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ORIGINAL RESEARCH ARTICLE

Synergistic Effects of Standardized *Moringa oleifera* Leaf Extract and a Probiotic Consortium on Intestinal Permeability and Environmental Enteric Dysfunction Biomarkers in Indonesian Children: A Randomized Controlled Trial

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ABSTRACT

Background: Environmental enteric dysfunction (EED) underlies much of the residual stunting and anemia that persist in Indonesian children despite nutrition programs. *Moringa oleifera* Lam. (Moringaceae) leaves contain quercetin, kaempferol, chlorogenic acid, β-sitosterol and glucomoringin-derived isothiocyanates that reinforce tight junctions and dampen mucosal NF-κB signaling, whereas *Lactobacillus* and *Bifidobacterium* probiotics generate short-chain fatty acids and displace enteropathogens.

Objective: To determine whether standardized *M. oleifera* leaf extract co-administered with a defined probiotic consortium reduces intestinal permeability and EED biomarkers in Indonesian children aged 12–59 months.

Methods: In a three-arm, double-blind, placebo-controlled randomized trial, 150 children aged 12–59 months with biomarker-confirmed EED received, for 12 weeks, either standardized 50% ethanolic *M. oleifera* leaf extract 500 mg/day plus a probiotic consortium of *Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* subsp. *lactis* Bb-12 and *Lactobacillus plantarum* Dad-13 (1×10^{10} CFU/day) [Moringa + Probiotic], *M. oleifera* plus placebo probiotic [Moringa alone], or double placebo [Placebo]. The primary outcome was the change in the urinary lactulose-to-mannitol (L:M) ratio at week 12.

Results: In intention-to-treat analysis the L:M ratio fell from 0.42 ± 0.09 to 0.18 ± 0.06 with Moringa + Probiotic, from 0.41 ± 0.10 to 0.27 ± 0.08 with Moringa alone and from 0.43 ± 0.09 to 0.39 ± 0.10 with placebo ($p < 0.001$; $d = 1.42$ for Moringa + Probiotic versus placebo). EED remission occurred in 62.0%, 36.0% and 18.0% of recipients, respectively (OR 4.82, 95% CI 2.31–10.06, $p < 0.001$). Fecal myeloperoxidase, alpha-1-antitrypsin and neopterin, serum zonulin, lipopolysaccharide-binding protein and hs-CRP, hemoglobin and height-for-age Z-score all improved more with the combination, and adverse events were comparable across arms.

Conclusion: Co-administration of standardized *M. oleifera* leaf extract with a multi-strain probiotic consortium restored intestinal barrier function and suppressed EED biomarkers in Indonesian children, supporting a scalable herbal–microbial complementary therapy.

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

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1. Introduction

Childhood stunting remains the most intractable nutritional disorder of Southeast Asia: Indonesia reports a national under-five stunting prevalence of approximately 21.6%, and this figure plateaus in urban-poor populations despite improvements in household income, food security and sanitation.[1] A major share of this residual stunting is now attributed

to environmental enteric dysfunction (EED), a subclinical small-bowel disorder defined by villous blunting, crypt hyperplasia, intra-epithelial lymphocytosis, increased paracellular permeability, and translocation of microbial products into the portal and systemic circulations.[2,3] Unlike acute diarrhea, EED is largely asymptomatic; it is identified through a panel of biomarkers that includes the urinary lactulose-to-mannitol (L:M) ratio, fecal myeloperoxidase (MPO), fecal alpha-1-antitrypsin (AAT), fecal neopterin, serum zonulin and lipopolysaccharide-binding protein (LBP).[4,5]

The pathophysiology of EED weaves together repeated enteric infection, dysbiotic microbiota, mycotoxin and environmental toxin exposure, and chronic inadequate nutrient intake. Each insult impairs claudin-1, occludin and zonula occludens-1 (ZO-1) expression, exposes sub-epithelial dendritic cells to luminal antigens, and amplifies NF-κB- and MAPK-driven cytokine production.[3,5] The resultant enteropathy increases energy expenditure, blunts vaccine responses, depletes micronutrients, and diverts amino acids from linear growth toward acute-phase protein synthesis.[2,6] Conventional nutritional therapy — therapeutic foods, multiple-micronutrient powders and zinc — only partially reverses these derangements.[7] Adjunctive strategies that can simultaneously dampen mucosal inflammation, reinforce tight junctions and restore a beneficial microbiota are urgently required.

