



# Formulation And Evaluation Of Phytosynbiotic Shrikhand

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**Abstract:-** The potential of Shrikhand, a classic fermented dairy dessert from India, as a functional meal is being investigated. In order to maximize health advantages, this research introduces the formulation of phytosynbiotic shrikhand, which combines probiotics, prebiotics, and bioactive plant chemicals. Inulin, a gut-friendly prebiotic, chakka made from A2 cow milk, and medicinal substances such jambul seed powder, Catharanthus roseus, and Clitoria ternatea (butterfly pea) were included because of their neuroprotective, anti-inflammatory, and antioxidant qualities. Over the course of its shelf life, the formulation sought to balance probiotic viability, stability, taste, and texture. Probiotic development was aided by inulin, and the therapeutic value was increased by plant phytoconstituents. The product's viability was validated by preliminary assessments, which included physicochemical testing, sensory analysis, and microbiological stability. A new, health-promoting dairy product that supports metabolic balance and digestive wellness is phytosynbiotic shrikhand. This dessert satisfies market need as customer interest in functional meals high in nutrients grows. Future studies will evaluate scalability, maximize shelf life, and confirm therapeutic effectiveness. This research demonstrates the creative use of phytochemicals and synbiotics to enhance conventional dairy products and provide sustainable, health-conscious substitutes.

**Keywords:-** Fermented products, yoghurt, value added shrikhand, Lactobacillus lacti.

## 1. INTRODUCTION

### 1.1. Immunity overview

As a sophisticated defensive network, the human immune system keeps the body safe from infections and promotes general well-being. The gut microbiota, a varied microbial population in the gastrointestinal system, is a major determinant affecting immune function. Recent research has established the gut as a central location for immune control by highlighting the critical relationship between the gut bacteria and the immune system. By modulating responses to pathogens and maintaining immunological homeostasis, the gut microbiota contributes to the development and control of both innate and adaptive immunity. Immune-related illnesses including autoimmune diseases, allergies, and inflammatory bowel disease have been linked to dysbiosis, or imbalances in gut microorganisms. Dietary strategies have shown potential for enhancing immune responses and modifying the gut microbiome. The immune-boosting properties of probiotics (healthy living microorganisms), prebiotics (indigestible substances that support good bacteria), and synbiotics (a mix of the two) are being investigated. According to research, these kinds of treatments might boost immunity and lower the risk of illness. The development and evaluation of "Symbiotic Frequency," a new synbiotic formulation intended to improve immunity by modifying gut flora, is the main focus of this thesis. The goal of this product is to provide a wholesome, immune-boosting dietary solution by using the complementary effects of certain probiotics and prebiotics.

### 1.2. IMMUNOMODULATOR

In vertebrates, the immune system is a highly developed defensive mechanism that protects the body from dangerous outside substances. It produces a diverse range of substances and cells that can identify and eliminate dangers. Any alteration in immune activity, such as the stimulation, augmentation, repression, or control of certain immunological responses, is referred to as immune modulation. Agents known as immunomodulators have an impact on immunological processes and may either increase or decrease immune activity. Immunostimulators, which increase immunological responses, and immunosuppressants, which decrease or block them, are the two basic groups into which they fall. These compounds support the immune system's readiness to fight against threats. The possible immunomodulatory effects of plant extracts have been the subject of much investigation in various areas of the globe. Isolating and researching bioactive substances having these properties has been the subject of several studies. For example, *Acorus calamus* rhizome extract has shown the capacity to promote the proliferation of many human and animal cell lines while suppressing the release of nitric oxide (NO), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-2 (IL-2). Additionally, it inhibits the CD25 marker's expression. Furthermore, a number of plant-based substances have been shown to be efficient immunomodulators, including lectins, glycoproteins, alkaloids, polysaccharides, flavonoids, sterols, and sterolins.

