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Synergistic gastrointestinal protection by a multi-component food bioactive formulation

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Abbreviations: AGS, adenocarcinoma gastric cell line; GI, gastrointestinal; *H. pylori*, *Helicobacter pylori*; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MUC5B, mucin 5 subtype B; PBMCs, peripheral blood mononuclear cells; SD, standard deviation; ZO-1, zonula occludens-1

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Abstract

This study investigated the in vitro gastrointestinal protective effects of a multi-component food bioactive formulation composed of turmeric, manuka honey, sea buckthorn, black pepper, and chocolate chili extracts. The formulation was evaluated in peripheral blood mononuclear cells, gastric epithelial cells, and esophageal epithelial cells for its influence on lipopolysaccharide-induced cytokine secretion, mucin (MUC5B) production, and tight-junction protein (ZO-1) expression, as well as its antimicrobial activity against *Helicobacter pylori*. Compared with single ingredients or partial combinations, the complete formulation demonstrated synergistic activity by attenuating proinflammatory cytokine release, reinforcing epithelial barrier integrity, normalizing mucin secretion, and inhibiting *H. pylori* growth. These findings provide mechanistic evidence that multi-component food bioactive formulations can contribute to gastrointestinal protection through complementary anti-inflammatory and antimicrobial mechanisms, supporting their potential as functional strategies for maintaining gut health.

Keywords: Food bioactives; Gastrointestinal protection; Functional formulation; Cytokine modulation; Epithelial barrier; *Helicobacter pylori*.

1. Introduction

The gastrointestinal (GI) tract serves not only as the site for digestion and nutrient absorption but also as a critical barrier protecting the body from dietary antigens, gastric acid, bile salts, and microbial pathogens. The integrity of the gastric and esophageal mucosa is essential for maintaining homeostasis, and its disruption is implicated in common disorders such as gastritis, peptic ulcers, gastroesophageal reflux disease (GERD), and *Helicobacter pylori* infection (Polk and Peek, 2010; Vakil, 2024). GI barrier dysfunction allows microbial components like lipopolysaccharide (LPS) to enter systemic circulation, triggering low-grade inflammation and contributing to obesity, type 2 diabetes, and metabolic syndrome (Bischoff et al., 2014; Camilleri, 2019). Increased intestinal permeability has been implicated in a wide range of chronic diseases, including autoimmune, neurodegenerative, and cardiovascular disorders, highlighting the far-reaching consequences of GI barrier impairment.

Against this background, dietary supplements containing bioactive natural compounds have gained increasing scientific interest as

complementary strategies to support gastrointestinal health (Martinsen et al., 2005). Natural compounds exert multi-level protective effects, including anti-inflammatory, mucosal defense enhancement, and antimicrobial activity. For instance, curcumin from turmeric has been shown to downregulate NF-κB activation and reduce pro-inflammatory cytokines such as TNF-α and IL-6, thereby alleviating mucosal inflammation in experimental gastritis and colitis models (Deguchi et al., 2007). Honey exhibits antimicrobial activity against *H. pylori* and promotes gastric ulcer healing (Nzeako et al., 2006). Sea buckthorn extract protects the gastric mucosa through antioxidant actions and stimulation of mucin production (Tritean et al., 2023). Black pepper extract provides piperine, its key bioactive constituent, known to enhance curcumin bioavailability and modulate inflammatory pathways (Shoba et al., 1998). These examples reflect increasing interest in GI barrier preservation and preventive gut health strategies. However, while many studies have explored the GI-protective effects of individual compounds, comprehensive evaluations of multi-component formulations designed for synergistic and multi-target protection remain limited.

The tested formulation is an oral liquid dietary supplement composed of turmeric (*Curcuma longa*) extract, manuka honey, sea buckthorn (*Hippophae rhamnoides*) extract, black pepper extract, chocolate chili extract, and other ingredients. It is designed to provide gastrointestinal support through multiple mechanisms, including modulation of inflammation, protection of the mucosal barrier, strengthening of epithelial integrity, and antibacterial activity. The formulation integrates these complementary properties to offer synergistic, multi-target support for gastrointestinal health. In this study, we evaluated the in *vitro* efficacy across four key aspects of gastrointestinal protection: suppression of LPS-induced proinflammatory cytokine secretion in peripheral blood mononuclear cells, modulation of mucin secretion in gastric epithelial cells, reinforcement of tight junction integrity in esophageal epithelial cells, and inhibition of *H. pylori* growth. These findings provide preliminary scientific evidence for the formulation's potential role in gastrointestinal health maintenance.

2. Material and methods

2.1. Test product

The test product was a commercially available oral liquid dietary supplement (BEE+ Golden Shield, BEE PLUS NEW ZEALAND LIMITED, Auckland, New Zealand) formulated for gastrointestinal health. The potent formula features turmeric extract, Bioperine® black pepper extract, chocolate chili extract, sea buckthorn extract, and UMF 20+ manuka honey, delivered using the proprietary DoubleNutri™ Liposomal Delivery System to enhance absorption and efficacy. Additional excipients present in the commercial product are inactive formulation components that do not influence the measured biological outcomes. The product was tested as supplied (100% concentration). For clarity, the complete oral liquid formulation containing all listed components is hereafter referred to as the complete formulation.

