) is widely reported as optimal for the growth and stability of probiotic bacteria, particularly *Lactobacillus plantarum*, while simultaneously inhibiting pathogenic microorganisms (Figure 2). Similar observations have been reported in earlier studies on fermented plant-based probiotic beverages, confirming that an acidic pH enhances both shelf stability and microbial safety [24,25].



**Figure 1.** Collection of raw materials for functional beverage preparation (a) Banana Stem, (b) Amla, (c) Aloe Vera, (d) Honey.



**Figure 2.** pH measurement of the prepared functional beverage

### Sensory Evaluation of Functional Beverage

The sensory characteristics of the prepared beverage were evaluated using a 9-point hedonic scale. The beverage showed good acceptability among panel members. The scores obtained for taste, aroma, and color are presented in Table 1. The results revealed that the beverage possessed a mildly sour taste with a pleasant sweetness, a fresh herbal aroma with slight fermentation notes, and an appealing light greenish-yellow color. The sensory evaluation results indicate that the developed functional beverage exhibited good overall acceptability, with favorable scores for taste, aroma, and appearance.

**Table 1:** Sensory Evaluation of the Developed Functional Beverage

Parameter	Score (out of 9)	Description
Taste	7.2 ± 0.4	Mildly sour with pleasant sweetness from honey
Aroma	6.8 ± 0.5	Fresh herbal scent with mild fermented note
Colour	7.0 ± 0.3	Light greenish-yellow, appealing appearance

### RBC Membrane Stabilization Test: Anti-inflammatory Activity

The anti-inflammatory activity of the beverage was assessed using the RBC membrane stabilization method. The composition of the control and test reactions is summarized in Table 2, and the appearance of the tubes after water-bath treatment is shown in Figure 3. The sample exhibited moderate anti-inflammatory activity, with membrane stabilization of approximately 50% (Table 3).

**Table 2:** Preparation of control and test sample for RBC assay

Test	RBC (ml)	Distilled water (ml)	Test drink (ml)
Control	1ml	1ml	-
Sample	1ml	-	1ml

**Table 3.** RBC Membrane Stabilization Test Results

Parameter	Value
Control OD [Ac]	1.99
Test OD [At]	1



**Figure 3.** Sample and control tubes after water-bath treatment (50°C for 15 minutes).

Membrane stabilization (%) =  $(Ac - At)/Ac \times 100 = (1.99 - 1.00)/1.99 \times 100 = 49.7\%$  ( $\approx 50\%$ ).

This ability to stabilize erythrocyte membranes suggests that the formulation may help prevent cell lysis under stressful conditions. The effect may be attributed to the presence of bioactive substances such as flavonoids and phenolic compounds derived from honey and the herbal ingredients. Comparable results have been reported in studies where plant-based extracts exhibited significant membrane stabilization owing to their protective effect on biological membranes [26,27].

#### Antioxidant Activity (DPPH Assay)

The antioxidant capacity of the beverage was evaluated using the DPPH radical scavenging assay. The beverage exhibited strong antioxidant activity, comparable to that of the common antioxidants rutin and BHT, with an  $IC_{50}$  value of 20.38  $\mu\text{g/mL}$  (Table 4). The percentage inhibition increased with concentration, confirming a dose-dependent antioxidant response (Table 5). The bioactive components of amla, aloe vera, and honey may act synergistically to enhance this effect. Fermentation by *Lactobacillus plantarum* may further improve antioxidant potential by releasing bound phenolic compounds, as reported in previous studies [28,29].

**Table 4.** Comparison of the antioxidant activity of the sample and standard compounds (DPPH assay).

Sample	Extract/Standard	$IC_{50}$ ( $\mu\text{g}/3 \text{ mL}$ )	$IC_{50}$ ( $\mu\text{g/mL}$ )
Sample	26–1006	61.14	20.38
Standard	Rutin	76.88	25.62
Standard	BHT	65.98	21.99

**Table 5.** DPPH radical scavenging activity (% inhibition) of the sample and standards.

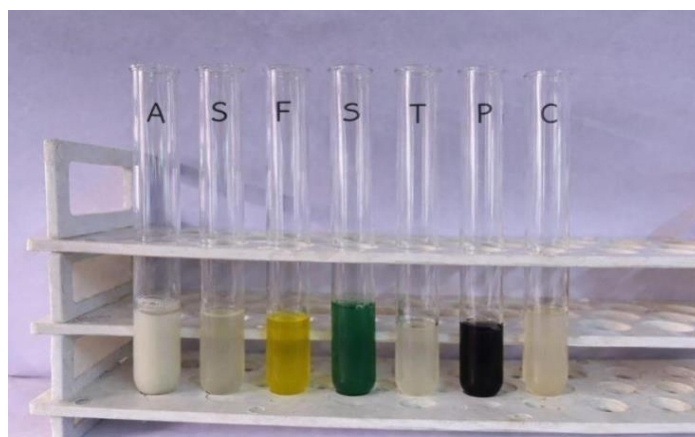
Sample Type	Extract/Standard	Concentration ( $\mu\text{g}/3 \text{ mL}$ )	% Inhibition	$IC_{50}$ ( $\mu\text{g}/3 \text{ mL}$ )	$IC_{50}$ ( $\mu\text{g/mL}$ )
Sample	26–1006	20	23.55	61.14	20.38
		40	31.52		
		60	51.38		
		80	63.97		
		100	76.67		
Standard	BHT	20	18.89	65.98	21.99
		40	34.27		
		60	44.71		
		80	63.84		
		100	70.89		
Standard	Rutin	20	8.00	76.88	25.62
		40	20.53		
		60	33.80		
		80	54.10		
		100	70.18		