### 1.3. COMPOSITION AND FUNCTIONS OF THE GUT MICROBIOTA

#### 1.3.1. IMMUNOMODULATION AND ITS ROLE IN THE IMMUNE SYSTEM

In vertebrates, the immune system is a highly developed defensive mechanism that protects the body from dangerous outside substances. It produces a vast array of chemicals and cells that can recognize and eliminate a variety of dangers. Any alteration in immunological responses, such as the stimulation, improvement, control, or repression of certain activities, is referred to as immune system modulation. The ability of certain plant extracts to alter immunological function has been studied. For example, it has been shown that *Acorus calamus* rhizome extract may promote the proliferation of several human and mouse cell lines while inhibiting the synthesis of nitric oxide (NO), interleukin-2 (IL-2), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Additionally, it lowers the CD25 surface marker's expression. Furthermore, a number of plant-

based substances have been identified as having interesting immunomodulatory qualities, including lectins, glycoproteins, alkaloids, polysaccharides, sterols, sterolins, and flavonoids.

### 1.3.2. DEVELOPMENT AND EARLY COLONIZATION OF GUT MICROBIOTA

Although prenatal exposure to germs is modest, microbial colonization and the formation of the gut microbiota start at birth. The immune system and homeostasis are significantly influenced by the gut microbiota, with early colonization being particularly important. Early-life microbial exposure activates important trophic and immunological functions, which may not develop efficiently if colonization is postponed until maturity, according to research on germ-free animals. The establishment of gut microbiota is influenced by many variables, such as:

- Delivery type: children delivered vaginally develop microbiota that resembles the mother's vaginal flora, whereas children born by cesarean section develop microbiota that resembles their skin and the surrounding environment.
- Gestational age: Preterm newborns usually have higher amounts of Enterobacteriaceae, which include possible pathogens like *Escherichia coli* and *Klebsiella pneumoniae*, but lower levels of helpful anaerobes like *Bifidobacterium* and *Bacteroides*.
- Use of antibiotics: The makeup of the microbiota may be significantly impacted by antibiotics used by the mother or child.
- Type of feeding: newborns who are breastfed have a microbiota that is enriched in *Bifidobacteria*, while newborns who are formula-fed have different microbial profiles, microbial diversity is also impacted by environmental exposures, such as siblings, pets, and rural vs urban environments.

### 1.3.3. MATURATION AND COMPOSITION OF ADULT GUT MICROBIOTA

The makeup of the gut microbiota changes significantly when breast milk is replaced with solid meals. The major phyla, Firmicutes and Bacteroidetes, have an impact on the structure of the microbiota throughout life. A child's gut microbiota by the age of three is essentially the same as an adult's, however certain bacteria communities continue to develop until puberty.

About 90% of the bacteria in the adult gut belong to the phyla Bacteroidetes and Firmicutes, with the remaining 10% being made up of:

- Proteobacteria
- Actinobacteria
- Fusobacteria
- Verrucomicrobia
- Some Archaea species

### 1.3.4. ADDITIONAL COMPONENTS OF GUT MICROBIOTA

The gut microbiota also includes protists, bacteriophages, and yeasts in addition to bacteria: One of the viral components mostly composed of bacteriophages, which target dominant species and encourage horizontal gene transfer to control microbial populations. However, because of their poor resemblance to existing reference genomes, many viral sequences are still unidentified. Yeasts are far less common than bacteria, yet they nonetheless serve a useful purpose in the gut environment. In the intestines of healthy adults, less than 20 yeast species have been found.

#### 1.4. INTRODUCTION OF DOSAGE FORM (SHRIKHAND)

A traditional Indian delicacy, shrikhand is made from strained yogurt (hanging curd), sweetened with sugar, and flavored with almonds, cardamom, or saffron. It is particularly well-liked in the Gujarati and Maharashtra regions and has a rich, creamy texture.

