



PREBIOTIC EFFECTS OF CASSIA AURICULATA EXTRACTS ON GROWTH, VIABILITY, AND METABOLITE PRODUCTION IN LACTOBACILLUS ACIDOPHILUS: AN IN VITRO STUDY

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ABSTRACT

This study investigates the prebiotic potential of *Cassia auriculata* and its effect on the growth and functional activity of *Lactobacillus acidophilus*. Probiotics such as *Lactobacillus acidophilus* play a crucial role in maintaining gut microbiota balance, enhancing immunity, and preventing gastrointestinal disorders. However, their survival and efficacy depend on suitable substrates, particularly prebiotics. *Cassia auriculata*, a medicinal plant rich in polyphenols, flavonoids, and dietary fibers, was evaluated for its ability to support probiotic growth. The plant extract was prepared and co-incubated with probiotic cultures under controlled conditions. Growth parameters were analyzed using optical density measurements, Relative Growth Index (RGI), and Prebiotic Index (PI). Results indicated a significant increase in probiotic growth in the presence of *Cassia auriculata* extract compared to control. The findings suggest a strong synbiotic interaction between the plant extract and probiotic bacteria. This study highlights the potential application of *Cassia auriculata* as a natural prebiotic

ingredient in functional foods and nutraceuticals.

KEYWORDS: Probiotics, Prebiotics, *Cassia auriculata*, *Lactobacillus acidophilus*, Synbiotics, Gut Health.

INTRODUCTION

The human gastrointestinal tract contains a highly complex and diverse population of microorganisms collectively known as the gut microbiota. These microorganisms play a major role in maintaining human health by supporting digestion, nutrient absorption, metabolism, immune regulation, and protection against pathogenic organisms. Among the beneficial microorganisms present in the gut, probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium species* are considered highly important because of their therapeutic and health-promoting properties. Probiotics are defined as live microorganisms that provide health benefits to the host when consumed in adequate amounts. They help maintain microbial balance in the intestine and improve gastrointestinal health by preventing the colonization of harmful pathogens.

Lactobacillus acidophilus is one of the most widely studied probiotic bacteria and is commonly found in fermented dairy products such as yogurt, curd, and probiotic beverages. This bacterium produces lactic acid through carbohydrate fermentation, which lowers intestinal pH and inhibits the growth of harmful microorganisms. In addition, *Lactobacillus acidophilus* produces antimicrobial compounds such as bacteriocins and hydrogen peroxide, which contribute to the protection of the gastrointestinal tract from infections. The bacterium also plays a significant role in improving lactose digestion, reducing constipation, enhancing immune responses, and maintaining intestinal epithelial integrity.

Despite the numerous health benefits associated with probiotics, their survival during food processing, storage, and gastrointestinal transit remains a major challenge. Probiotic bacteria are highly sensitive to acidic gastric conditions, bile salts, digestive enzymes, and environmental stress factors. As a result, many probiotic organisms lose viability before reaching the intestine, thereby reducing their therapeutic effectiveness. To overcome this problem, researchers have focused on the use of prebiotics to improve the survival, growth, and functional activity of probiotic bacteria.

Prebiotics are non-digestible food ingredients that selectively stimulate the growth and activity of beneficial microorganisms in the gut. Unlike probiotics, prebiotics are not living organisms but are mainly dietary fibers, oligosaccharides, and plant derived compounds that

resist digestion in the upper gastrointestinal tract and reach the colon intact. In the colon, these compounds serve as substrates for beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, promoting their proliferation and metabolic activity. The fermentation of prebiotics by probiotic bacteria leads to the production of short-chain fatty acids such as acetate, propionate, and butyrate, which are essential for intestinal health and immune regulation.

In recent years, there has been increasing interest in the use of medicinal plants as natural sources of prebiotics due to their rich nutritional and bioactive composition. Plant derived prebiotics are considered safer, more economical, and environmentally friendly compared to synthetic alternatives. Medicinal plants contain various bioactive compounds including flavonoids, polyphenols, tannins, dietary fibers, and polysaccharides that can support the growth of beneficial gut bacteria. These plant compounds not only improve probiotic survival but also provide antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties.

One such medicinal plant with significant therapeutic value is *Cassia auriculata*, commonly known as Tanner's *Cassia* or Avaram. *Cassia auriculata* belongs to the family *Caesalpinaceae* and is widely distributed in India and other Asian countries. The plant has been extensively used in traditional Ayurvedic medicine for the treatment of diabetes, skin diseases, urinary disorders, gastrointestinal problems, and inflammation. The flowers, leaves, roots, and bark of the plant possess medicinal properties due to the presence of several phytochemicals such as flavonoids, phenolic compounds, alkaloids, glycosides, and tannins.

