



DEVELOPMENT AND EVALUATION OF ANTIDIABETIC HERBAL PROBIOTIC CURD: NUTRITIONAL AND IN VITRO PROBIOTIC ASSESSMENT

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Abstract: The present study aims to develop a functional dairy product—herbal curd—fortified with antidiabetic medicinal plants and to evaluate its probiotic properties, phytochemical composition, nutritional profile and sensory acceptability. Herbs used in the study were *Glycyrrhiza glabra* (Mulethi), *Gymnema sylvestri* (Gudmar), *Stevia rebaudiana* (Madhuparni), *Beta vulgaris* (Beetroot), cinnamon, cardamom, dry ginger and nutmeg. Curds were prepared by supplementing milk with herb powders followed by fermentation using a curd starter culture. The prepared curd was evaluated for sensory properties, phytochemical composition, nutritional value and probiotic characteristics. Lactic acid bacteria (LAB) were isolated and characterized using morphological methods. The isolates exhibited tolerance to low pH (2–3), bile salts and showed antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenols and tannins. Nutritional analysis revealed compliance with FSSAI standards. The study demonstrates that herbal curd can serve as a potential functional food with antidiabetic and probiotic benefits.

Keywords: Herbal curd, Probiotics, Antidiabetic activity, Lactic acid bacteria, Phytochemicals, Functional food

1 INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and is associated with severe complications such as cardiovascular diseases, neuropathy, nephropathy and retinopathy [1]. The International Diabetes Federation has reported a rapid increase in diabetes cases globally, with India being among the most affected countries, highlighting the urgent need for effective and sustainable management strategies [2].

Although conventional antidiabetic therapies are widely used, they are often associated with adverse effects and high economic burden. This has led to increasing interest in alternative approaches, particularly dietary interventions and plant-based therapies. Medicinal plants are rich in bioactive compounds such as alkaloids, flavonoids, phenolics, terpenoids and glycosides, which exhibit antidiabetic activity through mechanisms including inhibition of carbohydrate-digesting enzymes (α -amylase and α -glucosidase), enhancement of insulin secretion, improvement of insulin sensitivity and antioxidant effects [3]. Several herbs, including *Gymnema sylvestri*, *Glycyrrhiza glabra*, *Stevia rebaudiana*, *Cinnamomum verum* and *Zingiber officinale*, have been reported for their hypoglycemic potential [4].

However, a major limitation of herbal therapies is poor palatability, as many antidiabetic plants possess a bitter or strong taste, which reduces patient compliance. To address this challenge, the present approach emphasizes the use of non-bitter, commonly consumed herbs and spices, many of which are

integral components of daily diets (such as cinnamon, cardamom, ginger and nutmeg). This strategy not only improves acceptability but also facilitates regular consumption without significant dietary modification.

In recent years, functional foods have gained considerable attention as dietary tools for managing chronic diseases. Probiotic foods, particularly fermented dairy products such as curd (yogurt), are widely recognized for their health benefits. Curd contains beneficial microorganisms, mainly *Lactobacillus* spp. and *Streptococcus thermophilus*, which contribute to gut health, immune modulation and metabolic regulation [5]. Probiotics have also been reported to influence glycemic control by modulating gut microbiota and reducing systemic inflammation [6].

Incorporation of medicinal herbs into probiotic dairy products represents a promising strategy to develop functional foods with enhanced therapeutic value. Additionally, the fermentation process, combined with the natural flavor of selected spices, can help mask undesirable tastes, enabling the development of a flavoured, palatable and sweet probiotic product. This is particularly important for individuals with diabetes, who often have restricted access to sweet food options.

Therefore, the present study was designed not only to develop an antidiabetic functional food but also to formulate a probiotic sweet herbal (flavoured) curd using non-bitter, diet-compatible herbs. The study includes selection and phytochemical analysis of herbs, optimization of herbal formulation for improved taste, preparation of herbal curd and evaluation of its sensory characteristics, probiotic properties and nutritional composition. This approach aims to bridge the gap between therapeutic efficacy and consumer acceptability, providing a nutritionally enriched and medicinally valuable dairy product suitable for regular consumption by individuals with diabetes.

