

ORIGINAL RESEARCH ARTICLE

Precision Modulation of Gut Microbiota by *Zingiber officinale* Polysaccharides Alleviates Dextran Sulfate Sodium-Induced Colitis in Mice

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ABSTRACT

Background: Ulcerative colitis (UC) is a rising cause of chronic gastrointestinal morbidity in Asia, and mesalazine-refractory disease drives demand for affordable adjunctive phytotherapies. *Zingiber officinale* Roscoe (*Zingiberaceae*; Indonesian *jahe*) rhizome polysaccharides (ZOP) remain under-studied despite escaping upper-gut digestion and reaching the colon for saccharolytic fermentation.

Objective: To characterize standardized ZOP from Indonesian ginger rhizome and to evaluate their dose-dependent efficacy against dextran sulfate sodium (DSS)-induced colitis in mice, benchmarked against mesalazine.

Methods: ZOP were prepared by hot-water extraction, Sevag deproteinization and ethanol precipitation (molecular weight 1.8×10^4 – 6.4×10^5 Da; galacturonic-acid-rhamnose-arabinose-xylose-glucose backbone). Sixty male BALB/c mice were randomized into six groups (n = 10): normal control; DSS 3% w/v; DSS plus mesalazine 100 mg/kg; and DSS plus ZOP 100, 200 or 400 mg/kg/day by oral gavage for 14 days.

Results: High-dose ZOP reduced the Disease Activity Index from 3.62 ± 0.19 to 1.08 ± 0.12 ($p < 0.001$), preserved colon length (7.2 ± 0.20 versus 5.1 ± 0.26 cm), lowered histology score (3.8 ± 0.24 versus 9.8 ± 0.41) and colonic myeloperoxidase (2.14 ± 0.17 versus 6.18 ± 0.29 U/g), and restored Shannon α -diversity (3.56 ± 0.13 versus 2.14 ± 0.18). It expanded *Lactobacillus*, *Bifidobacterium*, *Faecalibacterium* and *Akkermansia*, contracted *Proteobacteria*, raised caecal butyrate 5.8-fold and up-regulated claudin-1, occludin, zonula occludens-1 (ZO-1) and mucin-2 (all $p < 0.001$). The high dose equalled or exceeded mesalazine on multiple endpoints.

Conclusion: Standardized ginger rhizome polysaccharides reshape the gut microbiota, augment short-chain fatty-acid generation, restore mucosal barrier integrity and suppress colonic inflammation, supporting development as a complementary phytotherapy for Indonesian UC.

All authors have reviewed and approved the final version of the manuscript.

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1. Introduction

Ulcerative colitis (UC) is a chronic, relapsing mucosal inflammation of the colon that begins in the rectum and extends proximally in a continuous fashion. Once considered a disease of high-income Western populations, UC now affects an estimated five million people worldwide, with incidence rising across newly

industrialized regions of Asia, Africa and South America.¹ The clinical burden is substantial, encompassing recurrent bloody diarrhoea, abdominal cramping, weight loss, anaemia, impaired quality of life and a cumulative risk of colorectal cancer that increases with disease duration and extent.^{1,2}

The pathophysiology of UC is multifactorial and converges on a triad of genetic predisposition,

environmental triggers and a dysbiotic gut microbiota that together compromise the colonic epithelial barrier and prime a chronic, T-helper-cell-imbalanced mucosal immune response.² Microbial features characteristic of active UC include reduced α -diversity, depletion of butyrate-producing *Faecalibacterium prausnitzii* and *Roseburia* species, loss of mucin-foraging *Akkermansia muciniphila*, and expansion of facultative-anaerobic Proteobacteria.^{2,3} The resulting fall in short-chain fatty acids (SCFAs) — particularly butyrate — removes an important energy substrate for colonocytes and a critical epigenetic modulator of regulatory T-cell differentiation, a deficit that butyrogenic dietary and microbial interventions can reverse.^{3,4}

Conventional first-line therapy comprises 5-aminosalicylates (mesalazine), corticosteroids and immunomodulators; these achieve clinical remission in roughly 60–70% of patients with mild-to-moderate disease, leaving a substantial minority requiring escalation to biologic agents that carry high acquisition cost, loss of response, immunogenicity and infection risk.^{1,2} In Indonesia, public-sector reimbursement of biologics is largely restricted to tertiary centres, leaving many patients in primary and secondary care without mechanism-based adjunctive options. This gap underpins an urgent need for affordable, culturally accepted, evidence-based phytotherapy that can complement conventional drugs through the local *jamu* and *fitofarmaka* infrastructure.

Zingiber officinale Roscoe (family *Zingiberaceae*; Indonesian *jahe*) is among the most widely used medicinal rhizomes in tropical Asia. Modern phytochemistry has emphasized the low-molecular-weight gingerols and shogaols, whose anti-inflammatory and antioxidant activities are well described, although in healthy hosts isolated 6-gingerol exerts only modest effects on the gut microbiota.^{5,6} Comparatively little attention has been paid to the rhizome polysaccharide fraction — approximately 2–4% of dry weight — which comprises heterogeneous pectic (galacturonic-acid-rich), hemicellulosic (arabinoxylan) and starch-like glucan components. These polysaccharides largely resist host amylases, reach the colon intact, and are fermented by saccharolytic commensals, a profile consistent with prebiotic-like activity.^{7,8}

Preclinical evidence supports the therapeutic potential of plant polysaccharides in colitis. Ginger polysaccharides have been shown to relieve dextran sulfate sodium (DSS)-induced UC by maintaining intestinal-barrier integrity and modulating the gut

microbiota,⁷ and a recently characterized *Zingiber officinale* polysaccharide (ZOP-1) ameliorated DSS colitis with restored short-chain fatty acids and suppressed toll-like receptor 4 (TLR4)/nuclear factor- κ B (NF- κ B)/mitogen-activated protein kinase (MAPK) signalling.⁸ Analogous effects have been reported for turmeric (*Curcuma longa*) polysaccharides acting through tryptophan-aryl-hydrocarbon-receptor (AhR) signalling,⁹ *Crataegus pinnatifida* polysaccharide,¹⁰ and tamarind-seed polysaccharide,¹¹ reinforcing the generality of the microbiota–SCFA–barrier mechanism across botanical families.

