International Journal of Basic & Clinical Pharmacology
Dhabadi P et al. Int J Basic Clin Pharmacol. 2026 Sep;15(5):896-903
https://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20262859

Original Research Article

Evaluation of antidiarrheal, antiulcer, anti-collagenase and cholesterol lowering activity of *Heyndrickxia coagulans* MTCC 25308 (Bacospore®)

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Received: 22 May 2026
Revised: 17 June 2026
Accepted: 06 July 2026

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ABSTRACT

Background: Probiotics are increasingly recognized for their role in promoting gastrointestinal health and metabolic regulation. The present study investigated the multifunctional biological activities of *Heyndrickxia coagulans* (formerly *Bacillus coagulans*) MTCC 25308 (Bacospore®), focusing on its antiulcer, antidiarrheal, cholesterol-lowering, and anti-collagenase properties.
Methods: Antiulcer activity was evaluated in rats using an ethanol-induced gastric ulcer model. Bacospore® was administered orally at doses of 100 and 200 mg/kg body weight, and its effects were compared with ranitidine (20 mg/kg body weight). Antidiarrheal activity was assessed in a castor oil-induced diarrhea model using the same doses and compared with loperamide. Anti-collagenase activity was determined in vitro using enzyme inhibition assays, while cholesterol-lowering potential was assessed through bile salt hydrolase (BSH)-mediated cholesterol reduction assays.
Results: Bacospore® exhibited significant gastroprotective activity against ethanol-induced gastric ulcers, with the highest ulcer inhibition observed at 200 mg/kg body weight, surpassing the protective effect of ranitidine. In the castor oil-induced diarrhea model, Bacospore® demonstrated notable antidiarrheal activity comparable to the standard treatment. *In vitro* studies showed strong collagenase inhibition, reaching 85% at 200 µg/ml, indicating potential extracellular matrix-protective effects. Additionally, Bacospore® achieved 97.72% cholesterol reduction at 1000 µg/ml through BSH-mediated bile salt deconjugation, suggesting substantial hypocholesterolemic potential.
Conclusions: *Heyndrickxia coagulans* MTCC 25308 (Bacospore®) demonstrated significant antiulcer, antidiarrheal, anti-collagenase, and cholesterol-lowering activities. These findings suggest that Bacospore® exerts pleiotropic health benefits through modulation of gastrointestinal function, enzymatic activity, and lipid metabolism, supporting its potential as a multifunctional next-generation probiotic for managing gastrointestinal disorders and metabolic dysregulation.

Keywords: Bile salt hydrolase, Collagenase inhibition, Gastroprotective activity, *Heyndrickxia coagulans*, Lipid metabolism

INTRODUCTION

The World Health Organization defines probiotics as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.[1] Probiotics are live microorganisms that are marketed as having health advantages when ingested, usually by enhancing or replenishing the gut flora. Last few years, the role of that probiotics play in human health and diseases has fascinated the attention of researchers. This is due to an outstanding performance of probiotics in preventing and an attenuate disease, as well as the public's increasing

inclination for natural therapies. The term probiotic refers to food supplements formulated to enhance human health.[2] Lactobacillus, Bifidobacterium, and Bacillus species are examples of probiotic organisms that are utilized for human consumption in a variety of products, including infant meals, cultured milks, and pharmaceutical preparations (tablets, capsules, sachets, etc.).

H. coagulans is a Gram-positive, spore-forming, lactic acid-producing bacterium that has emerged as a highly stable and efficacious probiotic. Unlike conventional non-spore-forming probiotics such as Lactobacillus and Bifidobacterium, *H. coagulans* produces heat-resistant endospores, enabling it to withstand extreme environmental conditions including high temperature, gastric acidity, and bile salts. Metabolically, Bacillus species are highly active, and prior studies have found that they produce a variety of beneficial enzymes and antimicrobes. In addition to these secreted metabolites, *Heyndrickxia* exhibits greater stability in probiotic products, owing to its ability to form endospores [3]. This unique characteristic ensures improved shelf stability, enhanced survival through the gastrointestinal tract, and better colonization efficiency, thereby increasing its therapeutic potential.[4-6] Due to these advantageous properties, *H. coagulans* has been widely investigated for its role in metabolic and gastrointestinal disorders.