2 MATERIALS & METHODOLOGY

2.1 Study Design

The present study was designed to develop a probiotic sweet herbal (flavoured) curd using non-bitter medicinal herbs with reported antidiabetic potential and to evaluate its phytochemical, microbiological, probiotic, sensory and nutritional characteristics. The overall experimental workflow included selection of suitable herbs based on palatability and therapeutic relevance, preparation of herbal formulations, development of the curd product and subsequent evaluation using standard analytical and microbiological techniques.

2.2 Materials

2.2.1 Raw Materials

Fresh cow milk was procured from a local dairy and used as the primary substrate for curd preparation. A previously prepared curd sample was used as the starter culture to initiate fermentation.

2.2.2 Herbal Ingredients

The herbal ingredients selected for the study included *Glycyrrhiza glabra* (mulethi), *Gymnema sylvestre* (gudmar), *Stevia rebaudiana* (madhuparni), *Beta vulgaris* (beetroot), *Cinnamomum verum* (cinnamon), *Elettaria cardamomum* (cardamom), *Zingiber officinale* (dry ginger) and *Myristica fragrans* (nutmeg). These herbs were selected based on their reported antidiabetic properties and relatively acceptable taste profiles, especially those commonly used as dietary spices, to ensure better palatability.

2.2.3 Chemicals and Media

All chemicals and reagents used in the study were of analytical grade. Microbiological media such as de Man, Rogosa and Sharpe (MRS) agar and nutrient agar were used for the isolation and cultivation of lactic acid bacteria.

2.3 Preparation of Herbal Extracts

The procured herbal powders were cleaned, sieved and stored under dry conditions until use. For phytochemical screening, aqueous and methanolic extracts were prepared by mixing a known quantity of each herbal powder with the respective solvent and subjecting the mixture to continuous agitation for 24 hours. The extracts were then filtered using Whatman filter paper and the filtrates were collected and stored for further analysis. This method ensures efficient extraction of bioactive compounds from plant materials [7].

2.4 Phytochemical Analysis

Qualitative phytochemical screening of the prepared extracts was carried out to detect the presence of major bioactive constituents such as alkaloids, flavonoids, phenols, tannins, carbohydrates and phytosterols. Standard procedures described by Harborne (1998) [7] and Trease and Evans (2002) [8] were followed. The presence of these compounds was determined based on characteristic color changes or precipitate formation after the addition of specific reagents, indicating the therapeutic potential of the selected herbs.

2.5 Preparation of Herbal Probiotic Curd

Fresh milk was boiled and subsequently cooled to approximately 40°C under aseptic conditions. A standardized mixture of herbal powders was prepared by combining equal proportions (0.1 g each) of the selected herbs, while *Stevia rebaudiana* was added in a slightly higher amount (0.5 g) to impart natural sweetness and improve palatability. The herbal mixture was thoroughly mixed with the cooled milk, followed by the addition of 2–3% (v/v) starter culture. The mixture was incubated at 37°C for 8–12 hours to facilitate fermentation and curd formation. The incorporation of selected non-bitter herbs aimed to produce a flavoured curd with enhanced therapeutic value and improved sensory acceptance.

2.6 Sensory Evaluation

The prepared herbal curd was subjected to sensory evaluation using a semi-trained panel. The evaluation was carried out using a 9-point hedonic scale to assess parameters such as appearance, color, texture, taste and overall acceptability. The purpose of this analysis was to ensure that the inclusion of medicinal herbs did not adversely affect the organoleptic properties of the product and that the final formulation remained palatable [9].