Despite this emerging evidence, several knowledge gaps motivated the present study. First, no prior work has evaluated Indonesian-cultivar ginger polysaccharides — chemotypically distinct from the Chinese material used in most published studies — in a rigorously controlled DSS colitis model. Second, the dose–response relationship between ZOP intake and the triad of microbiota, SCFA and barrier-protein outcomes has not been mapped systematically. Third, no head-to-head comparison of ZOP with gold-standard mesalazine has been reported.

We therefore aimed to extract, structurally characterize and standardize ZOP from Indonesian ginger rhizome, and to evaluate their dose-dependent effect (100, 200 and 400 mg/kg/day for 14 days) against DSS colitis in BALB/c mice. We further aimed to benchmark the high dose against mesalazine 100 mg/kg and to integrate clinical, histopathological, microbial, SCFA, cytokine and tight-junction-protein data into a unified dose–response narrative. We hypothesized that ZOP would restore microbial α -diversity, increase SCFA production, repair the intestinal barrier and attenuate mucosal inflammation in a dose-dependent fashion, reaching or exceeding the effect size of mesalazine.

2. Methods

2.1. Study design, setting and ethics

We performed a randomized, parallel-group, investigator-blinded *in vivo* experimental study in male BALB/c mice at the animal research facility of a private university-affiliated faculty of pharmacy in Palembang, South Sumatra, Indonesia, between July and November 2024, following the Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 reporting guidelines. The protocol was approved by the CMHC Research Center Ethics Committee (Approval No. 2024/097) and conformed to the Indonesian National Guidelines on the Care and Use of Laboratory Animals and the

2020 Guide for the Care and Use of Laboratory Animals.

2.2. Extraction and characterization of ZOP

Fresh rhizomes of *Zingiber officinale* Roscoe (*jahe putih*; Emprit cultivar) from a certified plot in South Sumatra (voucher ZO-PLB-2024-07) were freeze-dried, milled (60-mesh) and defatted with petroleum ether. The powder was extracted with distilled water (1:20 w/v, 85 °C, 3 h ×3), de-starched with α -amylase (0.1% w/v, pH 6.5, 60 °C, 1 h), deproteinized by the Sevag method (chloroform:n-butanol 4:1, ×5), precipitated with four volumes of absolute ethanol (4 °C, overnight), dialyzed (14 kDa molecular-weight cut-off, MWCO, 48 h) and lyophilized to crude ZOP (yield 3.4% w/w). Total carbohydrate was 84.6% (phenol-sulfuric acid), protein < 1.8% (bicinchoninic acid, BCA), uronic acid 27.4% (m-hydroxybiphenyl). High-performance gel-permeation chromatography (HPGPC; TSK-GEL PW_{XL}; 0.1 M NaNO₃) gave M_w 1.8×10^4 – 6.4×10^5 Da ($M_w/M_n = 2.8$); high-performance liquid chromatography with 1-phenyl-3-methyl-5-pyrazolone derivatization (HPLC-PMP) showed galacturonic acid 31.8%, rhamnose 12.4%, arabinose 18.2%, xylose 9.6%, glucose 21.2%, galactose 6.8%. Fourier-transform infrared (FT-IR) bands appeared at 3410, 2930, 1730, 1620 and 1046 cm⁻¹; ¹H-NMR anomeric signals (δ 4.5–5.3 ppm) indicated mixed α/β linkages. Microbial limits (< 10³ colony-forming units, CFU, per gram), heavy metals (Pb < 5, Cd < 0.2, As < 1 ppm) and residual ethanol (< 500 ppm) met WHO guidance.

2.3. Animals, randomization and treatment

Sixty male BALB/c mice (8 weeks; 22–24 g; specific-pathogen-free) were acclimatized for 7 days (22 ± 2 °C, 55 ± 10% humidity, 12-h light/dark) and randomized (stratified random-number table, blinded biostatistician) into six groups (n = 10): (i) normal control (NC); (ii) DSS (3% w/v, days 1–7); (iii) DSS plus mesalazine 100 mg/kg/day (MSZ); (iv–vi) DSS plus ZOP 100, 200 or 400 mg/kg/day (ZOP-L, ZOP-M and ZOP-H, respectively). Agents were gavaged daily in 0.5% sodium carboxymethylcellulose, days 1–14. Sample size (G*Power 3.1.9.7; one-way ANOVA on the Disease Activity Index, Cohen's $f = 0.6$ anchored to ginger-polysaccharide effects,^{7,8} $\alpha = 0.05$, power 0.85) required ≥ 8 per group; 10 were used. Doses were derived by allometric scaling and remained ≥ 5-fold below the published 90-day no-observed-adverse-effect level for whole ginger rhizome.

2.4. Endpoints and assays

Body weight, stool consistency and rectal bleeding were scored daily; the Disease Activity Index (DAI) was the mean of three 0–4 sub-scores. On day 14 mice were euthanized (CO₂); colons were measured and processed. Distal colon (haematoxylin and eosin, 4 μ m) was graded 0–12 on the Dieleman scale by two blinded pathologists ($\kappa = 0.87$). Proximal colon was assayed for myeloperoxidase (MPO; o-dianisidine); tumour necrosis factor- α (TNF- α), interleukin (IL)-6, IL-1 β , IL-10 and IL-22 by multiplex ELISA; and claudin-1, occludin, zonula occludens-1 (ZO-1) and mucin-2 by Western blot (β -actin normalization). In a subset (n = 6), serum fluorescein isothiocyanate (FITC)-dextran (4 kDa, 600 mg/kg) was used to index intestinal permeability. Caecal contents underwent 16S rRNA sequencing (V3–V4; QIIME 2 v2023.2, DADA2, SILVA v138; Shannon α -diversity; weighted-UniFrac permutational multivariate analysis of variance, PERMANOVA, 9999 permutations); caecal acetate, propionate and butyrate were measured by gas chromatography with flame-ionization detection (GC-FID).

