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Research Article

Prebiotic Potential of *Gymnema sylvestre* Leaf Extract: An *In-Vitro* Evaluation Using Selected Probiotic Strains

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ABSTRACT

Plant-derived extracts are being investigated as potential sources of natural prebiotic ingredients because they provide carbohydrates and other bioactive constituents capable of supporting beneficial microorganisms. *Gymnema sylvestre* is a medicinal plant traditionally recognized for its pharmacological properties, but comparatively limited information is available regarding the in-vitro prebiotic potential of its aqueous leaf extract. Fresh *Gymnema sylvestre* leaves were authenticated, shade-dried and powdered, and an aqueous extract was prepared by maceration. The percentage yield of extract was 9.4%. The dried extract was subjected to qualitative phytochemical screening and FTIR characterization. Growth stimulation was evaluated in MRS medium supplemented with GSAE (*Gymnema sylvestre* Aqueous Extract) at 0.25% and 0.50% using viable colony counts for *Lactobacillus* sp. strain YVU and *Bacillus stercoris* strain VFT. Carbohydrates, reducing sugars, flavonoids, alkaloids, tannins, phenolic compounds and saponins were detected. FTIR analysis showed characteristic bands at 3395, 2924, 1734, 1632 and 1246–1074 cm^{-1} . In the growth assay, 0.25% GSAE produced the highest reported viable counts for both *Bacillus stercoris* (122 ± 8) and *Lactobacillus* sp. (115 ± 10), compared with their respective controls (80 ± 5 and 60 ± 5). Gastric hydrolysis ranged from $13.8 \pm 0.5\%$ at pH 1.0 to $3.9 \pm 0.2\%$ at pH 4.0, while intestinal hydrolysis was $5.6 \pm 0.3\%$ at pH 6.8 and $3.8 \pm 0.2\%$ at pH 7.4. GSAE demonstrated growth-promoting potential towards probiotic strains and relatively low hydrolysis under the reported simulated gastrointestinal conditions. These results indicate that GSAE may serve as a potential plant-derived prebiotic ingredient for promoting the growth of beneficial probiotic strains.

INTRODUCTION

The gastrointestinal tract contains a complex microbial community that contributes to digestion,

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metabolic processes, immune regulation and maintenance of intestinal homeostasis. Growing interest in the gut microbiota has increased research into probiotics, prebiotics, postbiotics and synbiotics as functional approaches for supporting host health. Prebiotics are substrates that are selectively utilized by host microorganisms and can produce beneficial changes in the composition or activity of the microbiota. Examples include inulin, fructo-oligosaccharides and galacto-oligosaccharides, recent research has expanded toward resistant starches, plant polysaccharides and selected plant-derived compounds.^[1,2,3]

Plant materials are attractive sources for prebiotic research because they contain dietary carbohydrates, fibers, polyphenols and other phytochemicals. Several plant, fruit, vegetable and herbal extracts have been reported to stimulate beneficial bacterial growth or demonstrate resistance to simulated gastrointestinal digestion. Such properties may allow plant-derived materials to reach the intestinal environment in a form that remains available for microbial utilization.^[4,5,6]

Gymnema sylvestre is a perennial woody climber widely distributed in tropical regions and is traditionally known as Gurmar. Leaves contain triterpenoid saponins, gymnemic acids, flavonoids, alkaloids, tannins and phenolic constituents. Previous research has extensively investigated its antidiabetic, antioxidant, antimicrobial and other pharmacological properties. However, the prebiotic potential of its aqueous leaf extract remains comparatively less explored.^[7,8,9,10]

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL AND EXTRACTION

Fresh leaves of *Gymnema sylvestre* were collected in the month of January, 2026 and authenticated by

the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh (Plant Code No. SVUH: 2610). The leaves were washed, shade-dried and powdered. 50g of powder were mixed with 500 mL distilled water (1:10 w/v) and subjected to maceration for 48 h with intermittent stirring. The mixture was filtered through Whatman No. 1 filter paper. The filtrate was concentrated at 35–40°C by using rotary vacuum evaporator and the dried extract was stored at 4°C in amber colored glass bottles for future use.^[11,12,13]

Percentage yield: Percentage yield (%) = (weight of dried extract / weight of plant powder) × 100.