2.2. PBMC culture and cytokine assay

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque (GE Healthcare, Chicago, IL, USA) density gradient centrifugation and cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS, 1% penicillin-streptomycin, 1% NEAA, 1 mM sodium pyruvate, and 2 mM L-glutamine (all from Gibco, Thermo Fisher Scientific, Waltham, MA, USA), and 50 μM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in 5% CO_2.

Cells (1×10^6 cells/well) were seeded into 24-well plates (Corning Inc., Corning, NY, USA) with 500 μL medium per well and pretreated for 1 h with turmeric extract only, manuka honey only, turmeric extract + manuka honey + sea buckthorn extract, or the complete formulation at a concentration of 2% (v/v) before stimulation with 100 ng/mL LPS (Sigma-Aldrich). After 16 h, culture supernatants were harvested by centrifugation at 400 g for 7 min.

Cytokine concentrations (IL-1β, IL-6, IL-18) were determined using the LEGENDplex™ Human Inflammation Panel 1 (13-plex) with filter plate (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. Data were acquired using a BD Accuri C6 Plus Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed using LEGENDplex Data Analysis Software (BioLegend, San Diego, CA, USA).

2.3. Gastric mucin secretion assay

AGS human gastric epithelial cells (ATCC CRL-1739) were cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (all from Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Cells (1×10^4/well) were seeded in 96-well plates (Corning Inc., Corning, NY, USA) and incubated for 24 h for attachment. Cells were then treated with the complete formulation at 2% (v/v) in 200 μL fresh medium in the presence or absence of 10 μg/mL LPS (Sigma-Aldrich, St. Louis, MO, USA) for 48 h. MUC5B secretion was measured in supernatants using a commercial ELISA kit (Elabscience, Houston, TX, USA; Cat. E-EL-H2280). Absorbance was measured at 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA).

2.4. Esophageal barrier integrity assay

CE146T/VGH human esophageal epithelial cells (CVCL_Y009) were cultured in DMEM (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 1% penicillin-streptomycin (all from Gibco, Thermo Fisher Scientific). Cells (2×10^5 cells/well) were seeded on coverslips (Corning Inc., Corning, NY, USA) in 24-well plates and treated with the complete formulation at 2% (v/v) in the presence or absence of 10 μg/mL LPS (Sigma-Aldrich, St. Louis, MO, USA) for 24 h.

Cells were fixed with Cytological fixative (Toyo; Cat. 02F021), permeabilized with 0.5% Triton X-100 (Merck, Darmstadt, Germany), and blocked with 2% BSA (Thermo Fisher Scientific). Cells were incubated overnight at 4°C with anti-ZO-1 antibody (Abcam, Cambridge, UK; Cat. Ab221547) and anti-ZO-2 antibody (Abnova, Taipei, Taiwan, China; Cat. H00009414-M01). Secondary detection was performed using Alexa Fluor 594 goat anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA; Cat. A11012) and Alexa Fluor 488 goat anti-mouse IgG (Invitrogen; Cat. A11001). Nuclei were counterstained with Hoechst 33342 (Thermo Fisher Scientific, Waltham, MA, USA). Images were acquired using an Olympus IX71 fluorescence microscope (Olympus, Tokyo, Japan) and analyzed with ImageJ (NIH, Bethesda, MD, USA).