2.7 Isolation of Probiotic Bacteria

For microbiological analysis, the prepared curd sample was serially diluted using sterile saline and appropriate dilutions were spread onto MRS agar plates. The plates were incubated at 37°C for 24–48 hours to allow the growth of lactic acid bacteria. Distinct colonies were selected based on morphological characteristics and subcultured to obtain pure isolates for further characterization.

2.8 Characterization of Isolates

The isolated bacterial cultures were characterized using morphological methods. Colony morphology, including size, shape, color and texture, was observed and Gram staining was performed to determine the Gram nature and cellular morphology. Lactic acid bacteria are typically Gram-positive, which served as important criteria for their identification [10].

2.9 Probiotic Properties

The probiotic potential of the isolates was evaluated by assessing their tolerance to acidic pH and bile salts. For acid tolerance, bacterial cultures were exposed to media adjusted to pH values ranging from 2.0 to 3.0 and incubated for a defined period, after which their viability was determined. For bile tolerance, the isolates were inoculated into media containing 0.3% bile salts and incubated under standard conditions. The ability of the isolates to survive under these conditions indicates their potential to withstand the gastrointestinal environment [11].

2.10 Antimicrobial Activity

The antimicrobial activity of the isolated lactic acid bacteria was evaluated using the agar well diffusion method against common pathogenic organisms, namely *Staphylococcus aureus* and *Escherichia coli*. The zones of inhibition around the wells were measured after incubation, indicating the antagonistic activity of the isolates.

2.11 Antibiotic Susceptibility Test

The antibiotic susceptibility pattern of the isolates was determined using the disc diffusion method. Antibiotic discs were placed on inoculated agar plates and the plates were incubated at 37°C for 24 hours. The diameter of the zones of inhibition was measured and interpreted according to standard guidelines [12].

2.12 Nutritional Analysis

The nutritional composition of the prepared herbal curd was analyzed using standard AOAC methods (AOAC, 2016). Parameters such as fat, protein, carbohydrates, moisture content, sodium, cholesterol and energy value were determined to evaluate the nutritional quality of the developed product.

2.13 Statistical Analysis

All experiments were conducted in triplicates and the results were expressed as mean \pm standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) and differences were considered statistically significant at $p < 0.05$.

3 RESULTS

3.1 Phytochemical Analysis

The qualitative phytochemical screening of the selected herbal extracts revealed the presence of several bioactive compounds responsible for their therapeutic potential. Both aqueous and methanolic extracts showed positive results for alkaloids, flavonoids, phenolic compounds and tannins, while phytosterols were detected predominantly in aqueous extracts. Carbohydrates were mainly observed in methanolic extracts.

Table 1. Phytochemical screening of selected herbs

Phytochemical	Methanolic Extract	Aqueous Extract
Alkaloids	+	+
Flavonoids	+	-
Phenols	-	+
Tannins	-	+
Carbohydrates	+	-
Phytosterols	-	+

The presence of flavonoids and phenolic compounds is particularly significant, as these compounds are known for their antioxidant and antidiabetic activities. They play a role in reducing oxidative stress and inhibiting carbohydrate-digesting enzymes, thereby contributing to glycemic control [3, 13]. The results confirm that the selected herbs possess bioactive constituents that support their inclusion in functional food formulations.

3.2 Sensory Evaluation

The developed herbal curd was evaluated for organoleptic properties using a hedonic scale. The product exhibited good sensory acceptance, indicating that the use of non-bitter herbs and natural sweetener (stevia) successfully improved palatability.

Table 2. Sensory evaluation of herbal curd (Mean \pm SD, n=3)

Parameter	Score
Appearance	6.2 \pm 0.3
Color	6.5 \pm 0.2
Texture	6.0 \pm 0.4
Taste	6.3 \pm 0.3
Overall acceptability	6.2 \pm 0.3

The results indicate moderate to good acceptability, with color and taste receiving relatively higher scores. The addition of commonly used spices such as cinnamon and cardamom contributed to a pleasant flavor profile, while stevia provided sweetness without increasing sugar content. This aligns with previous studies indicating that flavor masking using natural additives can improve acceptability of herbal formulations [14].