Hypercholesterolemia, a major contributor to cardiovascular diseases, is commonly managed using synthetic drugs such as statins (e.g., atorvastatin), which act by inhibiting HMG-CoA reductase. However, long-term statin use is frequently linked to adverse effects such hepatotoxicity, myopathy, and gastrointestinal disturbances.[7] In contrast, probiotics such as *H. coagulans* offer a safer and more natural approach to cholesterol reduction through mechanisms including cholesterol assimilation, bile salt deconjugation via bile salt hydrolase activity, and modulation of lipid metabolism pathways.[8] Therefore, evaluating the *in-vitro* cholesterol-lowering activity of *H. coagulans* essential to explore its potential as an alternative hypocholesterolemic agent. In addition to lipid regulation, *H. coagulans* exhibits potential in inhibiting collagenase, an enzyme responsible for the degradation of collagen in connective tissues. Excessive collagenase activity contributes to skin aging, arthritis, and extracellular matrix breakdown. Although synthetic collagenase inhibitors are available; their use is often limited by toxicity and lack of specificity.[9] Natural bioactive compounds derived from probiotics have shown promising anti-collagenase activity with improved safety profiles.[10] Hence, assessing the anti-collagenase activity of *H. coagulans* can provide insight into its role in tissue protection and anti-aging applications. Gastric ulcers are a common gastrointestinal condition brought on by an imbalance between the defensive mechanisms of the stomach mucosa and aggressive forces such Helicobacter pylori infection, oxidative stress, and gastric acid secretion. Conventional therapies, including proton pump inhibitors (e.g., omeprazole) and H_2-receptor antagonists, are effective but often associated with long-term complications such as nutrient malabsorption, rebound acid hypersecretion, and increased susceptibility to infections.[11] Probiotics like *H. coagulans* provide a promising alternative by enhancing mucosal defence, reducing inflammation, producing antimicrobial compounds, and inhibiting pathogenic bacteria.[12,13] Thus, *in-vivo* evaluation of its anti-ulcer activity is necessary to validate its gastroprotective effects. Diarrhea remains a serious worldwide health concern, especially in developing countries, and is often associated with infections and gut microbiota imbalance. Common antidiarrheal drugs such as loperamide provides symptomatic relief but do not restore the underlying microbial balance and may lead to adverse effects such as constipation and dependency with prolonged use. In contrast, *H. coagulans* exerts anti-diarrheal effects by restoring intestinal microbiota, enhancing gut barrier integrity, producing antimicrobial substances, and modulating immune responses.[5,14]

In this context, the present study aimed to extensively evaluate the multifunctional therapeutic potential of *H. coagulans* through *in-vitro* assessment of cholesterol-lowering and anti-collagenase activities, along with in-vivo investigation of its anti-ulcer and anti-diarrheal effects.

METHODS

Preparation of Bacospore®

Bacospore® (*Heyndrickxia coagulans*) is manufactured and registered by Star Hi Herbs Pvt. Ltd., Bangalore, Karnataka. The probiotic strain *H. coagulans* (MTCC 25308) was procured from the same source and maintained under standard microbiological conditions prior to experimental use. A spray-dried powder form of *H. coagulans*, with a potency of 390×10^9 CFU/gm, was used in this study. The powdered sample was inoculated into selective broth and incubated for 18-24 hours. After incubation, the culture was centrifuged at 6000 rpm at 4°C, and the supernatant was collected and filtered for further studies.

Animals and experimental design

Male Wister rats with average body weight from 150 to 250 gm were utilized in this study. The rats were kept in the animal house of the department of pharmacy at PES College of Pharmacy. The rats were housed in polypropylene cages and maintained under standard conditions (12 hours light and dark cycles at 25±3°C and 35-60% humidity). Standard pelletized feed and water were provided ad libitum. Animals were selected and divided into groups of six animals each as per study requirement. Animals were fasted for 24 hours for anti-ulcer and 18 hours for anti-diarrheal before the study, but they had free access to water.

Ethical approval

Ethical approval was obtained from the Institutional Animal Ethics Committee of PES College of Pharmacy, Bangalore, India (Reg. No. PESCP/IAEC/130/2022).

Anti-ulcer activity of Bacospore®

The gastro-protective activity of Bacospore® was evaluated using an ethanol-induced model in Wistar rats. Animals were divided into five groups (n=6 per group) as follows: group I (normal control): received normal saline. Group II (negative control): received absolute ethanol (1 ml/200 gm body weight, p.o.). Group III (standard): received ranitidine (20 mg/kg, p.o.). Group IV (low dose): received Bacospore® (100 mg/kg, p.o.). Group V (high dose): received Bacospore® (200 mg/kg, p.o.).