2.2 QUALITATIVE PHYTOCHEMICAL SCREENING

The obtained extract was designated as GSAE (*Gymnema sylvestre* Aqueous Extract). The aqueous extract was subjected to preliminary qualitative phytochemical screening. Carbohydrates were assessed by Molisch's test, reducing sugars by Benedict's test, proteins by the Biuret test, amino acids by the Ninhydrin test, alkaloids by Dragendorff's test, flavonoids by the Shinoda test, tannins and phenolic compounds by ferric chloride tests, saponins by the foam test, terpenoids by the Salkowski test, steroids by the Liebermann–Burchard test and glycosides by the Keller–Killiani test.^[14,15]

2.3 FTIR CHARACTERIZATION

The dried aqueous extract was finely powdered. Approximately 1–2 mg of extract was mixed thoroughly with 100–200 mg spectroscopic-grade potassium bromide (KBr). The mixture was compressed under high pressure using a hydraulic press to prepare a transparent KBr pellet. The pellet was immediately placed in the FTIR spectrophotometer, and the spectrum was recorded



over 4000–400 cm^{-1} . Characteristic absorption bands were interpreted with respect to their corresponding functional groups^[16, 17, 18]

2.4 PROBIOTIC STRAINS AND CULTURE CONDITIONS

The study used *Lactobacillus sp.* strain YVU and *Bacillus stercoris* strain VFT. *Lactobacillus sp.* strain YVU was identified by 16S rRNA gene sequencing and reported with GenBank accession PP587743.1; its isolation source was Bajra crop root soil. *Bacillus stercoris* strain VFT was identified by 16S rRNA gene sequencing and reported with GenBank accession PX992070.1; its isolation source was fermented tomatoes.

De Man–Rogosa–Sharpe (MRS) broth and MRS agar were used as culture media. Culture media were sterilized by autoclaving at 121°C and 15 psi for 15–20 min. Active cultures were prepared in sterile MRS broth and incubated at 37°C for 24 h.^[19,20]

2.5 GROWTH STIMULATION ASSAY

The growth stimulation assay was performed to evaluate the effect of GSAE on viable bacterial growth. MRS broth containing the bacterial culture without extract served as the control. Treatment groups contained GSAE at 0.25% or 0.50% (w/v). For *Bacillus stercoris*, 0.025 g and 0.05 g extract were added to 10 mL broth for the 0.25% and 0.50% treatments, respectively. The corresponding quantities were used for *Lactobacillus sp.* cultures. Cultures were incubated at 37°C for 24 h.

Following incubation, cultures were centrifuged at 3800 rpm for 10 min, the supernatant was discarded, and the bacterial pellet was washed with sterile PBS. The pellet was resuspended in PBS and serially diluted up to 10^{-7} . Appropriate

dilutions were spread on MRS agar and incubated at 37°C for 24 h. Colonies were counted and bacterial population was expressed as CFU/mL.^[21-26]

2.6 SIMULATED GASTRIC ACID HYDROLYSIS

Artificial gastric fluid was prepared using NaCl (8 g/L), $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (8.25 g/L), KCl (0.2 g/L), NaH_2PO_4 (14.35 g/L), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.18 g/L) and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1 g/L). The pH was adjusted to 1.0, 2.0, 3.0 and 4.0 using HCl, followed by addition of pepsin to a final concentration of 1000 U/mL. GSAE was prepared at 0.5% (w/v) and mixed with 2 mL artificial gastric fluid. The reaction mixtures were incubated at 37°C. Measurements were recorded after 24h of incubation. Reducing sugar and total carbohydrate contents were estimated and hydrolysis was calculated as:

$$\text{Hydrolysis (\%)} = \left(\frac{\text{reducing sugar released}}{\text{total carbohydrate content}} \right) \times 100 \quad [27,28]$$

2.7 SIMULATED INTESTINAL FLUID HYDROLYSIS

For simulated intestinal digestibility, the thesis describes artificial intestinal fluid consisting of 1× PBS containing 0.5% (w/v) bile salts and trypsin at a final concentration of 1000 U/mL. The methodology section reports adjustment to pH 8.0, whereas the Results section reports measurements at pH 6.8 and 7.4. Measurements were recorded after 24h of incubation. Reducing sugar and total carbohydrate contents were estimated and hydrolysis was calculated.^[29,30]

3. RESULTS & DISCUSSION

3.1 EXTRACTION YIELD



From 50 g of *Gymnema sylvestre* leaf powder, 4.7 g of dried aqueous extract was obtained. The calculated extraction yield was 9.4%.