Among the different parts of the plant, the flowers of *Cassia auriculata* are particularly valued for their nutritional and therapeutic properties. They are rich in dietary fibers and polyphenolic compounds, which are capable of functioning as natural prebiotics. These compounds are not digested by human enzymes but are selectively utilized by probiotic microorganisms, thereby promoting their growth and metabolic activity. In addition to their prebiotic potential, *Cassia auriculata* flowers exhibit antioxidant, antimicrobial, antihyperglycemic, hepatoprotective, and anti-inflammatory effects, making them suitable for incorporation into functional foods and nutraceutical products.

The combination of probiotics and prebiotics is referred to as synbiotics. Synbiotics enhance the survival and colonization of beneficial microorganisms by providing a suitable nutritional environment. The synergistic interaction between probiotics and prebiotics helps improve gut

microbial balance, digestive function, nutrient absorption, and immune responses. The use of plant-based prebiotics along with probiotic bacteria has gained considerable attention in the development of functional foods and therapeutic dietary supplements.

In the present study, the prebiotic potential of *Cassia auriculata* flower extract was evaluated on the growth and functional efficacy of *Lactobacillus acidophilus*. The probiotic bacterium was isolated from fermented dairy products and cultured under laboratory conditions. Different concentrations of *Cassia auriculata* extract were prepared and co-incubated with the probiotic culture to determine their effect on bacterial growth. The growth of *Lactobacillus acidophilus* was assessed using optical density measurements, Relative Growth Index (RGI), and Prebiotic Index (PI).

The rationale behind selecting *Cassia auriculata* for this study lies in its rich phytochemical composition and its traditional medicinal importance. Since the plant contains fermentable fibers and bioactive compounds, it is expected to selectively enhance probiotic growth and activity. Furthermore, the use of natural plant-based prebiotics is becoming increasingly important due to growing consumer demand for functional foods with minimal synthetic additives.

The significance of this study extends beyond microbiological research, as it contributes to the development of novel synbiotic products that can improve gastrointestinal health and overall well-being. The findings may help in formulating probiotic dairy products, fermented beverages, dietary supplements, and nutraceutical formulations enriched with plant derived prebiotics. Additionally, the use of medicinal plants such as *Cassia auriculata* may provide a cost-effective and sustainable approach to improving probiotic efficacy. Overall, this project aims to explore the synergistic relationship between *Cassia auriculata* and *Lactobacillus acidophilus* and to establish the role of the plant extract as a natural prebiotic. The study highlights the growing importance of probiotics and prebiotics in maintaining gut health and demonstrates the potential application of medicinal plants in functional food development and therapeutic nutrition.

MATERIALS AND METHODS

Isolation and Identification of Probiotic bacteria

Probiotic bacteria were isolated from curd samples using serial dilution, spread plate, and streak plate techniques on MRS agar medium. The isolates were identified through Gram staining, biochemical tests, and carbohydrate fermentation tests.

Collection and Extraction of *Cassia auriculata*

An aqueous extract of *Cassia auriculata* flower powder was prepared by mixing the powder with distilled water, followed by incubation and filtration. Different concentrations of the extract (1%, 2%, and 3%) were co-incubated with *Lactobacillus acidophilus* in MRS broth under controlled conditions. Bacterial growth was evaluated using optical density measurements through a microtiter plate assay.

Preparation of Probiotic Inoculum

The isolated *Lactobacillus acidophilus* culture was inoculated into sterile MRS broth and incubated for 2 hours under anaerobic conditions to obtain actively growing bacterial cells.

The turbidity of the culture was adjusted to 0.5 McFarland standard using sterile MRS broth, corresponding to approximately 1.5×10^8 CFU/mL. Standardization of inoculum concentration ensured uniform bacterial growth during all experimental procedures.

Collection of Prebiotic Material

The prebiotic material used in this study was *Cassia auriculata* flower powder. The powdered material was procured from a certified herbal supplier in Chennai, Tamil Nadu, India. The material was obtained in sealed packaging to ensure quality and prevent contamination. The powder was stored in airtight containers under dry conditions until further use.

Preparation of Aqueous Extract

The aqueous extraction method was selected for extraction of bioactive compounds from *Cassia auriculata* because it efficiently extracts water-soluble polysaccharides and phytochemicals while maintaining their biological activity. One gram of finely powdered *Cassia auriculata* flower material was accurately weighed and transferred into a sterile conical flask. Twenty milliliters of distilled water was added to the flask and mixed thoroughly to ensure uniform dispersion of the powder.