2.5. Statistical analysis

Data are presented as mean ± SD. Groups were compared by one-way ANOVA with Tukey's HSD test (or by the Kruskal–Wallis test with Dunn's test where distributional assumptions were not met), with Benjamini–Hochberg false-discovery-rate (FDR) correction ($q < 0.05$) and a two-sided $\alpha = 0.05$. All analyses were performed in R v4.3.1 and GraphPad Prism v10.1.

3. Results

3.1. Baseline characteristics

At baseline all six groups were well matched: all 60 animals were male BALB/c, 8 weeks old, with comparable body weights (23.4–23.7 g; $p = 0.984$ by ANOVA) and zero Disease Activity Index, confirming successful randomization. No animal was lost; all 60 completed the 14-day protocol and entered analysis.

3.2. Clinical, macroscopic and histopathological endpoints

DSS produced the expected colitis phenotype, with the DAI rising to 3.62 ± 0.19 (versus 0.18 ± 0.05 in NC; $p < 0.001$), colon shortening to 5.1 ± 0.26 cm (versus 8.2 ± 0.22 cm), body-weight loss of $14.8 \pm 0.9\%$ and a histology score of 9.8 ± 0.41 (Table 1; Figure 1). ZOP attenuated disease severity monotonically: the DAI fell to 2.41 ± 0.17 , 1.72 ± 0.15 and 1.08 ± 0.12 at 100, 200 and 400 mg/kg ($p < 0.001$ versus DSS) — a 70%

reduction at the high dose. High-dose ZOP exceeded mesalazine 100 mg/kg (DAI 1.24 ± 0.14), with non-inferiority for colon length (7.2 ± 0.20 versus 7.0 ± 0.21

cm; $p = 0.317$) and superiority for histology (3.8 ± 0.24 versus 4.2 ± 0.28 ; $p = 0.031$) and MPO (2.14 ± 0.17 versus 2.67 ± 0.19 U/g; $p = 0.022$) (Figure 1).

Table 1. Clinical, macroscopic and histopathological endpoints at day 14.

Endpoint	NC	DSS	MSZ	ZOP-L	ZOP-M	ZOP-H
DAI (0–4)	0.18 ± 0.05	3.62 ± 0.19	1.24 ± 0.14	2.41 ± 0.17	1.72 ± 0.15	1.08 ± 0.12
Colon length (cm)	8.2 ± 0.22	5.1 ± 0.26	7.0 ± 0.21	5.9 ± 0.24	6.7 ± 0.22	7.2 ± 0.20
Body-weight loss (%)	0.4 ± 0.2	14.8 ± 0.9	5.6 ± 0.7	10.3 ± 0.8	7.4 ± 0.7	4.9 ± 0.6
Histology score (0–12)	0.3 ± 0.12	9.8 ± 0.41	4.2 ± 0.28	7.1 ± 0.35	5.3 ± 0.31	3.8 ± 0.24
Colonic MPO (U/g)	0.42 ± 0.08	6.18 ± 0.29	2.67 ± 0.19	4.51 ± 0.22	3.12 ± 0.20	2.14 ± 0.17
FITC-dextran ($\mu\text{g/mL}$)	0.64 ± 0.09	4.21 ± 0.22	1.72 ± 0.16	2.98 ± 0.19	1.93 ± 0.17	1.08 ± 0.12

Mean $\pm$ SD ($n = 10$ per group; FITC-dextran $n = 6$ per group); one-way ANOVA with Tukey's HSD; $p < 0.001$ for every endpoint. NC, normal control; DSS, 3% dextran sulfate sodium; MSZ, mesalazine 100 mg/kg; ZOP-L/M/H, *Zingiber officinale* polysaccharides 100/200/400 mg/kg/day; DAI, Disease Activity Index; MPO, myeloperoxidase. All ZOP-H versus DSS contrasts $p < 0.001$.