#### 1.5. INTRODUCTION OF A2 COW MILK

Specialty dairy products like A2 cow milk are known for their better digestion and health benefits compared to ordinary A1 milk. It has  $\beta$ -casein A2, which is unable to synthesize the peptide beta-casomorphin-7 (BCM-7), which is linked to digestive problems. A2 milk thus supports gut health and is kinder to the digestive tract. Additionally, it is abundant in immunoglobulins, probiotics, and vital amino acids, all of which promote improved immunity, metabolism, and general health.

A2 cow milk serves as the foundation element in our recipe for Shrikhand, a classic fermented dairy treat from India. Its richer, more nutrient-dense texture is enhanced by the A2 protein concentration. Its probiotic potential is further increased by fermentation, which promotes intestinal balance and digestion. A2 milk provides additional health benefits while preserving the genuine taste of Shrikhand.

We have included certain herbal and functional components to further enhance the nutritional value. Essential bioactive chemicals are extracted and preserved using hexane, and *Catharanthus roseus* provides antioxidant and therapeutic benefits. Rich in anthocyanins, butterfly peas promote brain function and provide a vibrant natural color.

Our approach creates a wellness-focused Shrikhand by combining traditional dairy practices with modern nutritional knowledge. By combining strong herbal and functional ingredients with A2 milk, we preserve the dessert's traditional significance while boosting its nutritional value. This creative recipe turns Shrikhand into a tasty treat and a nutritious food option, satisfying the rising need for functional foods that promote overall health.

#### 1.6. INTRODUCTION OF HERBAL INGREDIENTS

- 1. Inulin:-** Inulin is a naturally occurring polysaccharide and prebiotic fiber present in various plants like chicory root, garlic, onions, and bananas. It consists of fructooligosaccharides (FOS) and long-chain fructans that support gut health by promoting the growth of beneficial gut bacteria. Inulin offers multiple health benefits, including prebiotic effects, blood sugar regulation, cholesterol reduction, and improved digestive function. It also enhances calcium absorption, aids in regular bowel movements, and supports weight management by increasing feelings of fullness. Due to these properties, inulin is widely incorporated into functional foods, dairy products, and pharmaceutical formulations to maintain gut microbiota balance and promote overall well-being.
- 2. Jambul seed powder (*Syzygium cumini* (L.) Skeels):-** Jambul seed powder, obtained from the seeds of the *Syzygium cumini* tree (commonly known as Jamun or Indian blackberry), is widely recognized in traditional medicine for its therapeutic properties. Rich in bioactive compounds such as alkaloids, flavonoids, tannins, glycosides, and ellagic acid, it offers a range of health benefits. Jambul seed powder is known for its antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective effects. Traditionally, it has been used to help regulate blood sugar levels, support digestion, enhance liver function, and promote overall metabolic well-being.

**3. Catharanthus roseus (Madagascar Periwinkle):-** Catharanthus roseus, also known as Madagascar periwinkle, is a well-known medicinal plant used in both traditional and modern medicine. It is rich in bioactive compounds, including powerful alkaloids like vincristine and vinblastine, along with flavonoids, tannins, and phenolic acids. The plant is recognized for its antidiabetic, anticancer, antioxidant, antimicrobial, and cardioprotective properties. Traditionally, it has been used to lower blood sugar levels, enhance blood circulation, and treat various infections and inflammatory conditions. Owing to its potent alkaloids, Catharanthus roseus plays a significant role in chemotherapy treatments for leukemia and several other types of cancer.

**4. Butterfly Pea (Clitoria ternatea):-** Clitoria ternatea, commonly known as Butterfly Pea, is a medicinal plant highly valued in both traditional and modern medicine. It is rich in bioactive compounds such as flavonoids, anthocyanins (particularly ternatins), alkaloids, and phenolic acids. This plant is known for its antioxidant, anti-inflammatory, neuroprotective, antimicrobial, and antidiabetic properties. Traditionally, Butterfly Pea has been used to enhance memory and cognitive function, promote relaxation, support eye health, and regulate blood sugar levels. Owing to its vivid blue anthocyanins, it is also popular as a natural food colorant, an ingredient in herbal teas, and in various skincare formulations.