Moringa oleifera Lam. (family Moringaceae) is a drought-tolerant tropical tree whose leaves are a staple of Indonesian traditional medicine, where they are called *kelor*. The leaves contain an unusually rich profile of bioactives: quercetin-3-O-glucoside, kaempferol glycosides, chlorogenic and ferulic acids, β-sitosterol, vitamin C and tocopherols, high concentrations of glucomoringin which liberates the isothiocyanate moringin upon myrosinase-catalyzed hydrolysis, and a family of antimicrobial peptides and lectins.[8] Experimental work has shown that standardized *M. oleifera* leaf preparations protect the intestinal epithelial barrier, reduce enteric bacterial translocation and reactivate tight-junction proteins under inflammatory and ischemic stress,[9] and that *M. oleifera* leaf polysaccharide restores villus architecture while suppressing mucosal inflammation in experimental colitis.[10] Mechanistically, the moringin moiety regulates the Nrf2 and NF-κB pathways during lipopolysaccharide-driven inflammation.[11]

Probiotics offer complementary biology. *Lactobacillus rhamnosus* GG (LGG), *Bifidobacterium animalis* subsp. *lactis* Bb-12, and the Indonesian indigenous strain *Lactobacillus plantarum* Dad-13 each produce short-chain fatty acids (SCFAs), upregulate mucin-2, displace enterotoxigenic *E. coli* and *Shigella* spp., augment secretory IgA, and expand FOXP3⁺ regulatory T-cells.[12,13] In Indonesian children, *L. plantarum* Dad-13 favorably remodels the gut microbiota and raises beneficial taxa,[12] and a probiotic chocolate delivering the same strain altered microbiota composition in undernourished children.[14] Probiotic supplementation has likewise been shown to improve intestinal permeability in children.[15] However, no trial has yet tested whether combined phytochemical and probiotic action produces synergistic restoration of the intestinal barrier in children with EED.

Within the Indonesian context, combining standardized *M. oleifera* leaf extract with probiotics offers several practical advantages: both inputs are produced locally; regulatory barriers to pediatric use are low; cultural acceptability is high; cost per course is a small fraction of proprietary medical nutrition therapy; and supply can be decentralized to primary health centers. Furthermore, Indonesia hosts a uniquely well-characterized indigenous probiotic — *L. plantarum* Dad-13 — already standardized for pediatric use.[14] The novelty of the present study lies in three features: the first rigorous randomized evaluation of this phytotherapy-plus-microbial intervention in EED; the use of an Indonesian indigenous strain within the probiotic consortium; and the integration of a contemporary biomarker panel covering paracellular permeability, neutrophilic inflammation, protein-losing enteropathy, immune activation, barrier regulation and systemic endotoxemia.

The aim of this study was to evaluate whether 12 weeks of standardized *M. oleifera* leaf extract co-administered with a defined probiotic consortium would significantly reduce intestinal permeability — measured by the L:M ratio — and secondary biomarkers of EED, relative to *M. oleifera* extract alone and matched placebo, in Indonesian children aged 12–59 months.

2. Methods

2.1. Study design and setting

We conducted a three-arm, double-blind, placebo-controlled, parallel-group randomized trial at the outpatient nutrition clinic of a private university-affiliated teaching hospital in Palembang, South Sumatra, Indonesia between March 2024 and February 2025. The protocol adhered to CONSORT 2025 reporting, ICH-GCP and the Declaration of Helsinki

(2013 revision), and was approved by the CMHC Ethics Committee, Indonesia (Approval No. 2024/159). Written informed consent was obtained from at least one legally authorized parent or guardian before any study procedure.

2.2. Participants

Children aged 12–59 months who were attending routine clinic visits were screened for EED. Inclusion criteria were age 12–59 months, height-for-age Z-score (HAZ) between −1 and −3, a screening urinary L:M ratio ≥ 0.25, and parental willingness to attend four in-person visits. Exclusion criteria were acute severe malnutrition (WHZ < −3 or bipedal edema), active diarrhea with dehydration, known HIV or tuberculosis, congenital gastrointestinal disease, systemic antibiotic use within 14 days, chronic probiotic or herbal use within 30 days, and documented allergy to Moringaceae plants, cow-milk protein or lactose intolerance. Of 287 children screened, 150 met eligibility and underwent randomization; 142 completed week 12 (attrition 5.3%).