The flask was placed in a shaking incubator maintained at 40°C with shaking at 40-50 rpm overnight for approximately 24 hours. After incubation, the extract was cooled to room temperature and filtered using Whatman No.1 filter paper to remove particulate matter. The clear filtrate obtained was used as the aqueous extract for further experimental procedures.

Co-incubation Study

The co-incubation method was employed to determine the effect of *Cassia auriculata* extract on probiotic growth. Different concentrations of the extract (1%, 2%, and 3%) were prepared and sterilized by filtration. Sterile MRS broth containing actively growing *Lactobacillus acidophilus* culture was supplemented with different concentrations of the extract. Control samples without plant extract were maintained simultaneously. The cultures were incubated under suitable anaerobic conditions at 37°C. After incubation, bacterial growth was evaluated by measuring optical density and observing turbidity. Increased turbidity in extract-treated samples compared to the control indicated enhanced bacterial growth and prebiotic activity of the plant extract.

Prebiotic Activity Assay

The prebiotic activity assay was performed using a sterile 96-well microtiter plate method. Different concentrations of *Cassia auriculata* extract were tested and compared with fructooligosaccharides (FOS), which served as the positive control. Glucose was used as the control carbohydrate, while blank wells without carbohydrate source were included to eliminate background interference. Each assay well contained 100 µL of substrate solution, 100 µL of sterile MRS broth, and 30 µL of standardized probiotic inoculum. The final volume of each well was maintained at 230 µL. The microtiter plates were incubated under suitable conditions, and bacterial growth was determined by measuring optical density at 600 nm using a microplate reader.

Determination of Prebiotic Index (PI)

The Prebiotic Index (PI) was calculated to evaluate the effectiveness of the extract in supporting probiotic growth relative to glucose control. The formula used for calculation was:

$$\text{PI} = \text{OD}_{600} (\text{prebiotic substrate}) / \text{OD}_{600} (\text{glucose control})$$

A PI value greater than 1 indicated enhanced probiotic growth compared to glucose. Higher PI values were observed at increased concentrations of *Cassia auriculata* extract, especially at 3% concentration, indicating strong prebiotic activity.

Determination of Relative Growth Index (RGI)

The Relative Growth Index (RGI) was calculated to evaluate the growth-promoting ability of the extract relative to glucose control. The formula used was:

$$\text{RGI (\%)} = \frac{\text{OD}_{600} \text{ prebiotic substrate}}{\text{OD}_{600} \text{ control carbohydrate (glucose)}} \times 100$$

Higher RGI values indicated improved bacterial growth in the presence of the plant extract. Increased turbidity observed in extract-treated wells confirmed concentration-dependent growth stimulation and demonstrated the prebiotic potential of *Cassia auriculata* extract.

RESULT AND DISCUSSION

To evaluate the prebiotic potential of *Cassia auriculata* flower extract on the growth and functional efficacy of *Lactobacillus acidophilus*. The probiotic organism was successfully isolated from fermented dairy products using serial dilution and spread plate techniques on MRS agar medium. After incubation, characteristic creamy white colonies were observed. Microscopic examination after Gram staining revealed Gram-positive rod-shaped bacteria arranged singly and in short chains. The isolate also showed a negative catalase reaction, confirming the identity of *Lactobacillus acidophilus*.

The carbohydrate fermentation test was performed using Bromocresol Purple (BCP) broth supplemented with different sugars to evaluate the metabolic activity of the isolated *Lactobacillus acidophilus*. The organism showed positive fermentation for carbohydrates such as glucose, lactose, and sucrose, indicated by the color change of the medium from purple to yellow due to acid production. Among the tested sugars, glucose showed maximum fermentation with intense yellow coloration, while lactose and sucrose also demonstrated significant acid production. The results confirm the ability of *Lactobacillus acidophilus* to efficiently utilize carbohydrates and produce lactic acid as the primary metabolic end product.

Table 1

Different concentrations of aqueous *Cassia auriculata* extract (1%, 2%, and 3%) were used to evaluate their effect on probiotic growth. The growth of *Lactobacillus acidophilus* was analyzed using a microtiter plate assay based on Optical Density (OD) measurements. The results showed a clear increase in bacterial growth in all extract-treated samples compared to the control. Among the tested concentrations, the 3% extract exhibited the highest OD value, indicating maximum probiotic proliferation. The 2% extract also showed significant enhancement, while the 1% extract demonstrated moderate growth stimulation. **Figure 1**

The Increase in bacterial growth may be attributed to the presence of bioactive compounds such as dietary fibers, flavonoids, phenolic compounds, and oligosaccharides present in

Cassia auriculata. These compounds act as fermentable substrates that support the metabolic activity and proliferation of beneficial probiotic bacteria. The findings indicate that the plant extract provides a favorable environment for probiotic growth.