Table 1: Extraction yield of GSAE

Parameter	Value
Plant powder used	50 g
Dried extract obtained	4.7 g
Extraction yield	9.4%

Table 2: Phytochemical screening of GSAE

Phytochemical constituents	Result
Saponins	Present
Tannins	Present
Phenolic compounds	Present
Carbohydrates	Present
Reducing sugars	Present
Flavonoids	Present
Alkaloids	Present

3.2 PHYTOCHEMICAL PROFILE

3.3 FTIR CHARACTERIZATION OF GSAE

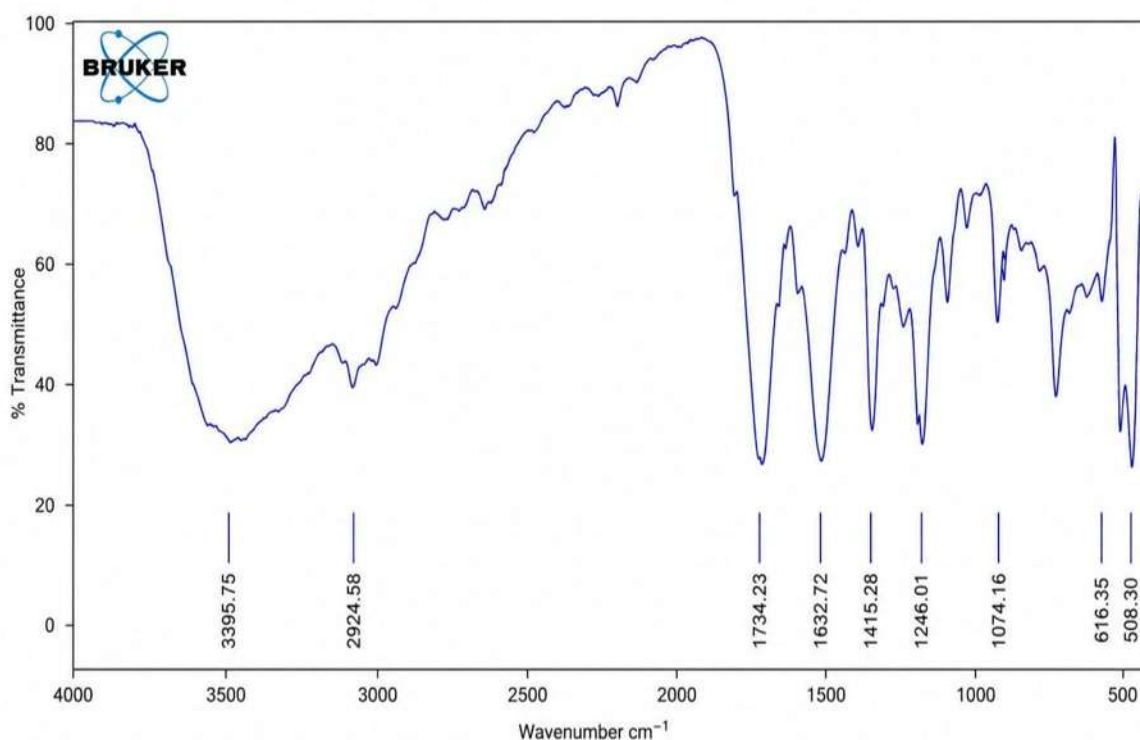


Figure 1: FTIR Spectrum of *Gymnema sylvestre* leaves aqueous extract (GSAE)

Table 3: FTIR Interpretation of GSAE

Peak (cm ⁻¹)	Observation	Significance
3395	Broad O–H stretching band	Indicates hydroxyl groups from phenols and polysaccharides.
2924	C–H stretching	Corresponds to aliphatic C–H stretching
1734	C=O stretching	Indicates the presence of carbonyl groups.
1632	C=C stretching	Confirm aromatic compounds and flavonoids.
1246–1074	C–O and C–O–C stretching	Indicates glycosides and carbohydrate-rich polysaccharides are associated with prebiotic properties.

From Figure 1, Table 3: The FTIR spectrum of the aqueous extract of *Gymnema sylvestre* leaves confirmed the presence of hydroxyl, carbonyl, aromatic, glycosidic, and carbohydrate functional

groups. The characteristic absorption bands in the carbohydrate region (1246–1074 cm⁻¹) indicate the presence of polysaccharides and glycosides, which may support the growth of beneficial

probiotic bacteria suggesting potential prebiotic activity.