All treatments were administered orally once daily for 7 consecutive days.

After the treatment period, animals were fasted for 24 hours with free access to water. 30mins post treatment Gastric ulcers were induced by oral administration of absolute ethanol (1 ml/200g body weight) to all groups except group I. One hour after ethanol administration, the animals were anesthetized and sacrificed.

The stomachs were excised immediately, opened along the greater curvature, and rinsed gently with cold normal saline to remove gastric contents. The gastric mucosa was examined for visible lesions such as hemorrhagic streaks, erosions, and ulcerations. The severity of gastric damage was assessed using a standard ulcer scoring system, and the ulcer index was calculated. The percentage of ulcer protection was determined by comparing treated groups with the ulcer control group.

Gastric tissues were further subjected to histopathological examination to evaluate mucosal integrity, epithelial damage, and inflammatory changes.

Ulcer index calculation formula: UI = UN +US + UP × 10-1

where, UI- ulcer index

UN- Average number of ulcers/animal

US- Average of severity score

UP- Percentage of animals with ulcers

Inhibition percentage was calculated by the formula

$$\%Inhibition = \frac{(control\ ulcer\ index) - (test\ ulcer\ index)}{(control\ ulcer\ index)} \times 100$$

Antidiarrheal effects of Bacospore®

The antidiarrheal activity of Bacospore® was evaluated using a castor oil-induced model in male Wistar rats (140-180 gm). Animals were fasted for 18 hours prior to experimentation with free access to water. After fasting, animals were randomly divided into five groups (n=6 per group): group I (normal control): received normal saline, group II (negative control): diarrhea induced (castor oil), group III (standard): diarrhea induced (castor oil) + treated with loperamide (1 mg/kg), group IV (low dose): diarrhea induced (castor oil) + treated with Bacospore® (100 mg/kg b.w.), group V (high dose): diarrhea induced (castor oil) + treated with Bacospore® (200 mg/kg b.w)

All treatments were administered orally at a constant volume of 1 ml/kg body weight. One hour after treatment, diarrhea was induced in groups II-V by administering castor oil (1 ml/animal, p.o.). Immediately after castor oil administration, each animal was placed in an individual cage lined with clean absorbent paper. Animals were observed for 4-6 hours after castor oil administration, and the wet weight of stool was recorded.[15]

Anti-collagenase activity of Bacospore®

The method of Moore and Stein [16] with slight modifications by Mandl, was used to determine the anti-collagenase activity.[17] Briefly, to the 2 ml storage tubes, add 25 µl of collagenase (1 mg/ml), TES buffer (50 mM) with 0.36 mM calcium chloride (pH 7.4), and the test samples with various concentrations. The blank control contained 75 µl of TES buffer, while the negative control contained 25µl of collagenase and 50 µl TES buffer. The positive control contained 25 µl collagenase, 25 µl of TES buffer, and 25 µl EDTA (1 mg/ml). The solvent control contained equal amounts (25 µl) of collagenase, TES buffer, and 0.3% DMSO (solvent in which the given sample dissolved completely).

Test tubes were incubated in a water bath at 37°C for 20 min. afterwards, 100 µl of FALGPA was added to all the tubes and incubated again for 30 mins in a water bath at 37°C. Further, to all tubes, 200 µl of a solution containing equal volumes of 200 mM citrate buffer (pH 5) and Ninhydrin solution was added, which is a modified ninhydrin-based detection of collagen degradation products. All the tubes were placed in a water bath at 100°C for 5 minutes and left to cool to room temperature by adding 200 µl of 50% isopropanol to each tube slowly. The whole contents of tubes were then transferred to a 48-well plate and the absorbance was read at 540 nm using a BioTek ELX-800 plate reader. The % of collagenase inhibition is calculated using the below formula,

$$\%\ of\ collagenase\ inhibition = 100 - \frac{[absorbance\ of\ control - absorbance\ of\ test]}{absorbance\ of\ control} \times 100$$

These are the samples used for anti-collagenase activity with concentrations

Blank – 75 µl of TES buffer

Negative control- 25 µl collagenase+50 µl TES buffer

Positive control- 25 µl collagenase+25 µl TES buffer+25 µl EDTA (1mg/ml)