The Relative Growth Index (RGI) was calculated to determine the efficiency of the extract in promoting bacterial growth relative to the standard substrate. The RGI values increased with increasing concentration of the plant extract, with the highest value observed in the 3% concentration. An RGI value greater than one indicates enhanced growth of the probiotic organism, confirming the strong prebiotic effect of *Cassia auriculata*. This suggests that the nutrients present in the extract were effectively utilized by *Lactobacillus acidophilus*. **Figure 2**

The Prebiotic Index (PI) analysis further supported these findings by demonstrating selective stimulation of probiotic growth. Positive PI values were observed in all treated samples, with the maximum value recorded in the 3% extract concentration. This confirms that *Cassia auriculata* possesses significant prebiotic properties capable of enhancing the viability and functional efficacy of probiotic bacteria. **Figure 3**

To demonstrate a strong synbiotic relationship between *Cassia auriculata* and *Lactobacillus acidophilus*. The plant extract acted as a natural prebiotic, while the probiotic organism utilized the bioactive compounds for enhanced growth and metabolic activity. Such synergistic interactions are beneficial for maintaining gut microbial balance, improving digestion, increasing nutrient absorption, and strengthening immune function.

Overall, the results of the *Cassia auriculata* can serve as an effective plant-based prebiotic ingredient. Its ability to significantly enhance the growth and activity of *Lactobacillus acidophilus* highlights its potential application in the development of functional foods, probiotic dairy products, nutraceuticals, and therapeutic formulations aimed at improving gastrointestinal health and overall well-being.

TABLE

Table No 1: Sugar Fermentation Test For *Lactobacillus Acidophilus*.

SUGAR NAME	<i>Lactobacillus acidophilus</i>
Glucose	+
Galactose	+
Sucrose	+

Mannitol	-
Raffinose	-

GRAPHICAL REPRESENTATION

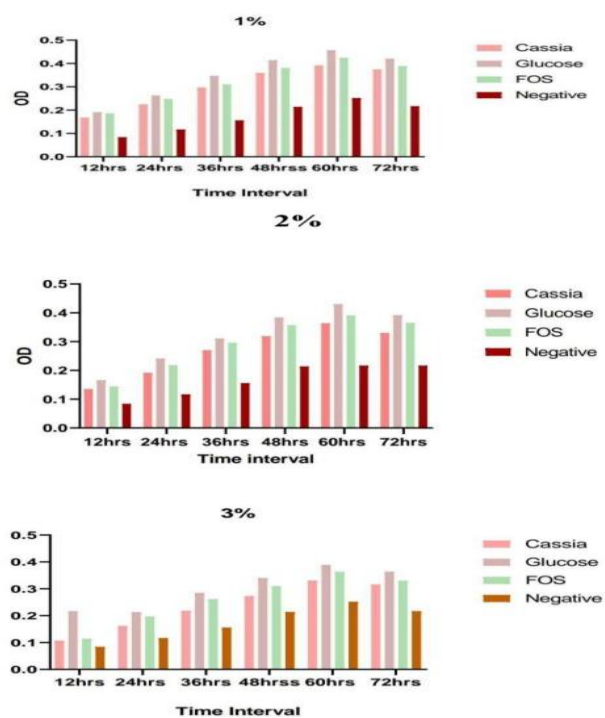


Figure 1: Sample.

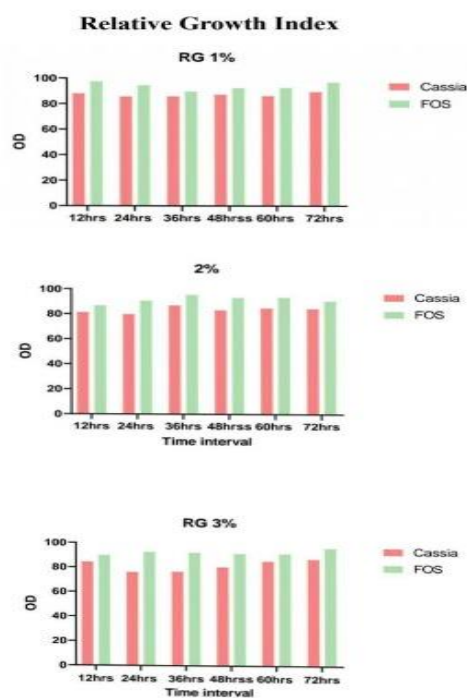


Figure 2: Relative Growth Index.