3.4 GROWTH STIMULATION ASSAY OF *Bacillus stercoris* strain VFT



Figure 2. Growth of *Bacillus stercoris* in control & treatment groups

Table 4: Effect of GSAE on *Bacillus stercoris* strain VFT on growth stimulation

<i>Bacillus stercoris</i> strain VFT	Mean colony count $\pm$ SD	Observation
Control	80 ± 5	Normal growth
GSAE 0.25%[w/v]	122 ± 8	Maximum growth
GSAE 0.50%[w/v]	100 ± 5	Good growth

S.D: Standard deviation

From Figure 2, Table 4: The control showed a mean colony count of 80 ± 5 , whereas 0.25% and 0.50% GSAE showed 122 ± 8 and 100 ± 5 , respectively. The highest growth was observed at 0.25% GSAE, followed by 0.50% GSAE. These results indicate that GSAE promoted the growth of *Bacillus stercoris* compared with the control under the tested conditions.

3.4 GROWTH STIMULATION ASSAY OF *Lactobacillus* sp. Strain YVU

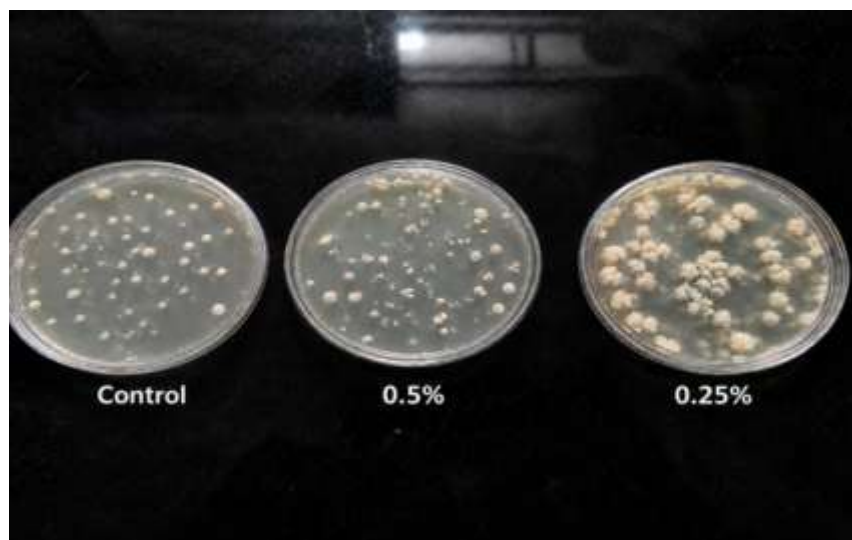


Figure 3. Growth of *Lactobacillus* sp. in control & treatment groups

Table 5: Effect of GSAE on *Lactobacillus* sp. strain YVU on growth stimulation

<i>Lactobacillus</i> sp. strain YVU	Mean colony count $\pm$ SD	Result
Control	60 ± 5	Normal growth
GSAE 0.25% [w/v]	115 ± 10	Maximum growth (large colony size)
GSAE 0.50% [w/v]	80 ± 7	Good growth

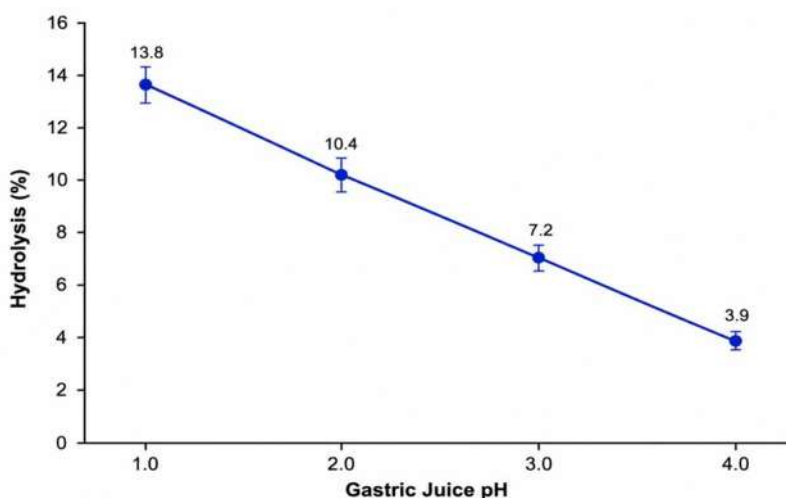
S.D: Standard deviation

From Figure 3, Table 5: The control showed a mean colony count of 60 ± 5 , whereas 0.25% and 0.50% GSAE showed 115 ± 10 and 80 ± 7 , respectively. The highest growth was observed with 0.25% GSAE, along with larger colony size, followed by 0.50% GSAE. These results indicate that GSAE promoted the growth of *Lactobacillus* sp. compared with the control under the tested conditions.