## 2. MATERIAL AND METHODOLOGY

### 2.1. Material:-

Table 1 Formula for Shrikhand

INGREDIENTS	QUANTITY
Chakka	100gm
Inulin	2gm
Jamun Seed Powder	1gm
Catharanthus roseus	2 gm
Clitoria ternatea	1 gm
Sugar	Quantity Sufficient
Elaichi Powder	Quantity Sufficient

## 2.2. Method of preparation

### 1. Ingredient selection:

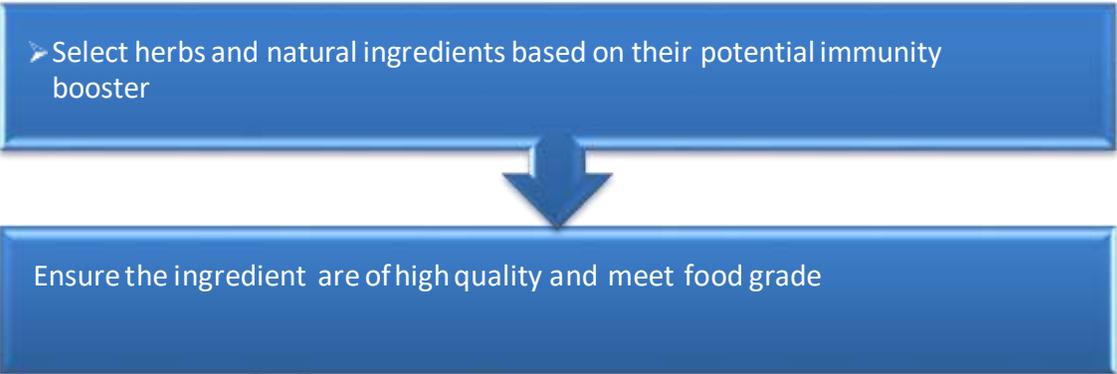


Figure 1 Inulin Powder



Figure 2 Butterfly pea



Figure 2 Jamun Seed Powder



Figure 1 Catharanthus Roseus Powder



Figure 5 Curd



Figure 6 Chakka

## 2. Preparation of Curd and Chakka:-

- **Preparation of curd :-** Boil 1L of A2 COW milk and allow it to cool to 40°C.
- Add a table spoon of curd (starter culture) and mix well.
- Let it set at room temperature for 6–8 hours or overnight, forming thick yogurt.

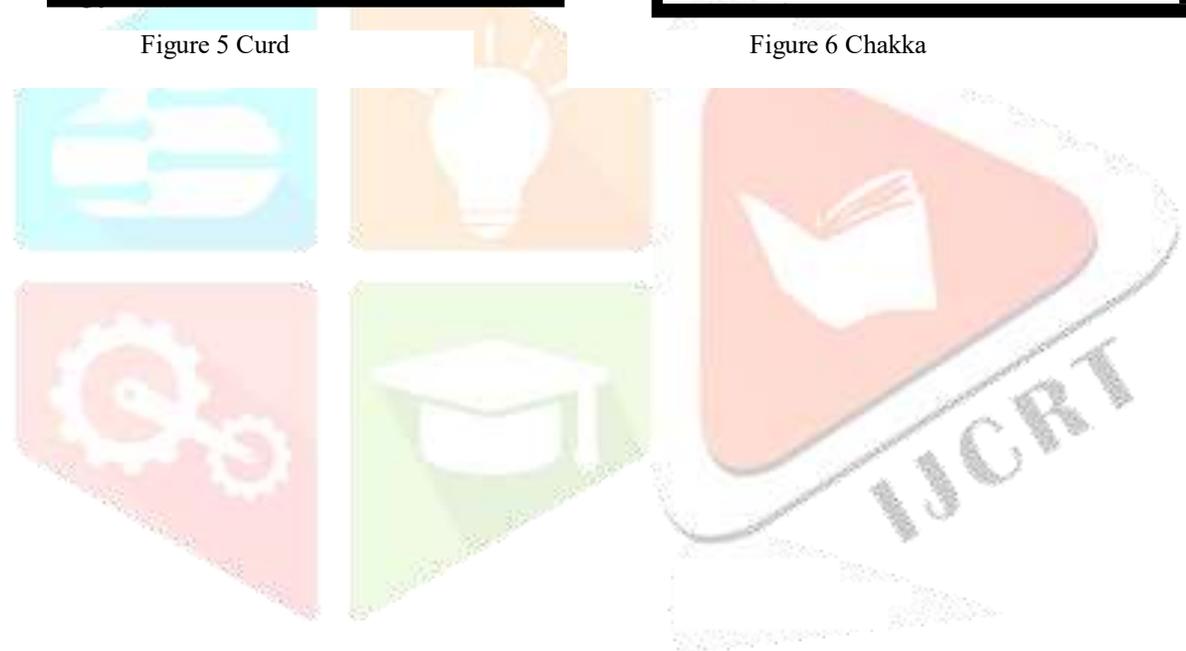
- **Strain the curd to make chakka :-** Place a cheese cloth or muslin cloth in a clean sieve and pour the yogurt into it.
- Allow the whey to drain out for 4–5 hours or until you get a thick, creamy consistency known as **chakka** (thickened curd).