2.3. Sample size justification

The primary effect size was anchored to the L:M ratio: a between-arm difference of 0.10 (SD 0.09, two-sided $\alpha = 0.05$, power 0.80, 10% attrition) required 46 participants per arm. To allow adjustment for HAZ stratum and sex and to accommodate interim loss, we enrolled 50 per arm (total n = 150). Post-hoc power at observed variance exceeded 0.95.

2.4. Randomization and blinding

An off-site biostatistician generated a stratified (by baseline HAZ and sex), block-randomized (block sizes 6 and 9) allocation sequence in a 1:1:1 ratio using the R blockrand package v1.5 in R v4.3.1. Allocation was concealed in serially numbered, opaque, sealed envelopes opened only after baseline assessment by an independent custodian. Active and placebo capsules and probiotic sachets were visually, tactually and organoleptically indistinguishable. Participants, caregivers, clinicians, outcome assessors, laboratory analysts and the data-analysis team remained blinded until database lock. At week 12, caregiver and clinician guess rates did not differ from chance (33.3%) (caregivers 35.4%, p = 0.61; clinicians 34.7%, p = 0.74).

2.5. Herbal intervention

Fresh, mature leaves of *Moringa oleifera* Lam. were harvested from a verified cultivar plot in South Sumatra; voucher specimens (MO-PLB-2024-003) were deposited at the university herbarium. Leaves were air-dried at ≤ 40 °C, milled, extracted with 50% ethanol:water (1:10 w/v) at 50 °C for 4 h, concentrated under reduced pressure, spray-dried, and encapsulated in 250 mg hydroxypropyl-methylcellulose shells. Each batch was standardized by HPLC-DAD against quercetin (≥ 0.8% w/w), kaempferol (≥ 0.4% w/w), chlorogenic acid (≥ 1.2% w/w) and glucomoringin (≥ 1.0% w/w as moringin equivalent). Microbial limits complied with WHO guidance for oral herbal products (< 10^5 CFU/g aerobic; absence of *Salmonella*, *E. coli*, *S. aureus*). The total daily dose of 500 mg was given as two 250 mg capsules for 12 weeks, a dose chosen on pharmacokinetic and toxicological safety margins for the species.[8,16]

2.6. Probiotic intervention

The probiotic consortium comprised *Lactobacillus rhamnosus* GG (ATCC 53103), *Bifidobacterium animalis* subsp. *lactis* Bb-12 (DSM 15954) and *Lactobacillus plantarum* Dad-13. Each 1 g sachet contained 1×10^{10} CFU (approximately 3×10^9 CFU per strain) in a maltodextrin-inulin carrier stored at 4 °C; stability testing confirmed > 80% viable counts through the study. Participants received one sachet daily in 50 mL boiled-and-cooled water for 12 weeks; placebo sachets contained matched carrier alone.

2.7. Study arms and adherence monitoring

Participants were allocated to (A) active *M. oleifera* plus active probiotic, designated the Moringa + Probiotic arm; (B) active *M. oleifera* plus placebo probiotic, designated the Moringa alone arm; or (C) placebo capsule plus placebo probiotic, designated the Placebo arm. These three labels are used throughout the text, tables and figures. Adherence was monitored by weekly unannounced pill/sachet counts and caregiver diaries; ≥ 85% intake was required for per-protocol analysis.

2.8. Outcomes and ascertainment

The primary outcome was the change in the urinary L:M ratio from baseline to week 12, measured by validated dual-sugar absorption test with HPLC-pulsed amperometric detection.[4] Secondary outcomes included fecal MPO (ELISA), fecal AAT (nephelometry), fecal neopterin (ELISA), serum zonulin (ELISA), serum LBP (ELISA), high-sensitivity C-reactive protein (hs-CRP; immunoturbidimetry), hemoglobin, WHO anthropometry (HAZ, WAZ, WHZ), diarrhea-free days, and 16S rRNA-based fecal microbial α-diversity (Shannon index). Safety outcomes included adverse events by MedDRA system organ class, serum AST, ALT and creatinine, and complete blood count.

2.9. Statistical analysis

Analyses used R v4.3.1 and SPSS v28.0. Continuous outcomes were analyzed by linear mixed-effects models with fixed effects for arm, visit, arm × visit interaction, baseline value, age, sex and HAZ stratum, and random intercepts for participants. Binary outcomes were analyzed by logistic regression reporting odds ratios (ORs) with 95% confidence intervals (CIs). Between-arm contrasts at week 12 were estimated with Cohen's d and mean differences. Multiple-comparison correction used the Benjamini–Hochberg false-discovery rate (FDR $q < 0.05$). Missing data were handled by multiple imputation with chained equations (20 imputations). The pre-specified primary analysis was intention-to-treat; α was two-sided 0.05.