Solvent control- 25 µl collagenase+25 µl TES buffer+0.3% DMSO (solvent)

Bacospore®- 25 µl collagenase+25 µl TES buffer+25 µl of test with different concentrations (12.5, 25, 50, 100, 200 µg/ml)

Bile salt hydrolase activity of Bacospore®

The Bacospore® strain was studied for cholesterol-lowering activity in MRS liquid broth supplied with cholesterol. MRS broth was initially prepared with 0.05% cysteine-HCl to create microaerophilic conditions (low-oxygen environment) and autoclaved, followed by the addition of cholesterol (CHO) dissolved in ethanol: Tween 80 (3:1, v/v) to a final concentration of 500 µg/ml, w/v, in the MRS medium. The ethanol concentration should not exceed 5%. The MRS-CHO broth was inoculated with log-phase bacterial culture (2% v/v of A600 =0.5 OD culture) with various concentrations of given Bacospore® as well as commercially available bile as a reference std and incubated at 37°C for 24 hours. After the incubation, bacterial cells were pelleted out by centrifugation at 1500 rpm at 4°C for 10 minutes. The cholesterol concentration in spent medium (supernatant) was estimated by enzymatic method using the Enzychrom Cholesterol Assay kit (Cat No.: ECCH-100, Bioassay Systems, CA, USA) following the manufacturer's protocol and compared with uninoculated MRS-CHO broth as control (0 µg/ml), and the percentage cholesterol reduction (lowering activity) was calculated.

Blank-assay buffer provided in kit

Blank-Assay buffer provided in kit. Negative control-MRS broth with probiotic strain induced with cholesterol. Positive control-MRS broth with probiotic strain induced with cholesterol+bile with different concentrations of 31.25-1000 ug/ml. Bacospore®-MRS broth with probiotic strain induced with cholesterol+Bacospore®with different concentrations of 31.25-1000 ug/ml.

Bacterial growth and cholesterol removal

The culture was cultivated in MRS broth at 37°C with 120 rpm shaking. Combine 40 µl of 300 mg/dl standard with 360 µl of assay buffer to create a 10-fold diluted standard (STD). The standard (STD) should next be diluted in assay buffer. Fill each well of the flat-bottom 96-well plate with 50 µl of diluted standards. Next, prepare the samples by diluting the supernatants of both treated and untreated broth cultures ten times in 2 ml Eppendorf tubes (e.g., 10 µl sample with 90 µl assay buffer). Fill each well with 50 µl of the diluted sample. The culture was grown in MRS broth at 37°C while being shaken at 120 rpm. To make a 10-fold diluted standard (STD), mix 40 µl of 300 mg/dl standard with 360 µl of assay buffer. The next step is to dilute the standard (STD) with assay buffer. Pour 50 µl of diluted standards into each well of the 96-well plate with a flat bottom. The supernatants of the treated and untreated broth cultures should then be diluted ten times in 2 ml Eppendorf tubes to produce the samples (e.g., 10 µl sample with 90 µl assay buffer). Pour 50 µl of the diluted sample into each well. Utilizing a 96-well plate ELISA reader, measure OD at 340 nm. Using the OD for the standard and sample wells, determine the substrate OD of the blank (assay buffer). To calculate the sample cholesterol concentration from the standard curve, use the OD values. Note that no dilution factor is needed because the standards and samples were both diluted ten times. Dilute the sample in water and repeat the experiment if the sample's OD value is greater than the 300 mg/dl standard's OD. The values should be multiplied by the dilution factor.

Statistical analysis

All data were expressed as mean±standard error of the mean (SEM). Statistical analyses were Performed using STATISTICA version 6.0 (StatSoft Inc., Tulsa, OK, USA). Cholesterol-lowering experiments were conducted in duplicate across three independent runs. Differences among multiple groups were analyzed using one-way analysis of variance. (ANOVA). For the anti-diarrheal study, fecal weight of treatment groups was compared with that of the negative control using ANOVA, while comparisons between the positive control and negative Control was performed using Student's t-test. For the cholesterol-lowering activity, analysis was carried out using Duncan's Multiple Range Test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Effects of Bacospore® on Ethanol induced ulcers