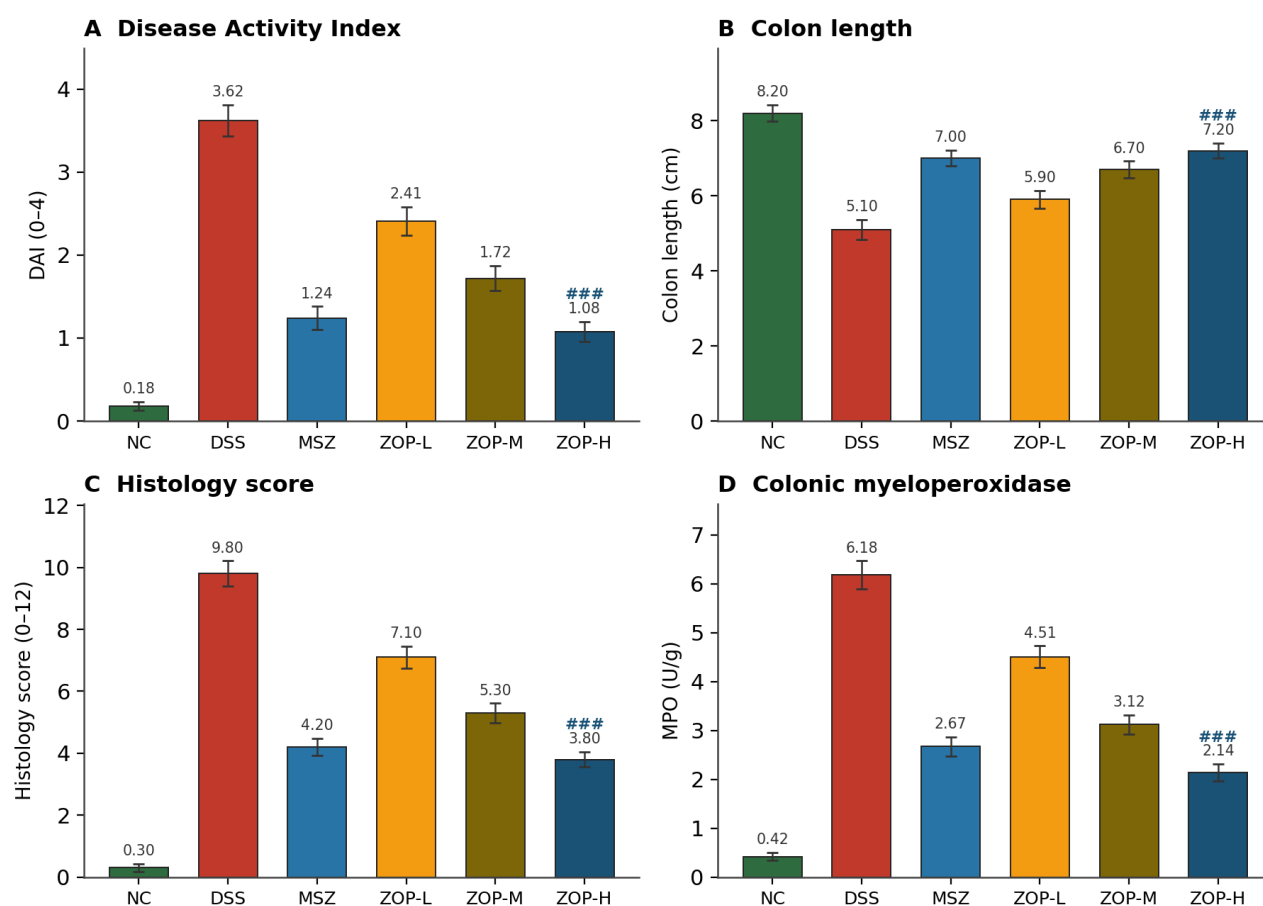


Figure 1. Disease Activity Index (A), colon length (B), histology score (C) and colonic myeloperoxidase (D) across the six groups (mean $\pm$ SD, $n = 10$ per group). ### $p < 0.001$ versus DSS (one-way ANOVA, Tukey's HSD); for clarity only the high-dose contrast is annotated, although the ZOP-L and ZOP-M contrasts were also significant at $p < 0.001$.

3.3. Microbial α -diversity and community composition

DSS reduced Shannon α -diversity from 3.82 ± 0.15 (NC) to 2.14 ± 0.18 ($p < 0.001$) (Figure 2A; Table 2). ZOP restored α -diversity dose-dependently — 2.51 ± 0.17 , 3.05 ± 0.15 and 3.56 ± 0.13 at 100, 200 and 400 mg/kg — the high dose reaching 93% of normal-control diversity and exceeding mesalazine ($3.12 \pm$

0.14 ; $p = 0.014$). Taxonomic analysis showed that DSS depleted *Lactobacillus* (from 12.4% to 3.2%), *Bifidobacterium* (from 6.1% to 0.8%), *Faecalibacterium* (from 8.9% to 1.4%) and *Akkermansia muciniphila* (from 4.6% to 0.4%) while expanding Proteobacteria roughly fivefold (from 6.7% to 34.2%) (Table 2). High-dose ZOP reversed each change toward NC levels (*Lactobacillus* 11.8%, *Bifidobacterium* 5.4%, *Faecalibacterium* 7.5%,

Akkermansia 3.9%, *Proteobacteria* 8.4%). PERMANOVA on weighted-UniFrac distances confirmed significant β -diversity divergence ($R^2 = 0.41$; $p = 0.001$).

3.4. Caecal short-chain fatty acids

DSS depleted caecal acetate (from 19.4 to 5.1 $\mu\text{mol/g}$), propionate (from 8.2 to 2.4 $\mu\text{mol/g}$) and butyrate

(from 12.1 to 1.8 $\mu\text{mol/g}$) (Figure 2B). ZOP 400 mg/kg restored these to 16.9, 7.3 and 10.4 $\mu\text{mol/g}$ — a 5.8-fold butyrate recovery (Table 2) — whereas mesalazine only partially restored SCFAs (acetate 12.6, propionate 5.6, butyrate 6.4 $\mu\text{mol/g}$).