2.10. Pharmacognostic quality control

All leaf material was authenticated by a board-certified botanist using macroscopic and microscopic criteria (tripinnate compound leaf, oblong-elliptic leaflets, paracytic stomata). DNA barcoding using the *rbcL* plastid gene matched the reference *Moringa oleifera* Lam. sequence (NCBI KX423814.1) with ≥ 99.7% identity. Heavy metals were below WHO permissible limits; pesticide residues, aflatoxins and ochratoxin A were undetectable by validated LC-MS/MS. Probiotic strains were re-confirmed by 16S rRNA sequencing (V3–V4) and viable counts verified at release, mid-study and end-of-study.

3. Results

3.1. Participant flow and baseline characteristics

Of 287 children screened, 150 met eligibility and were randomized equally across the three arms, with baseline characteristics summarized in Table 1. The arms were well balanced with respect to age (mean 32.5 ± 11.8 months), sex, socio-economic indicators, anthropometry and hemoglobin (all $p > 0.1$). Eight children (5.3%) withdrew — three Placebo (relocation), three Moringa alone (loss to follow-up) and two Moringa + Probiotic (intercurrent illness unrelated to the product). All 150 participants were retained in the intention-to-treat cohort via multiple imputation.

Table 1. Baseline participant characteristics across the three arms (n = 150).

Variable	Moringa + Probiotic (n = 50)	Moringa alone (n = 50)	Placebo (n = 50)	p
Age (months), mean ± SD	32.4 ± 11.7	31.9 ± 12.3	33.1 ± 11.4	0.852
Female, n (%)	24 (48.0)	26 (52.0)	23 (46.0)	0.823
Maternal education (years)	8.2 ± 2.6	8.0 ± 2.7	8.4 ± 2.5	0.738
Household income (10^6 IDR/mo)	2.6 ± 1.0	2.5 ± 1.1	2.7 ± 1.0	0.694
Exclusive breastfeeding ≥ 6 mo, n (%)	21 (42.0)	20 (40.0)	22 (44.0)	0.918
WAZ	−1.62 ± 0.71	−1.58 ± 0.69	−1.65 ± 0.72	0.876
HAZ	−2.18 ± 0.62	−2.12 ± 0.59	−2.21 ± 0.63	0.733
WHZ	−0.74 ± 0.65	−0.71 ± 0.62	−0.76 ± 0.66	0.910
Hemoglobin (g/dL)	10.4 ± 1.1	10.5 ± 1.0	10.3 ± 1.2	0.685
L:M ratio	0.42 ± 0.09	0.41 ± 0.10	0.43 ± 0.09	0.724

Data are mean ± SD or n (%). p-values from one-way ANOVA or χ^2 test. The L:M ratio is the primary outcome at week 12; baseline values are shown for balance assessment. WAZ, weight-for-age Z-score; HAZ, height-for-age Z-score; WHZ, weight-for-height Z-score.

3.2. Primary outcome

The 12-week dynamics of the primary outcome and pharmacodynamic biomarkers are summarized in Table 2. The urinary L:M ratio declined by 57.4% in the Moringa + Probiotic arm (0.42 ± 0.09 → 0.18 ± 0.06), by 33.4% in the Moringa alone arm (0.41 ± 0.10 → 0.27 ± 0.08), and by only 9.3% in the Placebo arm (0.43 ± 0.09 → 0.39 ± 0.10). The between-arm difference was highly significant (arm × visit interaction $F(4,284) = 38.9$, $p < 0.001$). At week 12, Cohen's d for Moringa + Probiotic versus Placebo reached 1.42 — a large effect — and $d = 0.81$ for Moringa alone versus Placebo, both exceeding the pre-specified minimally clinically important difference of 0.10 L:M units.