The anti-ulcerogenic activity of Bacospore® was evaluated *in-vivo* using an ethanol-induced gastric ulcer model in rats at doses of 100 mg/kg and 200 mg/kg body weight. The results obtained, as presented in Figure 1, demonstrated that Bacospore® treatment significantly reduced the ulcer index compared to the control group and negative control, indicating a marked attenuation of gastric mucosal damage. Furthermore, Bacospore® exhibited a high level of gastroprotection, with approximately 80.4% inhibition of ulcer formation, reflecting its strong protective efficacy under ulcerogenic conditions. The reduction in ulcer severity suggests that Bacospore® may effectively counteract the deleterious effects of ethanol on the gastric lining. This protective effect could be attributed to its ability to enhance mucosal defence mechanisms, such as

increased mucus secretion, maintenance of epithelial integrity, and reduction of oxidative stress and inflammation. These highlight the potent anti-ulcer potential of Bacospore® and support its role as a promising natural therapeutic agent for the prevention of gastric mucosal injury.

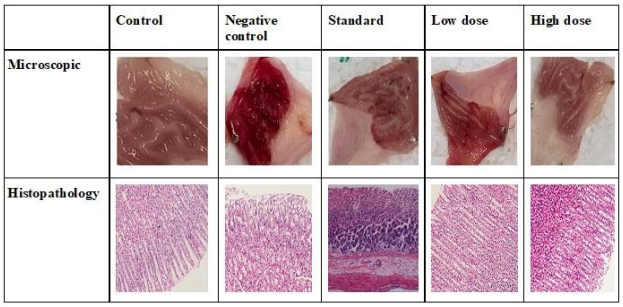

Figure 1: Effect of Bacospore® on ethanol-induced gastric ulcer healing in rats.

Effect of Bacospore® on fecal weight and percentage inhibition in a castor oil-induced diarrhea model

The anti-diarrheal activity of Bacospore® was evaluated in a castor oil-induced diarrhea model in rats and compared with the normal control group. Treatment with Bacospore® at doses of 100 and 200 mg/kg significantly ($p<0.05$) reduced fecal output at both 2 hours and 4 hours compared to the negative control group, indicating a dose-dependent protective effect against castor oil-induced intestinal hypersecretion and motility. At the 4 hours time point, the negative control group exhibited the highest fecal weight, reflecting severe diarrheal symptoms, whereas treatment groups showed a marked reduction in wet fecal weight, suggesting decreased intestinal fluid accumulation and secretion. The standard drug, loperamide, also produced a significant ($p<0.05$) reduction in stool weight at 4 hours, confirming the validity of the experimental model, Table 1.

Table 1: Fecal weight and percentage inhibition for anti-diarrheal activity of Bacospore®.

Groups	Wet weight of stool (2 hours)	Wet weight of stool (4 hours)	% inhibition of diarrhea
Normal control	0	0	0
Negative control	2.16±0.23	1.58±0.20	-
Standard loperamide 1 mg/kg	1.06±0.12	0.91±0.11	42.4
Bacospore® 100 mg/kg	1.6±0.08	1.36±0.14	13.9
Bacospore® 200 mg/kg	1.45±0.12	1.16±0.24	26.6

All the values are MEAN±SD, followed by one way ANOVA test by Dunnett's multiple comparison test.

Percentage inhibition for loperamide treated group was 42.2% while that of the highest dose of Bacospore® was 26.6%. Overall, these findings demonstrate that Bacospore® possesses notable anti-diarrheal activity, possibly through modulation of intestinal motility and enhancement of fluid absorption, thereby reducing the severity of diarrhea.

Effects of inhibition concentration of collagenase enzyme on Bacospore®

Aging is associated with increased collagenase activity, which leads to the degradation of collagen and loss of skin structural integrity. In this study, the anti-collagenase activity of Bacospore® was evaluated *in-vitro* by determining the percentage reduction of collagenase at different concentrations (12.5, 25, 50, 100, and 200 µg/ml). Bacospore® exhibited a dose-dependent inhibition of collagenase activity, with increasing concentrations showing progressively higher inhibitory effects (Figure 2). Notably, the highest inhibition of 85.40% was observed at 200 µg/ml, indicating strong anti-collagenase potential. Therefore, Bacospore® may have a significant role in protecting collagen from enzymatic degradation and could be beneficial in applications related to anti-aging and maintenance of skin integrity.