3.3 Isolation and Morphological Characterization of LAB

Lactic acid bacteria were successfully isolated from the prepared curd using MRS agar. The colonies appeared creamy white, smooth and mucoid, which are typical characteristics of LAB.

Table 3. Colony characteristics of isolated LAB

Characteristic	Observation
Shape	Rod
Size	Large
Margin	Smooth
Texture	Mucoid
Color	Creamy white
Gram reaction	Positive

Microscopic examination confirmed that the isolates were Gram-positive rods, consistent with *Lactobacillus* species. These findings are in agreement with standard descriptions of LAB used in fermented dairy products [10].

3.4 Probiotic Properties

The isolates demonstrated the ability to survive under acidic conditions and in the presence of bile salts, indicating their probiotic potential.

Table 5. Probiotic properties of LAB (Mean \pm SD, n=3)

Parameter	Result
Acid tolerance	7.4 \pm 0.5 %
Bile tolerance	3.33 \pm 0.2 %

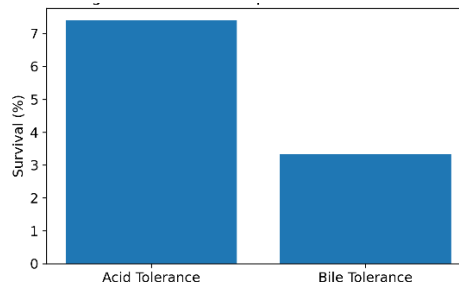


Figure 1. Acid and bile tolerance of LAB isolates

The ability of LAB to survive at low pH and in bile salts is essential for their survival in the gastrointestinal tract. The results indicate that the isolates possess characteristics required for probiotic functionality. Similar findings have been reported in studies evaluating LAB for probiotic applications (FAO/WHO, 2002; Kumar et al., 2012).

3.5 Antimicrobial Activity

The antimicrobial activity of LAB isolates was assessed against *Staphylococcus aureus* and *Escherichia coli*. The isolates exhibited inhibitory effects against both pathogens.

Table 6. Antimicrobial activity of LAB (Mean \pm SD, n=3)

Organism	Zone of Inhibition (mm)
<i>S. aureus</i>	8.5 \pm 2.1
<i>E. coli</i>	11.5 \pm 1.5

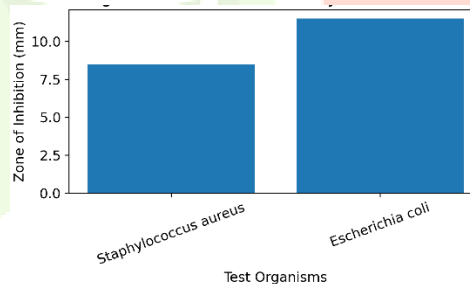


Figure 2: Antimicrobial activity of LAB

The higher inhibition observed against *E. coli* suggests that LAB metabolites such as organic acids and bacteriocins are effective against Gram-negative bacteria. This antimicrobial property enhances the functional value of the developed product and supports its role in improving gut health.

3.6 Antibiotic Susceptibility

The isolates showed varied responses to different antibiotics.

Table 7. Antibiotic susceptibility pattern

Antibiotic	Result
Erythromycin	Sensitive
Linezolid	Sensitive
Gentamicin	Sensitive
Vancomycin	Intermediate
Chloramphenicol	Intermediate
Streptomycin	Resistant
Ceftazidime	Resistant

The observed sensitivity pattern is important for assessing the safety of probiotic strains, as recommended by international guidelines [12].

3.7 Nutritional Analysis

The developed herbal curd was analyzed for its nutritional composition.