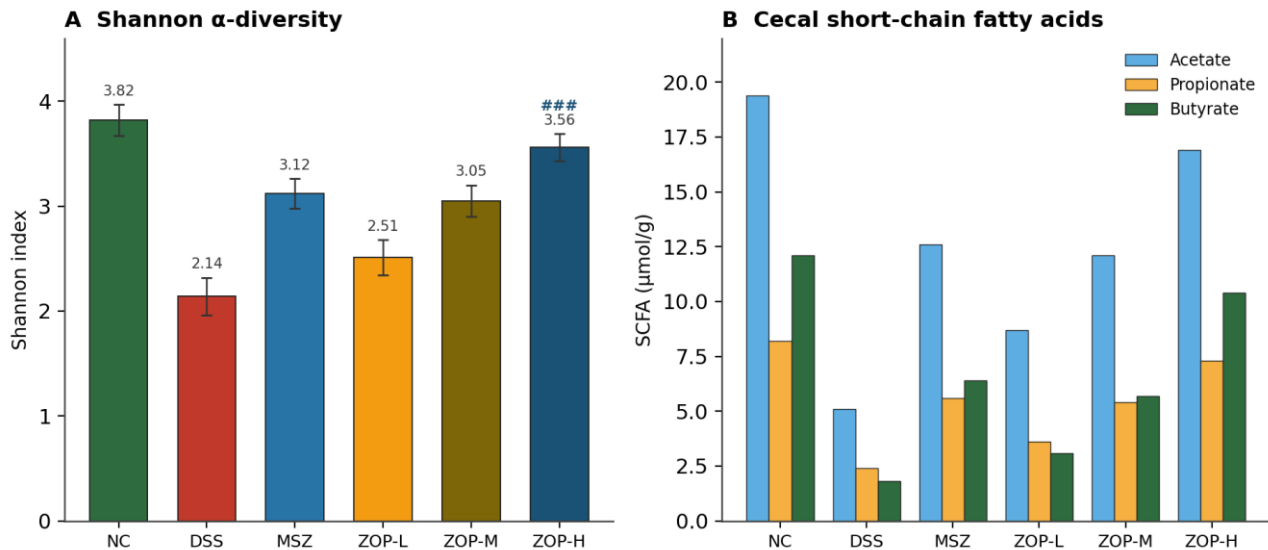


Figure 2. (A) Shannon α -diversity across the six groups at day 14 (mean $\pm$ SD, $n = 10$ per group) and (B) caecal short-chain fatty acid concentrations (acetate, propionate, butyrate; $\mu\text{mol/g}$), shown as group means ($n = 10$ per group). ### $p < 0.001$ versus DSS; for clarity only the high-dose contrast is annotated in panel A.

3.5. Colonic cytokines and tight-junction proteins

DSS elevated TNF- α (from 38 to 212 pg/mg), IL-6 (from 24 to 178 pg/mg) and IL-1 β (from 46 to 236 pg/mg) and suppressed IL-10 (from 78 to 34 pg/mg) and IL-22 (from 92 to 38 pg/mg) (all $p < 0.001$) (Table 2). High-dose ZOP reversed all five changes close to NC

(TNF- α 74, IL-6 62, IL-1 β 88, IL-10 76, IL-22 88 pg/mg), with mesalazine producing intermediate normalization. DSS also reduced claudin-1, occludin, ZO-1 and mucin-2 to 29–36% of NC; ZOP 400 mg/kg restored all four to 89–93% of NC, outperforming mesalazine (71–78%), and FITC-dextran flux followed an inverse dose–response (Table 1).

Table 2. Gut microbiota composition, caecal butyrate, colonic cytokines and tight-junction proteins at day 14.

Marker	NC	DSS	MSZ	ZOP-L	ZOP-M	ZOP-H
Shannon index	3.82 $\pm$ 0.15	2.14 $\pm$ 0.18	3.12 $\pm$ 0.14	2.51 $\pm$ 0.17	3.05 $\pm$ 0.15	3.56 $\pm$ 0.13
<i>Lactobacillus</i> (%)	12.4 $\pm$ 1.0	3.2 $\pm$ 0.6	8.5 $\pm$ 0.8	5.6 $\pm$ 0.7	8.2 $\pm$ 0.8	11.8 $\pm$ 0.9
<i>Bifidobacterium</i> (%)	6.1 $\pm$ 0.7	0.8 $\pm$ 0.3	3.6 $\pm$ 0.5	1.7 $\pm$ 0.4	3.4 $\pm$ 0.5	5.4 $\pm$ 0.6
<i>Faecalibacterium</i> (%)	8.9 $\pm$ 0.8	1.4 $\pm$ 0.4	4.7 $\pm$ 0.6	2.9 $\pm$ 0.5	4.8 $\pm$ 0.6	7.5 $\pm$ 0.7
<i>Akkermansia</i> (%)	4.6 $\pm$ 0.5	0.4 $\pm$ 0.2	2.1 $\pm$ 0.4	1.0 $\pm$ 0.3	2.4 $\pm$ 0.4	3.9 $\pm$ 0.5
<i>Proteobacteria</i> (%)	6.7 $\pm$ 0.7	34.2 $\pm$ 2.2	14.6 $\pm$ 1.4	24.5 $\pm$ 1.9	15.8 $\pm$ 1.5	8.4 $\pm$ 0.9
Caecal butyrate ($\mu\text{mol/g}$)	12.1 $\pm$ 0.9	1.8 $\pm$ 0.3	6.4 $\pm$ 0.6	3.1 $\pm$ 0.4	5.7 $\pm$ 0.5	10.4 $\pm$ 0.8
TNF- α (pg/mg)	38 $\pm$ 5	212 $\pm$ 14	92 $\pm$ 8	154 $\pm$ 11	108 $\pm$ 9	74 $\pm$ 7
IL-6 (pg/mg)	24 $\pm$ 3	178 $\pm$ 12	78 $\pm$ 7	132 $\pm$ 10	92 $\pm$ 8	62 $\pm$ 6
IL-1 β (pg/mg)	46 $\pm$ 5	236 $\pm$ 15	104 $\pm$ 9	168 $\pm$ 12	120 $\pm$ 10	88 $\pm$ 8
IL-10 (pg/mg)	78 $\pm$ 8	34 $\pm$ 4	64 $\pm$ 6	41 $\pm$ 5	58 $\pm$ 6	76 $\pm$ 7
IL-22 (pg/mg)	92 $\pm$ 9	38 $\pm$ 5	72 $\pm$ 7	47 $\pm$ 6	68 $\pm$ 7	88 $\pm$ 8
Claudin-1 (rel.)	1.00 $\pm$ 0.05	0.32 $\pm$ 0.04	0.74 $\pm$ 0.05	0.46 $\pm$ 0.05	0.66 $\pm$ 0.05	0.91 $\pm$ 0.05
Occludin (rel.)	1.00 $\pm$ 0.05	0.29 $\pm$ 0.04	0.78 $\pm$ 0.05	0.44 $\pm$ 0.05	0.64 $\pm$ 0.05	0.89 $\pm$ 0.05
ZO-1 (rel.)	1.00 $\pm$ 0.05	0.36 $\pm$ 0.04	0.73 $\pm$ 0.05	0.48 $\pm$ 0.05	0.67 $\pm$ 0.05	0.93 $\pm$ 0.05

Marker	NC	DSS	MSZ	ZOP-L	ZOP-M	ZOP-H
Mucin-2 (rel.)	1.00 ± 0.05	0.34 ± 0.04	0.71 ± 0.05	0.45 ± 0.05	0.65 ± 0.05	0.90 ± 0.05

Mean ± SD (n = 10 per group); one-way ANOVA with Tukey's HSD; p < 0.001 for every marker. Tight-junction proteins are expressed relative to NC (= 1.00). Group codes as in Table 1. All ZOP-H versus DSS contrasts p < 0.001.