2.5. H. pylori inhibition assay

The *H. pylori* reference strain ATCC 43504 was used in this

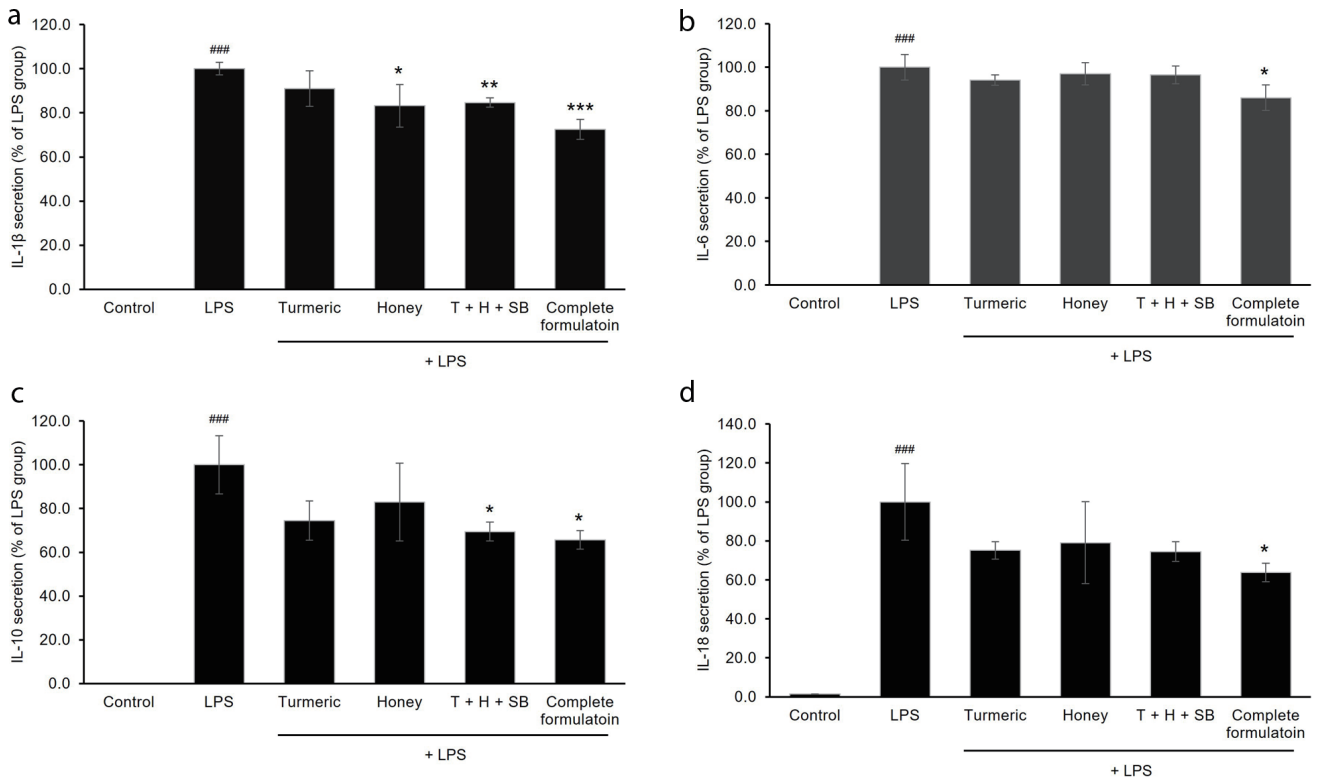

Figure 1. Effects of individual ingredients and the complete formulation on LPS-induced cytokine secretion in PBMCs. Cells were pretreated with turmeric (Turmeric), manuka honey (Honey), a combination of turmeric + honey + sea buckthorn (T+H+SB), or the complete formulation, followed by LPS stimulation for 16 h. Cytokine secretion (IL-1β, IL-6, IL-10, IL-18) was measured by ELISA. All treatments were applied at 2% (v/v). Data are expressed as mean ± SD (n=3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. LPS group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. control group.

study. The strain was obtained from the Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan, China). The bacterium was revived from cryogenic storage by inoculation into Brain Heart Infusion (BHI) broth (Tai Ding Biotech Co., Ltd., Taoyuan, Taiwan, China) and incubated at 37°C under microaerophilic conditions using AnaeroPack™-MicroAero (Mitsubishi Gas Chemical, Tokyo, Japan). Once revived, cultures were streaked onto solid BHI agar plates (Tai Ding Biotech Co., Ltd., Taoyuan, Taiwan, China) and incubated for 48 hours to establish colonies for downstream assays.

For the antibacterial activity assay, *H. pylori* cultures were grown in BHI broth to the logarithmic growth phase and adjusted to a standardized density. A 100 µL aliquot was spread on BHI agar plates using a sterile spreader (Corning Inc., Corning, NY, USA) to form a uniform lawn. After the plates were dried for 10–15 min under sterile conditions, a sterile borer (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was used to create a central well approximately 6 mm in diameter. Twenty microliters of the complete formulation (undiluted) was carefully pipetted into each well using a micropipette (Eppendorf, Hamburg, Germany). Each condition was tested in triplicate.

The plates were incubated at 37°C for 48–72 hours under microaerophilic conditions. After incubation, the diameter of the clear zone of inhibition was measured using a calibrated digital caliper (Mitutoyo, Kawasaki, Japan). The area of the inhibition zone was calculated and expressed in square millimeters (mm^2). Each experiment was independently repeated three times to ensure reproducibility.

2.6. Statistical analysis

All experiments were performed at least in triplicate. Results are expressed as mean ± standard deviation (SD). Data were analyzed using Student's t-test to compare treatment groups with either the control group or the LPS group, as appropriate for each analysis. Statistical analysis was performed using GraphPad Prism v9 (GraphPad Software, San Diego, CA, USA). A p-value < 0.05 was considered statistically significant. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, vs. LPS group; and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, vs. control group.