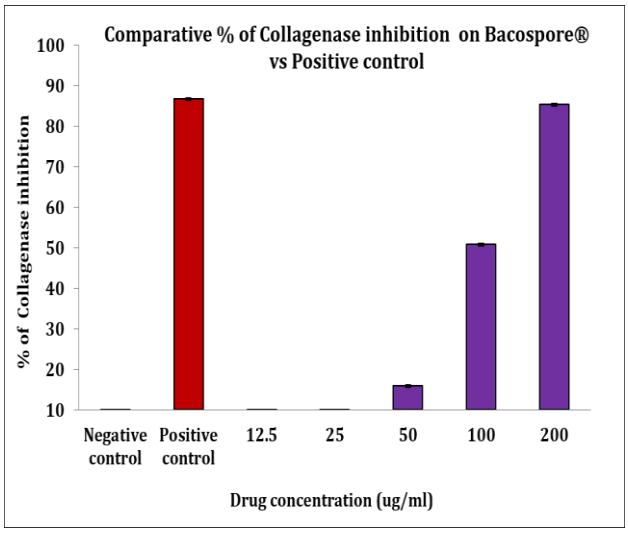

Figure 2: Effects of inhibition concentration of collagenase enzyme on Bacospore® versus positive control.

Reduction of cholesterol of Bacospore®

The cholesterol-lowering activity of Bacospore® MTCC 25308 was investigated *in-vitro* using a bile salt hydrolase (BSH) enzyme assay. The strain demonstrated the ability to remove cholesterol through multiple mechanisms, including BSH activity, in a strain-dependent manner. Cholesterol oxidase (CHO) activity and cholesterol levels in the medium were monitored throughout the growth period of the selected cultures. The results indicated that Bacospore® exhibited a dose-dependent reduction in cholesterol levels, comparable to the commercially available reference standard (bile salts). The percentage inhibition of cholesterol by Bacospore® was found to be 0.34%, 22.01%, 38.77%, 49.63%, 53.65%, and 97.72%, whereas the standard bile showed inhibition values of 17.04%, 36.17%, 56.78%, 83.89%, 95.03%, and 99.41% at corresponding concentrations of 31.25, 62.5, 125, 250, 500, and 1000 µg/ml, respectively. These findings demonstrate that Bacospore® possesses significant cholesterol-lowering potential, particularly at higher concentrations, approaching the efficacy of the standard. The detailed results of this evaluation are presented in Table 2.

Table 2: Comparison of % inhibition of cholesterol lowering activity of Bacospore® and bile salt.

Concentrations (µg/ml)	Bacospore® (%)	Bile salt (Ref. std.) (%)
0	0	0
31.25	0.34	17.04
62.5	22.01	36.17
125	38.77	56.78
250	49.63	83.89
500	53.65	95.03
1000	97.72	99.41

DISCUSSION

The present study demonstrates the broad-spectrum functional potential of Bacospore®, encompassing anti-ulcer and anti-diarrheal effects *in vivo*, along with anti-collagenase and anti-cholesterol activities *in-vitro*. The convergence of these biological properties highlights the organism's capacity to influence gastrointestinal integrity, metabolic homeostasis, and enzymatic regulation through multiple complementary mechanisms. The anti-ulcer activity observed in this study can be attributed to the cytoprotective and immunomodulatory effects of Bacospore® on the gastric mucosa. Probiotic-mediated enhancement of mucosal defence involves increased secretion of mucus and bicarbonate, stimulation of prostaglandin E2 synthesis, and reinforcement of tight junction integrity. Additionally, *B. coagulans* has been reported to attenuate oxidative stress by scavenging reactive oxygen species and enhancing endogenous antioxidant enzymes such as superoxide dismutase and catalase. The inhibition of ulcerogenic pathogens, particularly *Helicobacter pylori*, further contributes to the reduction of gastric inflammation and epithelial damage. Downregulation of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, represents another key mechanism underlying the observed gastroprotective effect.[18,19] Bacospore® demonstrated significant gastroprotective activity in an ethanol-induced gastric ulcer model in rats, reducing ulcer index and increasing ulcer inhibition, likely through enhancement of mucosal defence, preservation of epithelial integrity, and reduction of oxidative stress and inflammation. Additionally, Bacospore® exhibited anti-diarrheal activity by restoring gut microbial balance, inhibiting pathogenic bacteria such as *Escherichia coli* and *Salmonella enterica*, and enhancing intestinal barrier function through tight junction proteins. Production of antimicrobial compounds and short-chain fatty acids (SCFAs) further contributed to improved sodium and water absorption, thereby reducing stool frequency and improving stool consistency.[15,20] These findings are in agreement with earlier reports demonstrating significant improvements in diarrheal outcomes same as Bacospore®.