Figure 5 Curd



Figure 6 Chakka



### 3. Mixing Functional Ingredients into the Chakka:

• **Inulin**:- Weigh 2g **inulin** powder and add it to chakka. Stir gently to mix well. Inulin serves as a prebiotic, fostering healthy gut

• **Catharanthus roseus** :- Measure 2g of **Catharanthus roseus extract** .Add it to the curd chakka and mix thoroughly. The extract provides a range of bioactive compounds known for their antioxidant and immune-boosting

• **Butterfly pea** :- Add 5g of **butterfly pea extract** to the curd chakka. This will provide a vibrant color and anthocyanins with antioxidant properties.

• **Jamun seed powder**:- Add 1g of **jamun seed powder** to the curd chakka. This powder is rich in antioxidants and has antidiabetic and anti-

• **Sugar** :- Add **sugar** to the mixture. Adjust to taste based on the desired sweetness level.

• **Cardamom powder** :- Add 1g of **cardamom powder** for flavor. This will enhance the overall taste and provide digestive benefits.

#### 4. Final mixing & Blending

- **Blend the mixture:-** Using a spoon or electric hand mixer, blend all the ingredients thoroughly to ensure an even consistency.
- The resulting mixture should be smooth, creamy, and well-incorporated with the functional powders.



Fig 7 Mixing the Ingredient

#### 5. Refrigeration & Setting

**Chill the mixture:-** Transfer the final shrikhand mixture into a clean container and refrigerate for 4–6 hours. This step allows the flavors to blend well, and the consistency will firm up to the classic shrikhand texture.

#### 6. Optimization

Adjust the proportions of ingredients, mixing process and time to achieve the desired sensory and functional properties

### 3. EVALUATION PARAMETER

#### 3.1. Physiochemical Properties of Shrikhand

##### 3.1.1. pH Evaluation

A pH meter is an electronic device used to determine the acidity or alkalinity of a solution by measuring its pH level, using a sensitive glass electrode and a reference electrode. To measure the pH of shrikhand, begin by calibrating the pH meter using fresh buffer solutions with known pH values (typically pH 4.00, 7.00, and 10.00). Rinse the electrode with distilled water before immersing it into each buffer, adjusting the meter accordingly for accurate calibration.



Fig 8 Calibration of pH meter

Prepare the shrikhand sample by stirring thoroughly for uniformity, and if it is too thick, dilute slightly with distilled water. Before measurement, rinse the electrode again, blot gently, and immerse it into the sample, ensuring the sensing part is fully submerged. Stir gently and wait for a stable reading before recording the pH value. The ideal pH range for shrikhand lies between 4.5 and 5.5, which supports its taste, texture, and shelf-life; deviations may indicate issues with fermentation, ingredients, or storage.