3. Results

3.1. Suppression of LPS-induced proinflammatory cytokine secretion

This experiment aimed to evaluate the contribution of individual and combined functional components in modulating LPS-induced proinflammatory cytokine secretion in PBMCs. LPS stimulation markedly increased the secretion of IL-1β, IL-6, IL-10, and IL-18 compared to the untreated control group (all $p < 0.001$), confirming successful activation of the inflammatory response (Figure 1). Treatment with turmeric alone showed minimal effect across all cytokines, with IL-1β and IL-6 remaining >90% of LPS levels, and IL-10 and IL-18 modestly reduced to 74.5 ± 8.96% and 75.1

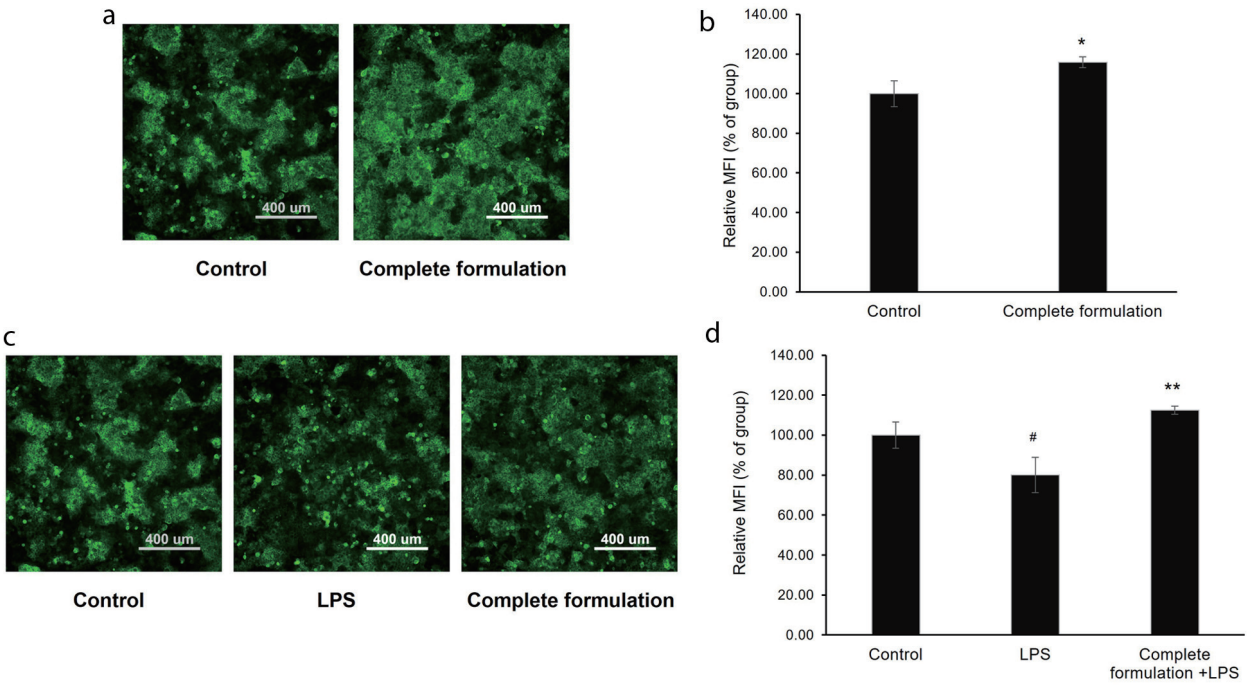

Figure 2. Effects of the complete formulation on ZO-1 expression in esophageal epithelial cells. Cells were treated with the formulation at 2% (v/v) with or without LPS for 24 h. ZO-1 expression was analyzed by immunofluorescence staining and quantified as mean fluorescence intensity (MFI). Data are presented as mean ± SD (n=3). * $p < 0.05$, ** $p < 0.01$ vs. LPS group; $^{\#}p < 0.05$ vs. control group.

± 4.50%, respectively. Manuka honey alone exhibited greater anti-inflammatory activity, particularly for IL-1β (83.2 ± 9.59%; $p <$ 0.05 vs. LPS), suggesting intrinsic cytokine-modulatory potential beyond antioxidant properties.

The partial formula combining turmeric, honey, and sea buckthorn (T + H + SB) achieved stronger and more consistent reductions, lowering IL-1β to 84.7 ± 2.10% ($p < 0.01$), IL-10 to 69.5 ± 4.26% ($p < 0.05$), and IL-18 to 74.5 ± 5.03%. However, IL-6 remained relatively unaffected at 96.5 ± 4.15%, indicating limited impact on this cytokine pathway at this formulation stage.

Notably, the complete formulation demonstrated the most comprehensive inhibitory effect across all proinflammatory cytokines. IL-1β was reduced to 72.5 ± 4.58% ($p < 0.001$), IL-6 to 86.0 ± 5.89% ($p < 0.05$), IL-10 to 65.7 ± 4.20% ($p < 0.05$), and IL-18 to 63.8 ± 4.78% ($p < 0.05$) compared to LPS alone. These results highlight the enhanced efficacy of the full formulation and support the potential role of its multi-component design in synergistically modulating the inflammatory cascade.

These findings indicate that while turmeric and honey individually contribute partial anti-inflammatory effects, maximal suppression of LPS-induced cytokine production in PBMCs requires the full formulation, supporting a synergistic, multi-pathway mechanism of action.

3.2. Enhancement of esophageal epithelial barrier integrity

This experiment aimed to evaluate the protective and restorative effects of the complete formulation on esophageal epithelial barrier integrity by assessing ZO-1 expression under normal and LPS-induced inflammatory conditions.