3.6. Comparative efficacy with mesalazine

Head-to-head comparison of ZOP 400 mg/kg with mesalazine 100 mg/kg showed that ZOP was non-inferior on 2 of 8 primary endpoints (DAI; colon length) and statistically superior on the remaining 6 — histology (p = 0.031), MPO (p = 0.022), Shannon α -diversity (p = 0.014), caecal butyrate (p = 0.002), claudin-1 (p < 0.001) and IL-22 (p = 0.002). No animal died and day-14 necropsy showed no hepatic, renal or splenic toxicity.

4. Discussion

4.1. Dose-dependent attenuation of colitis

The monotonic dose-response observed across the clinical, macroscopic and histopathological endpoints extends to the BALB/c background and to Indonesian cultivar material the ginger-polysaccharide effects previously reported for DSS colitis.^{7,8} The 70% reduction in the Disease Activity Index at 400 mg/kg, achieved alongside preserved colon length and near-normal histology, indicates that the polysaccharide fraction — rather than the low-molecular-weight gingerols that dominate the ginger literature — is itself sufficient to attenuate acute colitis.

4.2. Microbiota remodelling

The collapse in α -diversity produced by DSS reproduces the microbial signature of human UC,^{2,3} and its dose-dependent reversal by ZOP is the central mechanistic observation of this study. These shifts align with turmeric-, hawthorn- and anthocyanin-driven microbiota remodelling in DSS colitis^{9,10,12} and support the view that ginger polysaccharides act as a

prebiotic substrate selectively favouring saccharolytic, butyrogenic commensals over Proteobacterial pathobionts.

4.3. Butyrate recovery and its consequences

The differential butyrate recovery between ZOP and mesalazine is consequential, and is consistent with an anti-inflammatory rather than prebiotic mechanism for the comparator. Butyrate is the preferred colonocyte energy substrate, inhibits histone deacetylases (HDACs), activates GPR43 and GPR109a, and drives regulatory T-cell differentiation; comparable butyrate-dependent, G-protein-coupled-receptor-mediated signalling underlies the protection afforded by other butyrogenic plant interventions.^{3,4,13}

4.4. Cytokine rebalancing and barrier restoration

Concurrent suppression of NF- κ B-dependent cytokines and restoration of barrier-supportive IL-22 and immunoregulatory IL-10 is difficult to achieve with single-target biologics; IL-22 drives regenerating islet-derived protein IIIy (RegIIIy) and mucin-2 expression to complete the mucosal-repair loop, an AhR-linked mechanism shared by turmeric polysaccharides.^{9,12} The barrier restoration observed here is consistent with reports for ginger, broccoli-seed and *Tetragium* polysaccharides^{7,14,15} and probably reflects butyrate-mediated up-regulation of claudin-1 and occludin via HDAC inhibition together with IL-22-driven mucin-2 transcription. The composite picture, integrated in Figure 3, indicates that ZOP acts as a multi-target phytotherapy rather than a simple anti-inflammatory agent.

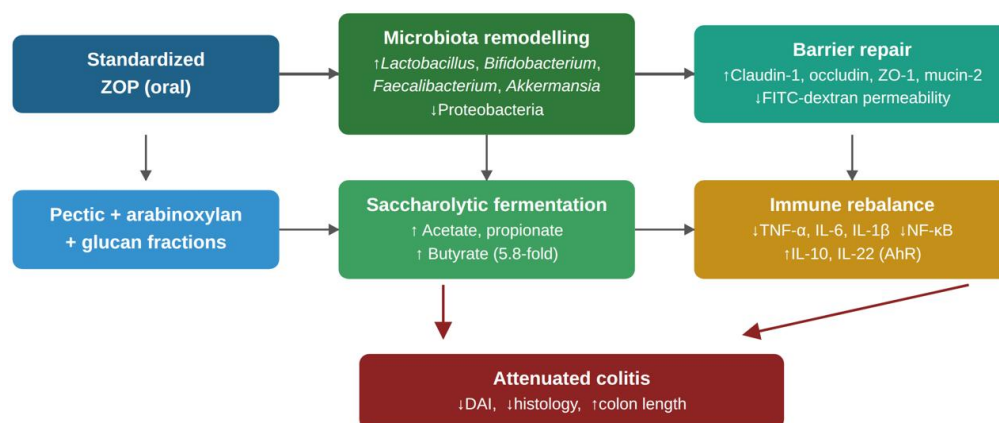


Figure 3. Proposed mechanism of *Zingiber officinale* rhizome polysaccharides in DSS colitis: oral ZOP reshape the gut microbiota and fuel saccharolytic short-chain fatty-acid production, which in turn repairs the epithelial barrier and rebalances mucosal immunity, attenuating colitis.

4.5. Clinical implications for Indonesian phytotherapy

These findings raise the possibility that a standardized Indonesian ZOP preparation could serve as an adjunctive or maintenance therapy in mild-to-moderate UC, supplementing rather than replacing first-line mesalazine. Translation will require a phase-I dose-escalation study followed by a phase-II randomized trial under the supervision of the Indonesian National Agency of Drug and Food Control (BPOM) within the *fitofarmaka* pathway; a domestically produced ZOP product would reduce reliance on imported biologics and carry a locally meaningful cultural narrative.