### 3.1.2. Ash Value

The principle of ash determination in food involves heating food samples to high temperatures (550–600 °C), where moisture and volatile compounds evaporate, and organic matter combusts in the presence of oxygen, producing carbon dioxide, nitrogen oxides, and water vapor. The remaining inorganic residue, composed of oxides, phosphates, sulphates, and chlorides, is referred to as ash. For ash analysis, pre-weighed empty crucibles are first heated at 600 °C for one hour in a muffle furnace, cooled in a desiccator, and weighed (W1). A 2.0 g food sample is then added to the crucible and weighed (W2), charred over a flame, and incinerated in the furnace at 600 °C for 3 hours.



Fig9 Ash Value

After complete ashing, the crucible is cooled in a desiccator and reweighed (W3). Incineration is repeated until the difference between two successive weights is less than 1.0 mg to ensure completeness. The final constant weight is recorded, and ash content is calculated using the formula:  
**Ash (%) = [(W3 - W1) / (W2 - W1)] × 100.**

### 3.1.3. Moisture Content

Moisture content is a crucial parameter in food analysis, as it directly affects the dry matter content, which has economic implications for both consumers and processors. Additionally, moisture levels influence the storage stability and overall quality of food products. To estimate the moisture content in shrikhand, a pre-weighed empty petridish with its lid (W1) is used. Approximately 2.0 g of the sample is weighed into the dish (W2) and spread evenly. The dish is then placed in a hot air oven at 102 °C with the lid open for 2 hours to allow for uniform drying.



Fig 10 Moisture content

After drying, the dish is transferred to a desiccator to cool before reweighing (W3). This process is repeated until a constant weight is achieved, indicating complete removal of moisture. The moisture content is then calculated using the formula: **Moisture (%) = [(W2 - W1) - (W3 - W1)] / (W2 - W1) × 100**, where W1 is the weight of the empty petridish, W2 is the weight with the sample before drying, and W3 is the weight after drying.

### 3.1.4. Determination of Titratable Acidity

The acidity of shrikhand is an important quality parameter that reflects its freshness, flavor, and microbial stability. It is determined through titration using 0.1 N sodium hydroxide (NaOH) and phenolphthalein as an indicator. For the procedure, 10.0 g of shrikhand is mixed with 30.0 ml of warm water and 1.0 ml of phenolphthalein indicator. The mixture is shaken thoroughly and titrated with 0.1 N NaOH until a faint pink color persists, indicating the endpoint.



Fig 11 Acid Titrability

The titration is completed within 20 seconds for accuracy. A blank is prepared using 10.0 g of shrikhand and 30.0 ml of water (without indicator or NaOH) for color comparison. The titratable acidity, expressed as a percentage of lactic acid, is calculated using the formula: **Titratable acidity (% LA) = (9 × A × N) / W**,

where *A* is the volume of NaOH used (in ml), *N* is the normality of NaOH, and *W* is the weight of the shrikhand sample in grams.

### 3.1.5. Determination of Reducing Sugars

Reducing sugars in shrikhand were estimated using Fehling's method, which involves the titration of sugars with Fehling's solutions A and B in the presence of an indicator. First, 25.0 g of shrikhand is weighed and transferred into a 250 ml volumetric flask. To this, 10.0 ml of neutral lead acetate solution is added, diluted to volume with water, and filtered. From the filtrate, 25.0 ml is taken into a 500 ml flask containing 100 ml of distilled water, followed by the addition of potassium oxalate until no further precipitation occurs, and made up to volume.