Under normal conditions, treatment with the complete formulation significantly enhanced ZO-1 expression by 15.8% compared to the control group (115.84 ± 2.71 vs. control; $p < 0.05$; Figure 2). This finding suggests that the complete formulation can strengthen tight junctions and promote basal barrier function even in the absence of inflammatory insult.

In the LPS-injured model, LPS exposure significantly reduced ZO-1 expression by 20% compared to the control ($p < 0.05$), confirming disruption of barrier integrity. Notably, co-treatment with the complete formulation restored ZO-1 expression to levels significantly higher than both the LPS group and untreated controls (112.45 ± 2.05; $p < 0.01$ vs. LPS). This substantial recovery not only reversed the LPS-induced damage but also suggested a barrier-fortifying effect beyond basal levels.

These results highlight the dual role of the complete formulation in both preserving esophageal epithelial barrier function under homeostatic conditions and restoring integrity following inflammatory injury. The consistent increase in ZO-1 expression supports the potential of the formulation to enhance epithelial resilience.

3.3. Normalization of gastric mucin secretion under inflammatory conditions

This experiment aimed to assess whether the complete formulation could normalize gastric mucin (MUC5B) secretion disrupted by LPS-induced inflammation in gastric epithelial cells. LPS stimulation significantly increased relative MUC5B secretion to 122.64 ± 0.83% of the control ($p < 0.01$), reflecting a typical inflammatory compensatory response (Figure 3). Treatment with the complete formulation restored MUC5B secretion to near baseline levels (101.36 ± 2.40% of control; $p < 0.001$ vs. LPS), suggesting effective normalization of mucin production under inflammatory conditions. These results indicate that the complete formulation not only mitigates LPS-induced mucin overproduction but also supports the

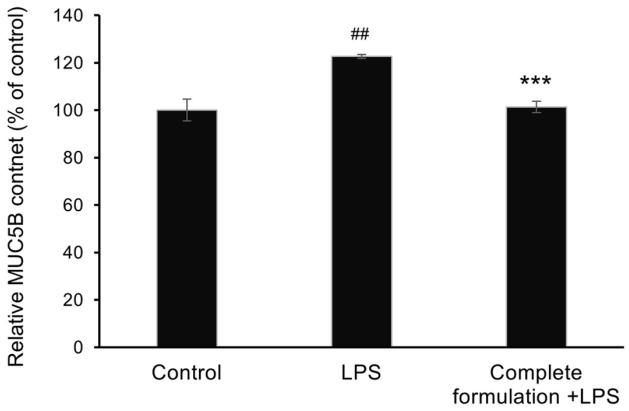

Figure 3. Effects of the complete formulation on relative MUC5B secretion in AGS cells following LPS stimulation. Cells were treated with the formulation at 2% (v/v) for 48 h. MUC5B levels in culture supernatants were quantified by ELISA. Data are expressed as mean ± SD (n=3). *** $p < 0.001$ vs. LPS group; ## $p < 0.01$ vs. control group.

maintenance of gastric mucosal homeostasis.

3.4. Inhibition of Helicobacter pylori growth

This experiment aimed to evaluate the antimicrobial activity of the complete formulation against *H. pylori in vitro*. The complete formulation produced a clear inhibition zone with an area of approximately 2,165 mm^2, compared to 754.8 mm^2 in the control group (Figure 4). This represented a 34.9% inhibition rate, supporting the antibacterial potential of the formulation against *H. pylori*. These findings suggest that the complete formulation possesses direct antimicrobial properties that may contribute to its gastrointestinal protective effects.

4. Discussion

This study provides in vitro evidence demonstrating the multi-target gastrointestinal protective potential of a complex oral dietary supplement formulation. The formulation, integrating turmeric, manuka honey, sea buckthorn, black pepper extract, chocolate chili, and other components, exhibited complementary biological activities, including suppression of proinflammatory cytokine secretion, reinforcement of gastrointestinal epithelial barrier integrity, normalization of mucin secretion, and inhibition of *H. pylori* growth. Collectively, these findings suggest that the supplement may support gastrointestinal health through multiple mechanisms relevant for integrative management strategies.

The suppression of LPS-induced cytokine secretion in PBMCs highlights the broad-spectrum anti-inflammatory potential of the formulation. The complete formulation reduced IL-1β secretion by 27.5% ($p < 0.001$), IL-6 by 14% ($p < 0.05$), IL-10 by 34.3% ($p < 0.05$), and IL-18 by 36.2% ($p < 0.05$) compared to LPS alone, consistently outperforming turmeric or honey administered individually. While the partial combination of turmeric, honey, and sea buckthorn showed intermediate efficacy, only the full formulation suppressed all measured cytokines, suggesting a synergistic advantage. The individual components are well-documented for their anti-inflammatory effects. Curcumin modulates NF-κB and MAPK pathways, leading to downregulation of proinflammatory cytokines (Aggarwal and Sung, 2009; Cho et al., 2007). Manuka