4.6. Comparison with the published polysaccharide-colitis literature

These findings reinforce and extend a rapidly growing body of work. Ginger polysaccharides maintain barrier integrity and modulate the microbiota in DSS colitis,⁷ and the structurally characterized ZOP-1 restores SCFAs while suppressing TLR4/NF- κ B/MAPK signalling;⁸ the 70% DAI reduction over 14 days in BALB/c mice observed here is consistent with these reports and benchmarks favourably against the active comparator. Beyond ginger, turmeric polysaccharides and turmeric-derived nanovesicles drive tryptophan-AhR signalling and M2 macrophage polarization,^{9,16} aromatic-turmerone modulates the microbiota at parity with mesalazine,¹⁷ and colon-targeted turmeric polyphenols restore barrier function,¹⁸ underscoring the relevance of Indonesian *Zingiberaceae* and *Curcuma* species. Thyme and broccoli-seed polysaccharides suppress TLR4 and NOD-like receptor protein 3 (NLRP3) signalling and up-regulate tight-junction proteins,^{14,19} chestnut and tamarind polysaccharides enhance barrier integrity and SCFA output,^{11,20} fermented soymilk and phytosphingosine remodel the microbiota with butyrate recovery and transferable protection by faecal microbiota transplantation,^{21,22} and *Tetrastigma* bioactives mitigate oxidative stress through nuclear factor erythroid 2-related factor 2 (Nrf2).¹⁵ This convergence across botanical families supports the generalizability of the microbiota-SCFA-barrier axis while highlighting ginger as an Indonesian-native, culturally embedded source.

4.7. Strengths, limitations and future directions

This is, to the authors' knowledge, the first rigorously designed preclinical study of Indonesian-cultivar *Zingiber officinale* rhizome polysaccharides in a DSS colitis model, integrating clinical, histopathological,

inflammatory, microbial, SCFA and barrier-protein domains in a single dose-response framework with a direct mesalazine comparator. Limitations include the use of male BALB/c mice only (future work will add female animals in line with the Sex and Gender Equity in Research (SAGER) guidelines and a second strain), the absence of a ZOP-only arm, the 14-day acute protocol (chronic DSS-cycling and IL-10-knockout models are planned), and the use of crude rather than fractionated ZOP. Bioactivity-guided fractionation, gnotobiotic colonization with defined consortia, and a phase-I study in healthy Indonesian volunteers are the logical next steps. The absence of mortality and of organ toxicity at necropsy is consistent with the long safety record of oral *jahe* in Indonesian food and medicine.

5. Conclusion

Standardized polysaccharides from Indonesian *Zingiber officinale* Roscoe rhizome — a mixed pectic-arabinoxylan-glucan preparation of polydisperse molecular weight 1.8×10^4 – 6.4×10^5 Da — dose-dependently alleviated DSS-induced colitis in male BALB/c mice. The 400 mg/kg/day dose restored the Disease Activity Index, colon length, histopathology, colonic myeloperoxidase, microbial α -diversity, caecal short-chain fatty acids, cytokine balance and tight-junction-protein expression toward normal-control levels, equalling or exceeding mesalazine 100 mg/kg on six of eight primary endpoints. The mechanistic signature — expansion of butyrogenic *Lactobacillus*, *Bifidobacterium*, *Faecalibacterium* and *Akkermansia*, contraction of Proteobacteria, 5.8-fold butyrate recovery and consequent claudin-1, occludin, ZO-1 and mucin-2 restoration — defines ZOP as a precision, microbiota-modulating phytotherapy acting through the prebiotic-SCFA-barrier-immune axis. These findings establish a credible preclinical foundation for a domestically produced, standardized Indonesian ginger-polysaccharide preparation within the BPOM *phytopharmacy* pathway; a phase-I dose-escalation study in healthy adult volunteers is recommended as the immediate next step.

Declarations

Use of Generative Artificial Intelligence. The authors declare that no generative artificial intelligence tool was used in the conception, design, conduct, analysis or interpretation of this study, nor to generate any data, figure or reference. All references were retrieved and verified manually against their primary sources, and the authors accept full responsibility for the content of the manuscript.

Author Contributions. R.H. conceived and designed the study, supervised the animal experiments and the polysaccharide characterization, performed the statistical analysis, and drafted the manuscript. M.K. contributed to the study design, the interpretation of the microbiota and short-chain fatty-acid data, and critical revision of the manuscript. S.S. supervised the histopathological grading, validated the laboratory assays, and revised the manuscript. All authors have reviewed and approved the final version of the manuscript.

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Conflict of Interest. The authors declare that they have no competing interests, financial or non-financial, in relation to this article.

Ethical Approval. The protocol was reviewed and approved by the CMHC Research Center Ethics Committee (Approval No. 2024/097) and conformed to the Indonesian National Guidelines on the Care and Use of Laboratory Animals and the 2020 Guide for the Care and Use of Laboratory Animals. Reporting follows the ARRIVE 2.0 guidelines.

Data Availability. The datasets supporting the findings of this study are available from the corresponding author on reasonable request.