Fig 12 Reducing Sugar

After thorough mixing, the solution is filtered through Whatman No. 1 filter paper and the clear filtrate is taken in a 50 ml burette. For titration, 5 ml each of Fehling's A and B solutions are added to a conical flask along with 10 ml of distilled water, heated to boiling, and 3 drops of methylene blue indicator are added. The sample is titrated from the burette until a brick-red endpoint appears, and the titre volume is recorded. For determining the Fehling factor, 4.75 g of analytical-grade sucrose is dissolved in 50 ml water, hydrolyzed with 5.0 ml concentrated HCl for 24 hours, neutralized with NaOH, and made up to 500 ml. From this, 50 ml is diluted to 100 ml and titrated similarly. The Fehling factor is calculated as: **Fehling factor = (Titrate value × Weight of sucrose) / 500** and the percentage of reducing sugars is calculated using:

**Reducing sugars (%) = (Dilution × Fehling factor) / (Weight of sample × Titre value).**

## 3.2. Preliminary Test

### 3.2.1. Inulin (Carbohydrate):-

#### 3.2.1.1. Molisch's Test:

Procedure: To 2 mL of the sample solution, add 2–3 drops of Molisch's reagent ( $\alpha$ -naphthol in ethanol). Carefully introduce 1 mL of concentrated sulfuric acid along the inner wall of the test tube to form two separate layers. Observation: A positive result is indicated by the formation of a purple or reddish-purple ring at the interface of the two layers, confirming the presence of carbohydrates.

### 3.2.1.2. Fehling's Test:

Procedure: Mix equal volumes of Fehling's solution A and B. Add 1 mL of the sample solution to this mixture and heat it in a boiling water bath for 5 minutes. Observation: The appearance of a brick-red precipitate indicates the presence of reducing sugars.

### 3.2.1.3. Benedict's Test:

Procedure: Combine 5 mL of Benedict's reagent with 8 drops of the sample solution in a test tube. Heat the mixture in a boiling water bath for 5–10 minutes. Observation: A color change from blue to green, yellow, or brick-red, accompanied by the formation of a precipitate, indicates the presence of reducing sugars.

## 3.2.2. Jamun Powder (*Syzygium Cumini*):-

### 3.2.2.1. Tannin Detection (Ferric Chloride Test):

Procedure: Add a few drops of 5% ferric chloride solution to 2 mL of the sample solution. Observation: The formation of a green to blue-black precipitate confirms the presence of tannins.

### 3.2.2.2. Tannin Detection (Ferric Chloride Test):

Procedure: Add a few drops of 5% ferric chloride solution to 2 mL of the sample solution. Observation: The appearance of a green to blue-black precipitate confirms the presence of tannins.

### 3.2.2.3. Shinoda's Test for Flavonoids:

Procedure: Dissolve 100 mg of the plant extract in 5 mL of hydrochloric acid. Add a few small pieces of magnesium metal to the solution. Observation: The development of a reddish-crimson or orange color indicates the presence of flavonoids.

## 3.2.3. Butterfly Pea (*Clitoria ternatea*):

### 3.2.3.1. Saponin Detection (Foam Test):

Procedure: Shake 1 mL of the sample extract with 5 mL of water vigorously for 15 minutes. Observation: The formation of a persistent blue foam layer confirms the presence of saponins.

### 3.2.3.2. Tannin Detection (Ferric Chloride Test):

Procedure: Add a few drops of ferric chloride solution to 2 mL of the sample solution. Observation: A blue-black coloration indicates the presence of tannins.

## 3.2.4. *Catharanthus roseus*:-

### 3.2.4.1. Alkaloid Detection: Hager's Test

Procedure: To 1 mL of the plant extract, add 1 mL of Hager's reagent (saturated picric acid solution). Observation: The formation of a yellow precipitate confirms the presence of alkaloids.

### 3.2.4.2. Flavonoid Detection: Shinoda Test

Procedure: To 1 mL of the plant extract, add 1 mL of 10% magnesium turnings in alcohol. Then, carefully add 1 mL of concentrated hydrochloric acid dropwise.  
Observation: The appearance of a pink, scarlet, or red color confirms the presence of flavonoids.

### 3.3. Microbial Testing:-

#### 3.3.1. 1. Total Plate Count (TPC) Procedure

##### Purpose:

Measures the total number of microorganisms, including both viable (living) and non-viable (dead) bacteria, in the Shrikhand sample.