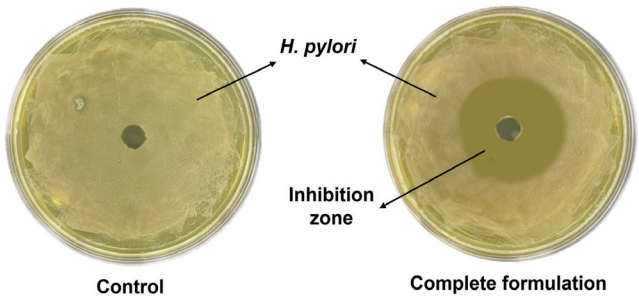

Figure 4. Inhibition of *H. pylori* growth by the complete formulation as assessed by disc diffusion assay. Twenty microliters of undiluted formulation (100%) was applied to each well. Data are presented as inhibition zone area (mm^2).

honey reduces cytokine production *via* antioxidant action and toll-like receptor modulation (Majtan, 2014; Minden-Birkenmaier et al., 2019; Tonks et al., 2007). Sea buckthorn provides flavonoids that inhibit inflammatory mediator release (Suryakumar and Gupta, 2011). Black pepper extract provides piperine, its primary bioactive constituent, which enhances curcumin bioavailability by inhibiting hepatic and intestinal glucuronidation, and has also been reported to independently reduce inflammatory cytokines through modulation of NF-κB signaling (Bang et al., 2009; Tharmalingam et al., 2014). Capsaicin-like compounds in chocolate chili have been reported to reduce IL-6 and TNF-α secretion (Jamornwan et al., 2022; Tang et al., 2015). The observed synergy aligns with prior reports that multi-component formulations can exert additive or synergistic effects on inflammation (Ulrich-Merzenich et al., 2010; Wagner and Ulrich-Merzenich, 2009).

Tight junction proteins such as ZO-1 are critical for maintaining epithelial barrier integrity by regulating paracellular permeability and preserving the selective barrier function of the gastrointestinal tract (Turner, 2009). ZO-1 links transmembrane proteins (e.g., claudins, occludins) to the actin cytoskeleton, providing structural support that prevents paracellular leakage of antigens, toxins, and microbes that could trigger mucosal inflammation (Luissint et al., 2016). Impaired or redistributed ZO-1 expression is a hallmark of barrier dysfunction observed in gastrointestinal diseases such as reflux esophagitis, inflammatory bowel disease, and functional dyspepsia (Camilleri, 2019). Disruption of ZO-1 compromises barrier function, leading to increased intestinal permeability ("leaky gut"), antigen translocation, and subsequent immune activation.

In this study, the complete formulation significantly increased ZO-1 expression under basal conditions (115.84% of control; $p < 0.05$) and reversed the LPS-induced reduction (112.45% vs. 80.02% in LPS alone; $p < 0.01$). These findings suggest that the formulation not only strengthens epithelial barrier integrity under normal conditions but also mitigates barrier damage induced by inflammatory stimuli. This dual action may have therapeutic relevance for preventing or managing gastrointestinal disorders associated with epithelial barrier dysfunction.

Gastric mucins, particularly MUC5B and MUC5AC, are essential components of the gastric mucus barrier, forming a viscoelastic gel that protects the epithelium from acid, digestive enzymes, and microbial pathogens (Johansson and Hansson, 2013). Mucins trap and neutralize luminal aggressors, facilitate epithelial repair, and contribute to innate defense mechanisms (Linden et al., 2008). However, dysregulated mucin production, characterized by either excessive or deficient secretion, has been implicated in the pathogenesis of gastritis, peptic ulcers, and *H. pylori* persistence (McGuckin et al., 2011). Excessive MUC5B secretion, often seen in

response to inflammatory stimuli such as LPS, may represent a compensatory attempt to reinforce the barrier but can disrupt mucosal homeostasis and impair normal gastric function (Hattrup and Gendler, 2008; McGuckin et al., 2011).

In our model, LPS stimulation significantly increased MUC5B secretion (122.64% of control; $p < 0.01$), whereas treatment with the complete formulation normalized mucin levels to near baseline (101.36% of control; $p < 0.001$). These findings suggest that the formulation helps restore mucosal balance by modulating mucin secretion in the face of inflammatory stress. This aligns with reports that honey and polyphenol-rich plant extracts can regulate mucin expression and contribute to gastric mucosal protection (Gharzouli et al., 2002; Yu et al., 2022). The ability of the formulation to normalize rather than suppress mucin secretion may be particularly advantageous for maintaining protective mucus layer integrity without compromising defense capacity.

H. pylori is a major gastric pathogen associated with chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and an increased risk of gastric cancer (Suerbaum and Michetti, 2002). Successful colonization by *H. pylori* depends on its ability to adhere to the gastric epithelium, evade host defenses, and neutralize gastric acidity through urease activity (Cover and Blaser, 2009). Eradication of *H. pylori* is a cornerstone of managing related diseases, but rising antibiotic resistance has spurred interest in adjunctive therapies, including plant-derived compounds and functional food components (Dore et al., 2016).