The IC<sub>50</sub> value represents the concentration required to scavenge 50% of DPPH radicals; a lower IC<sub>50</sub> value indicates superior antioxidant capacity, whereas a higher percentage inhibition indicates stronger antioxidant action

### Phytochemical Analysis

Qualitative phytochemical screening revealed the presence of several important secondary metabolites, including alkaloids, flavonoids, phenols, and steroids, whereas tannins and saponins were absent in the analyzed sample (Figure 4; Table 6).

Since these substances are known to support antibacterial, anti-inflammatory, and antioxidant activities, their presence enhances the beverage's functional qualities. Similar phytochemical profiles have been reported in herbal formulations containing plant extracts, indicating their therapeutic significance [30,31].



**Figure 4.** Qualitative phytochemical screening of the developed functional beverage

**Table 6.** Qualitative phytochemical analysis of the developed functional beverage.

S. No.	Phytochemical Test	Reagent Used	Observation (Color Change)	Result
1	Alkaloids	Mayer's reagent	Creamy white precipitate	+
2	Saponins	Distilled water (shaking)	No persistent foam formation	-
3	Flavonoids	NaOH solution	Intense yellow color turning colorless on acidification	+
4	Steroids	Liebermann-Burchard reagent	Green coloration	+
5	Tannins	5% FeCl <sub>3</sub> solution	No blue-black coloration observed	-
6	Phenols	FeCl <sub>3</sub> solution	Deep blue-black/black coloration	+
7	Control	No reagent	No color change	-

(+ = Present, - = Absent)

### Isolation and Growth of Probiotic Bacteria

The probiotic strain *Lactobacillus plantarum* was successfully isolated on MRS agar medium. The colonies appeared small, creamy, and circular, which is characteristic of *Lactobacillus* species (Figure 5). This successful growth

confirms that the formulated beverage provides a favorable environment for probiotic viability, and is consistent with previous findings in which fermented plant-based substrates supported the growth and activity of lactic acid bacteria under controlled conditions [32].



**Figure 6.** Isolation and colony morphology of *Lactobacillus plantarum* on MRS medium.

The findings of the present study show that the developed symbiotic beverage possesses important functional characteristics. The acidic pH supports probiotic stability, while the observed antioxidant and anti-inflammatory activities indicate potential health benefits. Fermentation appears to increase the bioavailability of phenolic compounds, as reflected by the low  $IC_{50}$  value obtained in this study, which is comparable to those reported for other plant-based probiotic beverages. The formulation's medicinal potential is further highlighted by its mild anti-inflammatory action. Phytochemical analysis confirms the presence of bioactive constituents responsible for these biological activities. The combined effect of herbal ingredients and probiotic fermentation appears to produce a synergistic enhancement in functional properties.

These findings are consistent with earlier research on non-dairy probiotic beverages, supporting the growing interest in plant-based functional foods as alternatives to conventional dairy products [33,34].

### **Conclusion**

This study presents the development of a novel plant-based symbiotic beverage formulated with *Lactobacillus plantarum* and bioactive-rich extracts of banana stem, aloe vera, amla, and honey. The integration of probiotic functionality with phytochemical constituents resulted in a formulation exhibiting enhanced functional and therapeutic attributes. The beverage maintained an optimal acidic pH (3.7), ensuring probiotic stability while suppressing undesirable microbial growth. The synergistic action of phenolic compounds and vitamin-rich plant components is responsible for its significant antioxidant capacity, demonstrated by a low  $IC_{50}$  value, highlighting its effectiveness in scavenging free radicals. In addition, the observed membrane stabilization activity demonstrates its capability to mitigate inflammatory responses at the cellular level. The presence of secondary metabolites, such as flavonoids, phenols, alkaloids, and steroids, was verified by phytochemical

profiling, supporting the formulation's functional importance. The combined effects of probiotic microorganisms and plant-derived bioactives suggest a synergistic mechanism that enhances the overall efficacy of the beverage. In summary, the developed symbiotic beverage represents a promising non-dairy functional food with potential applications in promoting gut health, strengthening immune function, and managing oxidative stress-related disorders. These findings support its prospective role in the functional food industry. Further investigations focusing on in vivo validation, mechanistic insights, and process optimization are recommended to facilitate its translation into commercial-scale production.

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