Table 8. Nutritional composition of herbal curd (Mean \pm SD, n=3)

Parameter	Value
Fat	4.68%
Protein	5.88 g/100g
Carbohydrates	4.45 g/100g
Moisture	84.99%
Sodium	88 mg/100g
Cholesterol	11 mg/100g
Energy	83.44 kcal

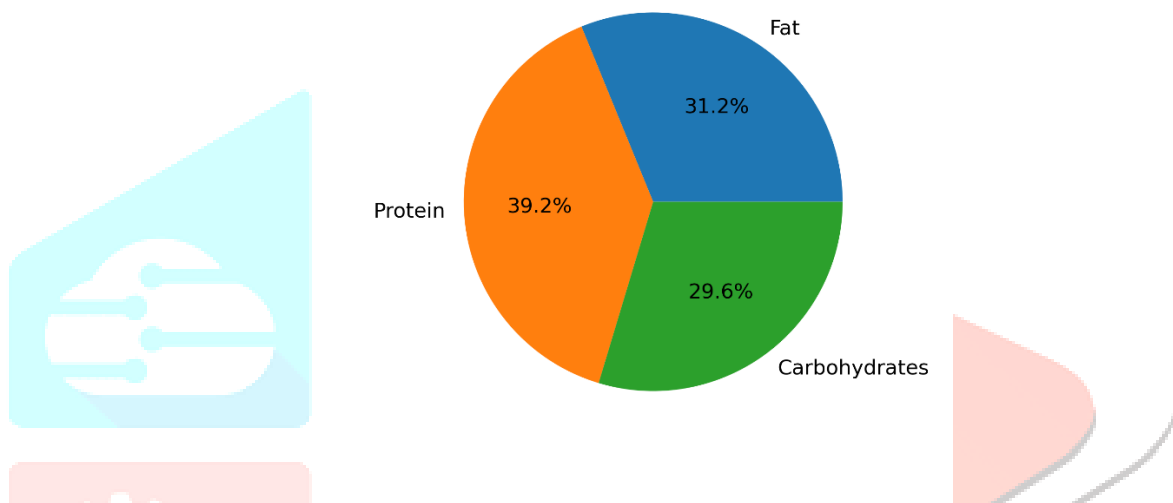


Figure 2. Nutritional profile of herbal curd

The nutritional analysis indicates that the developed product is a good source of protein with moderate fat content and low carbohydrate levels, making it suitable for diabetic individuals. The use of stevia as a natural sweetener further enhances its suitability as a low-sugar functional food.

3.8 Statistical Analysis

The results obtained were statistically analyzed using one-way ANOVA. Significant differences ($p < 0.05$) were observed in antimicrobial activity and probiotic survival rates, indicating reliability and reproducibility of the findings.

4 DISCUSSION

The present study successfully demonstrates the development of a probiotic sweet herbal (flavoured) curd incorporating non-bitter medicinal herbs with antidiabetic potential, while maintaining acceptable sensory attributes. The findings highlight an important advancement in functional food design, where therapeutic efficacy is integrated with palatability, addressing a key limitation of conventional herbal formulations.

The phytochemical screening of selected herbs confirmed the presence of bioactive compounds such as alkaloids, flavonoids, phenolics, and tannins, which are well documented for their antidiabetic and antioxidant properties. These compounds are known to regulate glucose metabolism through multiple mechanisms, including inhibition of α -amylase and α -glucosidase enzymes, enhancement of insulin secretion, and reduction of oxidative stress [3, 13]. The presence of phenolic compounds and flavonoids in particular plays a crucial role in improving insulin sensitivity and protecting pancreatic β -cells from oxidative damage. The results obtained in this study are consistent with earlier reports highlighting the therapeutic potential of *Gymnema sylvestre*, *Glycyrrhiza glabra*, and other medicinal plants used in traditional systems of medicine [4].

One of the major challenges in the development of herbal functional foods is poor palatability due to the inherent bitterness of many medicinal plants. In this study, the strategic selection of non-bitter, commonly consumed spices such as cinnamon, cardamom, ginger, and nutmeg, along with the use of *Stevia rebaudiana* as a natural sweetener, resulted in a product with acceptable sensory characteristics. The

sensory evaluation results indicated moderate to good acceptability, particularly in terms of taste and color. This suggests that flavor masking through natural ingredients and fermentation can significantly improve consumer acceptance of herbal formulations. Similar approaches have been reported to enhance palatability and compliance in functional food products [9].