In this study, the complete formulation exhibited antimicrobial activity against *H. pylori*, producing an inhibition zone of approximately 2,165 mm^2 compared to 754.8 mm^2 in controls. This suggests a substantial direct antibacterial effect. Bioactives present in the formulation, such as piperine from black pepper extract and polyphenols from plant extracts, are known to disrupt bacterial membranes, inhibit key bacterial enzymes, interfere with quorum sensing, and prevent adhesion to host tissues (Mandal and Domb, 2024; Tharmalingam et al., 2014). The multi-component nature of the formulation may enhance antibacterial efficacy by targeting several bacterial survival mechanisms simultaneously. These findings support the potential of the formulation as part of an integrative gastrointestinal health strategy, complementing standard *H. pylori* eradication protocols.

This study has several limitations. First, it was conducted entirely *in vitro*, and the findings cannot be directly extrapolated to in vivo or clinical outcomes. Second, only a single concentration of the complete formulation was tested, and no dose–response relationship was evaluated. Third, cytotoxicity or safety assessments were not performed, which would be important to establish the formulation's tolerability in biological systems. Future studies should therefore include dose–response experiments, *in vivo* validation, and clinical trials to confirm the translational relevance of these findings.

5. Conclusion

The complete formulation demonstrated synergistic, multi-target effects in promoting gastrointestinal health. Its bioactive components acted through complementary mechanisms, including suppression of proinflammatory cytokine secretion, reinforcement of epithelial barrier integrity, normalization of mucin secretion, and antimicrobial activity against *Helicobacter pylori*. These findings provide mechanistic evidence that multi-component food bioactive formulations can contribute to gastrointestinal protection and homeostasis. While the current study was conducted in vitro, future in vivo and clinical investigations are warranted to further validate efficacy and elucidate underlying pathways under physiological conditions.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest

The authors declare that this research was conducted objectively and without commercial influence on data interpretation or conclusions.

Author contributions

YK.L. contributed to study conception, experimental design, and overall supervision of the research project. Z.H. contributed to formulation development, sample characterization, and scientific interpretation. YH.L. and YK.L. provided conceptual input and guidance on study design. ST.C. and CF.C. performed experimental work, data collection, and statistical analysis, and critically revised the manuscript. YW.M. supervised the research, conducted statistical analyses, and was responsible for drafting and finalizing the manuscript. All authors have read and approved the final version of the manuscript and agree to be accountable for its contents.

References

Aggarwal, B.B., and Sung, B. (2009). Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol. Sci. 30(2): 85–94.

Bang, J.S., Oh, D.H., Choi, H.M., Sur, B.J., Lim, S.J., Kim, J.Y., Yang, H.I., Yoo, M.C., Hahm, D.H., and Kim, K.S. (2009). Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1beta-stimulated fibroblast-like synoviocytes and in rat arthritis models. Arthritis Res. Ther. 11(2): R49.

Bischoff, S.C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J.D., Serino, M., Tilg, H., Watson, A., and Wells, J.M. (2014). Intestinal permeability--a new target for disease prevention and therapy. BMC Gastroenterol. 14: 189.

Camilleri, M. (2019). Leaky gut: mechanisms, measurement and clinical implications in humans. Gut 68(8): 1516–1526.

Cho, J.W., Lee, K.S., and Kim, C.W. (2007). Curcumin attenuates the expression of IL-1beta, IL-6, and TNF-alpha as well as cyclin E in TNF-alpha-