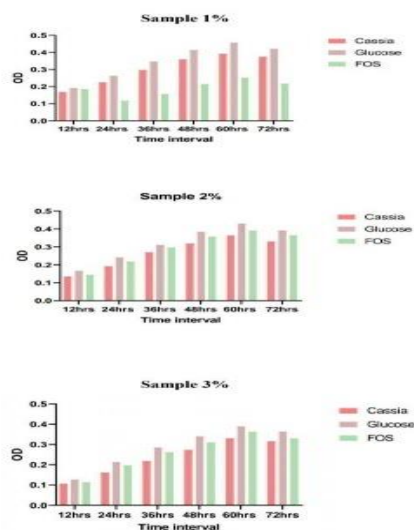


Figure 3: Prebiotic Index.

The graphs represent the growth pattern of *Lactobacillus acidophilus* in the presence of different concentrations (1%, 2%, and 3%) of *Cassia auriculata* extract. The bacterial growth was analyzed using optical density (OD) values measured at different time intervals such as 12, 24, 36, 48, 60, and 72 hours. Optical density indicates the turbidity of the culture medium, which directly reflects bacterial growth. Higher OD values indicate increased bacterial proliferation and better probiotic activity.

In the 1% concentration graph, the OD values gradually increased from 12 hours to 60 hours, showing steady growth of *Lactobacillus acidophilus* in the presence of *Cassia auriculata* extract. Although the growth was lower than glucose and fructooligosaccharides (FOS), the extract still supported probiotic growth effectively. The highest growth was observed at 60 hours, followed by a slight decrease at 72 hours, which may be due to nutrient depletion and accumulation of acidic metabolic products. The negative control showed very low OD values throughout the experiment, confirming that bacterial growth depended on the availability of a suitable carbon source.

In the 2% concentration graph, improved bacterial growth was observed compared to the 1% concentration. The OD values increased continuously up to 60 hours, indicating better utilization of the plant extract by the probiotic bacteria. The growth pattern was closer to the FOS control, suggesting enhanced prebiotic activity at this concentration. A slight decline in growth at 72 hours was also observed, likely due to reduced nutrient availability in the medium.

The 3% concentration graph showed the highest probiotic growth among all tested concentrations. The OD values increased significantly from 12 hours to 60 hours, indicating strong stimulation of *Lactobacillus acidophilus* growth by the plant extract. The growth pattern was almost comparable to glucose and FOS controls, demonstrating the effectiveness of *Cassia auriculata* extract as a natural prebiotic source. Similar to the other concentrations, a small reduction in OD was observed at 72 hours because of nutrient exhaustion and accumulation of inhibitory metabolites.

The sample graphs further confirmed that glucose supported maximum bacterial growth because it is an easily fermentable carbohydrate source. FOS also showed high growth-promoting activity as it is a standard prebiotic. *Cassia auriculata* extract demonstrated significant probiotic growth enhancement, especially at 2% and 3% concentrations.

The Relative Growth Index (RGI) graphs showed high percentage growth values for all concentrations of the extract. The RGI values increased with increasing concentration of the extract, indicating concentration-dependent probiotic stimulation. Among all concentrations, the 3% extract showed the highest RGI values, confirming maximum prebiotic activity.

Overall, the graphical analysis clearly demonstrates that *Cassia auriculata* extract effectively promotes the growth of *Lactobacillus acidophilus* and possesses significant prebiotic potential. The growth enhancement was concentration dependent, with the 3% concentration showing the best probiotic growth and highest Relative Growth Index. These findings suggest that *Cassia auriculata* can be used as a natural prebiotic source to support probiotic bacterial growth and improve gut microbial health.

CONCLUSION

The study provides compelling evidence that *Cassia auriculata* extract has significant prebiotic activity, promoting the growth of *Lactobacillus acidophilus*. The 3% concentration of the extract yielded the best results, emphasizing the concentration-dependent and time-dependent nature of its prebiotic effects. This extract showed growth stimulation similar to glucose, suggesting that plant-based prebiotics can be as effective as traditional carbohydrate-based sources. The findings support the potential of *Cassia auriculata* extract as a natural, sustainable prebiotic that could be incorporated into functional foods and dietary supplements, benefiting gut health and overall wellness. Future studies could further explore the specific bioactive components responsible for these effects, potentially leading to more targeted applications in health and nutrition.

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