3.6 SIMULATED GASTRIC HYDROLYSIS

Table 6: Effect of gastric pH on hydrolysis of *G. sylvestre* leaves aqueous extract

Sr. No	pH of Gastric Juice	Incubation time (h)	% Hydrolysis	Observation
1.	1.0	2	13.8 ± 0.5	Maximum hydrolysis observed under highly acidic condition
2.	2.0	2	10.4 ± 0.4	Moderate
3.	3.0	2	7.2 ± 0.3	Lower
4.	4.0	2	3.9 ± 0.2	Minimum hydrolysis, better stability

**Figure 4: Effect of gastric pH on hydrolysis of GSAE**

From Table 6, Figure 4: The percentage hydrolysis decreased with increasing pH of the simulated gastric juice. The highest hydrolysis ($13.8 \pm 0.5\%$) was observed at pH 1.0, indicating greater susceptibility to acid hydrolysis under highly acidic conditions. As the pH increased to 4.0,

hydrolysis decreased to $3.9 \pm 0.2\%$, suggesting improved stability of the sample under milder gastric conditions.

3.7 :SIMULATED INTESTINAL FLUID HYDROLYSIS

Table 7: Effect of intestinal pH on hydrolysis of GSAE

Sr. No	pH of Intestinal Juice	Incubation time (h)	% Hydrolysis	Observation
1.	6.8	2	5.6 ± 0.3	Slight hydrolysis observed
2.	7.4	2	3.8 ± 0.2	Low hydrolysis and better stability



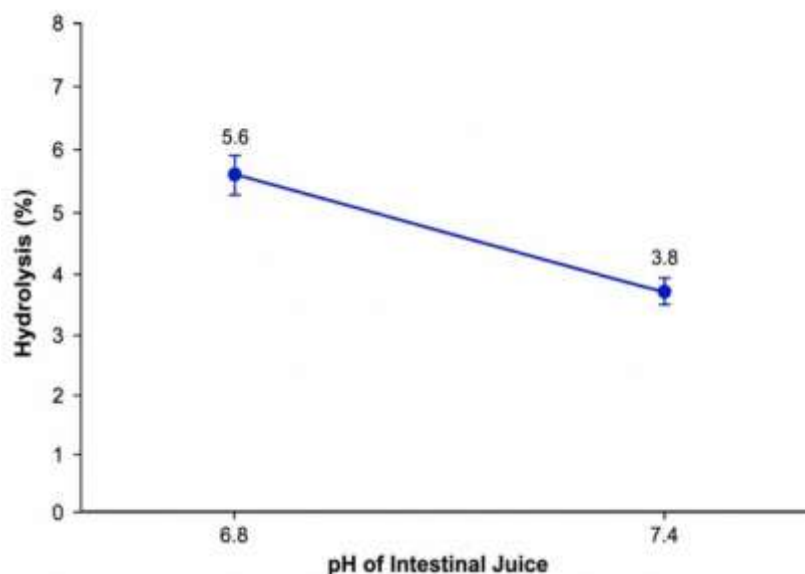


Figure 5. Effect of intestinal pH on hydrolysis of GSAE

From Table 7, Figure 5: The sample exhibited low hydrolysis under simulated intestinal conditions. At pH 6.8, the percentage hydrolysis was $5.6 \pm 0.3\%$, indicating slight degradation. At pH 7.4, hydrolysis decreased to $3.8 \pm 0.2\%$, demonstrating greater stability under near-neutral intestinal conditions. The low degree of hydrolysis suggests that the sample remained relatively resistant to intestinal digestion, supporting its potential prebiotic activity.

4. CONCLUSION

The present study evaluated the *in-vitro* prebiotic potential of *Gymnema sylvestre* leaves aqueous extract on probiotic strains (*Lactobacillus* spp. and *Bacillus stercoris*). The extract demonstrated the ability to promote the growth of probiotic bacteria, indicating its potential as a natural prebiotic. The gastric acid and intestinal fluid resistance studies suggested that the extract remained relatively stable under simulated gastrointestinal conditions, which is an important characteristic of an effective prebiotic.

FTIR analysis confirmed the presence of functional groups associated with carbohydrates

and other bioactive compounds that may contribute to its prebiotic efficacy. Overall, the study suggests that *Gymnema sylvestre* leaves aqueous extract has promising potential as a natural prebiotic ingredient and may be explored further for its application in functional foods and nutraceutical formulations.

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