treated HaCaT cells; NF-kappaB and MAPKs as potential upstream targets. Int. J. Mol. Med. 19(3): 469–474.
Cover, T.L., and Blaser, M.J. (2009). Helicobacter pylori in health and disease. Gastroenterology 136(6): 1863–1873.
Deguchi, Y., Andoh, A., Inatomi, O., Yagi, Y., Bamba, S., Araki, Y., Hata, K., Tsujikawa, T., and Fujiyama, Y. (2007). Curcumin prevents the development of dextran sulfate Sodium (DSS)-induced experimental colitis. Dig. Dis. Sci. 52(11): 2993–2998.
Dore, M.P., Lu, H., and Graham, D.Y. (2016). Role of bismuth in improving Helicobacter pylori eradication with triple therapy. Gut 65(5): 870–878.
Gharzouli, K., Amira, S., Gharzouli, A., and Khennouf, S. (2002). Gastroprotective effects of honey and glucose-fructose-sucrose-maltose mixture against ethanol-, indomethacin-, and acidified aspirin-induced lesions in the rat. Exp. Toxicol. Pathol. 54(3): 217–221.
Hattrup, C.L., and Gendler, S.J. (2008). Structure and function of the cell surface (tethered) mucins. Annu. Rev. Physiol. 70: 431–457.
Jamornwan, S., Chokpanuwat, T., Uppakara, K., Laorob, T., Wichai, U., Ketsawatsomkron, P., and Saengsawang, W. (2022). Nitro Capsaicin Suppressed Microglial Activation and TNF-alpha-Induced Brain Microvascular Endothelial Cell Damage. Biomedicines 10(11): 2680.
Johansson, M.E., and Hansson, G.C. (2013). Mucus and the goblet cell. Dig. Dis. 31(3-4): 305–309.
Linden, S.K., Sutton, P., Karlsson, N.G., Korolik, V., and McGuckin, M.A. (2008). Mucins in the mucosal barrier to infection. Mucosal. Immunol. 1(3): 183–197.
Luissint, A.C., Parkos, C.A., and Nusrat, A. (2016). Inflammation and the Intestinal Barrier: Leukocyte-Epithelial Cell Interactions, Cell Junction Remodeling, and Mucosal Repair. Gastroenterology 151(4): 616–632.
Majtan, J. (2014). Honey: an immunomodulator in wound healing. Wound Repair Regen. 22(2): 187–192.
Mandal, M.K., and Domb, A.J. (2024). Antimicrobial Activities of Natural Bioactive Polyphenols. Pharmaceutics 16(6): 718.
Martinsen, T.C., Bergh, K., and Waldum, H.L. (2005). Gastric juice: a barrier against infectious diseases. Basic Clin. Pharmacol. Toxicol. 96(2): 94–102.
McGuckin, M.A., Linden, S.K., Sutton, P., and Florin, T.H. (2011). Mucin dynamics and enteric pathogens. Nat. Rev. Microbiol. 9(4): 265–278.
Minden-Birkenmaier, B.A., Meadows, M.B., Cherukuri, K., Smeltzer, M.P., Smith, R.A., Radic, M.Z., and Bowlin, G.L. (2019). The Effect of Manuka Honey on dHL-60 Cytokine, Chemokine, and Matrix-Degrading Enzyme Release under Inflammatory Conditions. Med One 4(2): e190005.
Nzeako, B.C., and Al-Namaani, F. (2006). The antibacterial activity of honey on helicobacter pylori. Sultan Qaboos Univ Med J 6(2): 71–76.
Polk, D.B., and Peek, R.M. Jr (2010). Helicobacter pylori: gastric cancer and beyond. Nat. Rev. Cancer 10(6): 403–414.
Shoba, G., Joy, D., Joseph, T., Majeed, M., Rajendran, R., and Srinivas, P.S. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med. 64(4): 353–356.
Suerbaum, S., and Michetti, P. (2002). Helicobacter pylori infection. N. Engl. J. Med. 347(15): 1175–1186.
Suryakumar, G., and Gupta, A. (2011). Medicinal and therapeutic potential of Sea buckthorn (Hippophae rhamnoides L.). J. Ethnopharmacol. 138(2): 268–278.
Tang, J., Luo, K., Li, Y., Chen, Q., Tang, D., Wang, D., and Xiao, J. (2015). Capsaicin attenuates LPS-induced inflammatory cytokine production by upregulation of LXRalpha. Int. Immunopharmacol. 28(1): 264–269.
Tharmalingam, N., Kim, S.H., Park, M., Woo, H.J., Kim, H.W., Yang, J.Y., Rhee, K.J., and Kim, J.B. (2014). Inhibitory effect of piperine on Helicobacter pylori growth and adhesion to gastric adenocarcinoma cells. Infect. Agent. Cancer 9(1): 43.
Tonks, A.J., Dudley, E., Porter, N.G., Parton, J., Brazier, J., Smith, E.L., and Tonks, A. (2007). A 5.8-kDa component of manuka honey stimulates immune cells via TLR4. J. Leukoc. Biol. 82(5): 1147–1155.
Tritean, N., Dimitriu, L., Dima, S.O., Stoica, R., Trica, B., Ghiurea, M., Moraru, I., Cimpean, A., Oancea, F., and Constantinescu-Aruxandei, D. (2023). Cytocompatibility, Antimicrobial and Antioxidant Activity of a Mucoadhesive Biopolymeric Hydrogel Embedding Selenium Nanoparticles Phytosynthesized by Sea Buckthorn Leaf Extract. Pharmaceuticals (Basel) 17(1): 23.
Turner, J.R. (2009). Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9(11): 799–809.
Ulrich-Merzenich, G., Panek, D., Zeitler, H., Vetter, H., and Wagner, H. (2010). Drug development from natural products: exploiting synergistic effects. Indian. J. Exp. Biol. 48(3): 208–219.
Vakil, N. (2024). Peptic Ulcer Disease: A Review. JAMA 332(21): 1832–1842.
Wagner, H., and Ulrich-Merzenich, G. (2009). Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine 16(2-3): 97–110.
Yu, S., Duan, Z., Li, P., Wang, S., Guo, L., Xia, G., and Xie, H. (2022). Protective Effect of Polyphenols Purified from Mallotus oblongfolius on Ethanol-Induced Gastric Mucosal Injury by Regulating Nrf2 and MAPKs Pathways. Antioxidants (Basel) 11(12): 2452.