The successful isolation and characterization of lactic acid bacteria (LAB) from the prepared curd further confirm the probiotic nature of the developed product. The isolates exhibited typical characteristics of LAB, including Gram-positive nature and negative catalase activity. These findings are consistent with previous studies on fermented dairy products, where *Lactobacillus* spp. are predominant and contribute to probiotic functionality [10]. The presence of viable LAB is essential for conferring health benefits associated with probiotic foods.

The probiotic potential of the isolates was validated through acid and bile tolerance tests, which are critical parameters for assessing the survival of probiotic bacteria in the gastrointestinal tract. The isolates demonstrated the ability to survive under acidic conditions (pH 2–3) and in the presence of bile salts, indicating their potential to withstand gastric and intestinal environments. These results align with the guidelines established by FAO/WHO (2002), which emphasize acid and bile tolerance as essential criteria for probiotic selection. Furthermore, previous studies have shown that LAB capable of surviving such conditions can modulate gut microbiota and contribute to improved metabolic health, including glycemic control.

The antimicrobial activity exhibited by the LAB isolates against *Staphylococcus aureus* and *Escherichia coli* highlights another important functional attribute of the developed product. The observed inhibition may be attributed to the production of organic acids, hydrogen peroxide, and bacteriocins by LAB, which are known to suppress pathogenic microorganisms. The relatively higher inhibition observed against *E. coli* suggests effective antagonistic activity against Gram-negative bacteria. This antimicrobial property enhances the safety and shelf-life of the product and contributes to improved gut health upon consumption.

The antibiotic susceptibility profile of the isolates revealed sensitivity to commonly used antibiotics such as erythromycin, gentamicin, and linezolid, while showing resistance to certain antibiotics. This pattern is important from a safety perspective, as probiotic strains should not harbor transferable antibiotic resistance genes. The results obtained are in accordance with standard safety assessment guidelines for probiotic strains [12], although further molecular studies would be required for comprehensive safety evaluation.

Nutritional analysis of the developed herbal curd indicated that it is a nutritionally balanced product, with adequate protein content, moderate fat levels, and relatively low carbohydrate content. The use of *Stevia rebaudiana* as a natural sweetener significantly reduces sugar content, making the product suitable for diabetic individuals. The presence of essential nutrients, along with probiotic microorganisms and bioactive phytochemicals, positions the developed curd as a multifunctional food with both nutritional and therapeutic benefits.

An important aspect of this study is the integration of traditional medicinal knowledge with modern food technology to develop a product that is both functional and consumer-friendly. Unlike conventional herbal formulations, which often suffer from poor taste and compliance issues, the developed flavoured curd offers a practical dietary alternative that can be easily incorporated into daily diets. This approach aligns with the growing trend toward functional foods as preventive and supportive strategies for chronic diseases such as diabetes.

However, despite the promising results, the study has certain limitations. The antidiabetic potential of the developed product was inferred based on phytochemical composition and literature reports, and no in vivo or clinical studies were conducted to validate its efficacy. Additionally, the probiotic characterization was limited to basic parameters, and advanced molecular identification of LAB strains was not performed. Therefore, future studies should focus on in vivo evaluation, clinical trials, and detailed characterization of probiotic strains to establish the therapeutic efficacy and safety of the product.

5 CONCLUSION

The study successfully developed a probiotic sweet herbal (flavoured) curd using non-bitter, antidiabetic herbs, combining therapeutic potential with improved palatability. The product exhibited acceptable sensory qualities, probiotic characteristics, antimicrobial activity, and a favorable nutritional profile, making it suitable as a functional food for diabetic individuals. Further studies are recommended to validate its clinical efficacy.

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