The anti-collagenase activity observed *in-vitro* suggests an additional protective role of Bacospore® in preventing extracellular matrix degradation. Collagenases, particularly matrix metalloproteinases (MMPs), are critically involved in connective tissue breakdown associated with inflammation, aging, and chronic diseases. The inhibition of collagenase activity may be mediated by probiotic-derived bioactive compounds, including peptides, exopolysaccharides, and phenolic metabolites with antioxidant properties. These compounds may directly inhibit enzyme activity or indirectly suppress MMP expression via modulation of inflammatory signalling pathways such as NF-κB. This indicates potential applications of Bacospore® not only in gut health but also in tissue protection and anti-aging strategies.[21,22]

The cholesterol-lowering effect may involve multiple mechanisms, including direct assimilation of cholesterol into bacterial cell membranes, co-precipitation with deconjugated bile salts, and bile salt hydrolase (BSH)-mediated deconjugation of bile acids. These processes enhance bile acid excretion, increase hepatic cholesterol utilization for de novo bile acid synthesis, and ultimately reduce circulating cholesterol levels.[23-26] Additionally, probiotics may modulate the expression of genes involved in lipid metabolism, further contributing to improved lipid profiles. These findings are consistent with previous reports demonstrating the hypocholesterolemic effects of probiotic *Bacillus* strains.[7,8,27-29] Overall, the reported *in-vitro* anti-cholesterol efficacy highlights Bacospore®'s metabolic advantages.

Importantly, the integration of these biological activities suggests that *B. Coagulans* functions through a unified mechanism involving modulation of the gut microbiota-host axis. By restoring microbial balance, enhancing barrier integrity, reducing inflammation, and producing

bioactive metabolites, the probiotic exerts systemic effects that extend beyond the gastrointestinal tract.[30] The observed anti-collagenase and anti-cholesterol activities indicate that its benefits are not restricted to gut health but also encompass metabolic and cellular protective functions.

Overall, the findings of this study provide compelling evidence that Bacospore® is a multifunctional probiotic with significant therapeutic potential. Its combined gastroprotective, anti-diarrheal, enzyme inhibitory and lipid-lowering properties make it a promising candidate for the development of functional foods, nutraceuticals, and adjunct therapeutic formulations. Further studies focusing on molecular pathways and clinical validation are warranted to fully elucidate its mechanisms and translational applications.

CONCLUSION

The present study collectively demonstrates the multifunctional therapeutic potential of Bacospore®. Bacospore® exhibited a highly significant anti-ulcerogenic effect in the ethanol-induced ulcer model at a dose of 200 mg/kg body weight, as evidenced by a marked reduction in ulcer index and substantial percentage inhibition. In addition, it showed potent anti-diarrheal activity, with treated animals demonstrating reduced fecal output within 4 hours at the same dose. A maximum inhibition of 85.40% was recorded at 200µg/ml, indicating strong anti-collagenase activity. Furthermore, Bacospore® demonstrated effective cholesterol-lowering activity through multiple mechanisms, including cholesterol assimilation and bile- and CHO-mediated processes, independent of bile salt hydrolase (BSH) activity, suggesting a culture-dependent mode of action. Overall, these results confirm that Bacospore® possesses significant gastroprotective, anti-diarrheal, and hypocholesterolemic properties, highlighting its promise as a multifunctional probiotic for therapeutic and nutraceutical applications. However, further studies are warranted to elucidate its precise molecular mechanisms.

ACKNOWLEDGEMENTS

The authors are thankful to PES College of Pharmacy, Bangalore, Karnataka, India, helped us with *in-vivo* study and *in-vitro* study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee PES College of Pharmacy, Bangalore, Karnataka, India, with Reg. No. PESCP/IAEC/130/2022

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Cite this article as: Dhabadi P, Shreya, Tom A, Shivashankara S, Hussain ZF, Mirza FHH, et al. Evaluation of antidiarrheal, antiulcer, anti-collagenase and cholesterol lowering activity of Heyndrickxia coagulans MTCC 25308 (Bacospore®). Int J Basic Clin Pharmacol 2026;15:896-903.