Fig 13 Total plate count

##### Procedure:

1. Sample Preparation: Take 10 g of Shrikhand and mix it with 90 mL of sterile saline to make a 1:10 dilution. Perform serial dilutions up to  $10^{-6}$  by transferring 1 mL from the previous dilution into 9 mL of sterile saline in a new test tube.
2. Plating (Pour Plate Method): Transfer 1 mL of the diluted sample into a sterile Petri plate. Pour 15–20 mL of molten Plate Count Agar (PCA), cooled to approximately  $45^{\circ}\text{C}$ , into the plate. Swirl gently to mix and allow it to solidify.
3. Incubation: Invert the plates and incubate at  $37^{\circ}\text{C}$  for 24–48 hours.
4. Colony Counting & Calculation: Count all visible colonies (both small and large). Choose plates with 30–300 colonies for accurate results.

Calculation Formula:

$$\text{CFU/g} = (\text{Number of Colonies} \times \text{Dilution Factor}) / \text{Volume Plated (mL)}$$

### 3.3.2. Total Viable Count (TVC) Procedure

**Purpose:** Counts only viable (living) microorganisms capable of growth under specified conditions in the Shrikhand sample.



Fig 14 Total Viable Count

#### Procedure:

1. Sample Preparation: Take 10 g of Shrikhand and mix it with 90 mL of sterile saline (1:10 dilution). Perform serial dilutions up to  $10^{-6}$ .
2. Plating (Spread Plate Method): Take 0.1 mL of the diluted sample and place it on a pre-solidified PCA plate. Spread evenly using a sterile glass spreader.
3. Incubation: Invert the plates and incubate at  $37^{\circ}\text{C}$  for 24–48 hours.
4. Colony Counting & Calculation: Count only viable colonies (dead cells do not grow).

Calculation Formula:

$$\text{CFU/g} = (\text{Number of Colonies} \times \text{Dilution Factor}) / \text{Volume Plated (mL)}$$

## 4. RESULT

The sample analyzed showed a pH value of 4.75, indicating moderate acidity, along with an ash content of 10% and a relatively high moisture content of 52.5%, which may impact its shelf stability. The titratable acidity was measured at 0.89%, and the formulation contained 2.01% reducing sugar and 14.35% fat. Microbial examination revealed a notable increase in both total plate count and mould count over a seven-day period, with values rising from  $3.5 \times 10^7$  to  $5.8 \times 10^9$  log cfu/g for plate count and  $2.1 \times 10^6$  to  $3.8 \times 10^8$  log cfu/g for mould count, highlighting the importance of addressing microbial preservation for shelf life extension.

Preliminary phytochemical investigations confirmed the presence of several bioactive compounds across the ingredients. Inulin responded positively to carbohydrate and reducing sugar detection tests, while jamun powder was found to contain tannins and flavonoids. Butterfly pea demonstrated the presence of saponins, phenolic compounds, and flavonoids, and *Catharanthus roseus* likewise contained detectable carbohydrates and flavonoids. These findings indicate that the formulation is rich in beneficial phytochemicals and nutrients, though effective preservation strategies are needed to ensure microbial stability during storage.

## 5. CONCLUSION

The goal of the study was to develop a functional shrikhand incorporating dahi, inulin, *Catharanthus roseus*, butterfly pea, and jamun, aiming to enhance immune function. The resulting product demonstrated a favorable nutritional profile, offering a balanced composition of proteins, carbohydrates, and essential minerals. The inclusion of inulin contributed to dietary fiber content, while *Catharanthus roseus* and butterfly pea provided bioactive compounds with potential health benefits. Jamun addition not only enriched the flavor but also introduced beneficial antioxidants. Sensory evaluations indicated high palatability, and the product maintained acceptable quality over a reasonable shelf life. Despite these promising findings, further research and clinical trials are necessary to confirm the safety and efficacy of this formulation as a dietary intervention for immune enhancement.

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