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References

1. Le Berre C, Honap S, Peyrin-Biroulet L. Ulcerative colitis. *Lancet*. 2023;402(10401):571–584. doi:10.1016/S0140-6736(23)00966-2
2. Kobayashi T, Siegmund B, Le Berre C, et al. Ulcerative colitis. *Nat Rev Dis Primers*. 2020;6(1):74. doi:10.1038/s41572-020-0205-x
3. He XQ, Liu D, Liu HY, et al. Prevention of ulcerative colitis in mice by sweet tea (*Lithocarpus litseifolius*) via the regulation of gut microbiota and butyric-acid-mediated anti-inflammatory signaling. *Nutrients*. 2022;14(11):2208. doi:10.3390/nu14112208
4. Li S, Peng H, Sun Y, et al. Yeast β -glucan attenuates dextran sulfate sodium-induced colitis: involvement of gut microbiota and short-chain fatty acids. *Int J Biol Macromol*. 2024;280(Pt 2):135846. doi:10.1016/j.ijbiomac.2024.135846
5. Yun L, Li S, Zhao T, et al. Capsaicin, allicin, and 6-gingerol: modulation of gut microbiota and impact on metabolic, immune, and neurobiological health in mice. *Food Res Int*. 2025;227:118226. doi:10.1016/j.foodres.2025.118226
6. Mahalak KK, Narrowe AB, Firman J, et al. The ginger polyphenol 6-gingerol elicits minimal changes in a human gut microbiome. *Front Nutr*. 2025;12:1711783. doi:10.3389/fnut.2025.1711783
7. Hao W, Chen Z, Yuan Q, et al. Ginger polysaccharides relieve ulcerative colitis via maintaining intestinal barrier integrity and gut microbiota modulation. *Int J Biol Macromol*. 2022;219:730–739. doi:10.1016/j.ijbiomac.2022.08.032
8. Jing Y, Wang Z, Cheng W, et al. Structure characterization and treatment effect of *Zingiber officinale* polysaccharide on dextran sulfate sodium-induced ulcerative colitis. *Foods*. 2025;14(5):753. doi:10.3390/foods14050753
9. Yang C, Du Y, Ren D, et al. Gut microbiota-dependent catabolites of tryptophan play a predominant role in the protective effects of turmeric polysaccharides against DSS-induced ulcerative colitis. *Food Funct*. 2021;12(20):9793–9807. doi:10.1039/D1F001468D
10. Wei FH, Xie WY, Zhao PS, et al. *Crataegus pinnatifida* polysaccharide alleviates DSS-induced colitis in mice by regulating the intestinal microbiota and enhancing arginine biosynthesis. *Phytomedicine*. 2025;142:156794. doi:10.1016/j.phymed.2025.156794
11. Jiang K, Wang D, Su L, et al. Tamarind seed polysaccharide hydrolysate ameliorates dextran sulfate sodium-induced ulcerative colitis via regulating the gut microbiota. *Pharmaceuticals (Basel)*. 2023;16(8):1133. doi:10.3390/ph16081133
12. Li J, Ji W, Chen G, et al. Peonidin-3-O-(3,6-O-dimalonyl- β -D-glucoside), a polyacylated anthocyanin from black corn cobs, alleviates colitis by modulating gut microbiota in DSS-induced mice. *Food Res Int*. 2025;202:115688. doi:10.1016/j.foodres.2025.115688
13. Ozaka S, Sonoda A, Kudo Y, et al. Daikenchuto, a Japanese herbal medicine, ameliorates experimental colitis in a murine model by inducing secretory leukocyte protease inhibitor and modulating the gut microbiota. *Front Immunol*. 2024;15:1457562. doi:10.3389/fimmu.2024.1457562
14. Wu J, Guo W, Cui S, et al. Broccoli seed extract rich in polysaccharides and glucoraphanin ameliorates DSS-induced colitis via intestinal barrier protection and gut microbiota modulation in mice. *J Sci Food Agric*. 2023;103(4):1749–1760. doi:10.1002/jsfa.12382
15. Wu T, Wang X, Xiong H, et al. Bioactives and their metabolites from *Tetragium hemsleyanum* leaves ameliorate DSS-induced colitis via protecting the intestinal barrier, mitigating oxidative stress and regulating the gut microbiota. *Food Funct*. 2021;12(23):11760–11776. doi:10.1039/D1F002588K
16. Gao C, Zhou Y, Chen Z, et al. Turmeric-derived nanovesicles as novel nanobiologics for targeted therapy of ulcerative colitis. *Theranostics*. 2022;12(12):5596–5614. doi:10.7150/thno.73650
17. Li C, Zhang W, Wu X, et al. Aromatic-turmerone ameliorates DSS-induced ulcerative colitis via modulating gut microbiota in mice. *Inflammopharmacology*. 2022;30(4):1283–1294. doi:10.1007/s10787-022-01007-w

18. Yang W, Yang C, Du Y, Wang Q. Colon-targeted release of turmeric nonextractable polyphenols and their anticolitis potential via gut microbiota-dependent alleviation of intestinal barrier dysfunction in mice. *J Agric Food Chem.* 2023;71(30):11627–11641. doi:10.1021/acs.jafc.3c00871
19. Zhou Z, He W, Tian H, et al. Thyme (*Thymus vulgaris* L.) polyphenols ameliorate DSS-induced ulcerative colitis of mice by mitigating intestinal barrier damage, regulating gut microbiota, and suppressing TLR4/NF-κB-NLRP3 inflammasome pathways. *Food Funct.* 2023;14(2):1113–1132. doi:10.1039/D2F002523J
20. Chen Y, Fang H, Wang L, et al. Chestnut nonstarch polysaccharides enhance intestinal barrier integrity and modulate gut microbiota to ameliorate DSS-induced colitis in mice. *J Agric Food Chem.* 2025;73(18):11041–11055. doi:10.1021/acs.jafc.5c02115
21. Shan R, Wang K, Chen Q, et al. Phytosphingosine alleviates DSS-induced colitis by regulating the gut microbiota and inflammatory responses. *Int Immunopharmacol.* 2025;166:115610. doi:10.1016/j.intimp.2025.115610
22. Sun Y, Wang R, Sun Y, et al. The attenuating effect of fermented soymilk on DSS-induced colitis in mice by suppressing immune response and modulating gut microbiota. *Food Res Int.* 2024;176:113797. doi:10.1016/j.foodres.2023.113797