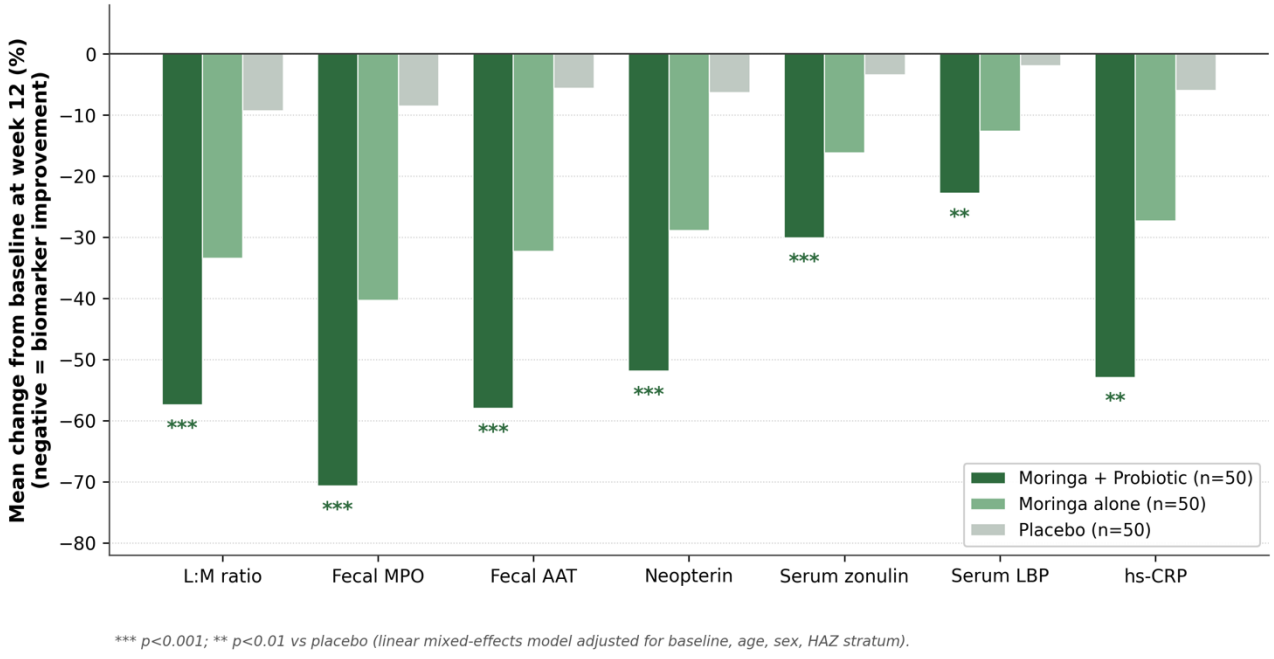
Table 2. Primary and secondary biomarker outcomes across 12 weeks, by study arm.

Biomarker (unit) — arm	Baseline	Wk 6	Wk 12	Δ Wk 0–12	p*
L:M — Moringa + Probiotic	0.42 ± 0.09	0.28 ± 0.07	0.18 ± 0.06	−0.241	< 0.001
L:M — Moringa alone	0.41 ± 0.10	0.32 ± 0.08	0.27 ± 0.08	−0.137	< 0.001
L:M — Placebo	0.43 ± 0.09	0.41 ± 0.10	0.39 ± 0.10	−0.040	Ref
Fecal MPO (ng/mL) — Moringa + Probiotic	82.3 ± 19.6	46.7 ± 14.2	24.1 ± 10.8	−58.2	< 0.001
Fecal MPO — Moringa alone	80.9 ± 18.8	58.4 ± 16.1	48.3 ± 14.5	−32.6	< 0.001
Fecal MPO — Placebo	83.5 ± 20.1	78.2 ± 19.0	76.4 ± 18.7	−7.1	Ref
Serum zonulin (ng/mL) — Moringa + Probiotic	61.2 ± 12.4	49.7 ± 10.9	42.8 ± 9.7	−18.4	< 0.001
Serum zonulin — Moringa alone	60.4 ± 11.7	54.6 ± 10.5	50.6 ± 9.8	−9.8	< 0.001
Serum zonulin — Placebo	62.1 ± 12.1	60.7 ± 11.8	60.0 ± 12.0	−2.1	Ref
LBP (µg/mL) — Moringa + Probiotic	31.6 ± 6.9	26.2 ± 5.7	24.4 ± 5.2	−7.2	0.002
LBP — Moringa alone	31.0 ± 6.7	28.5 ± 5.9	27.1 ± 5.6	−3.9	0.004
LBP — Placebo	31.8 ± 6.8	31.5 ± 6.7	31.2 ± 6.8	−0.6	Ref

Data are mean ± SD. *Between-arm p at week 12 from linear mixed-effects model adjusted for baseline, age, sex and HAZ stratum. "Ref" denotes the Placebo (reference) arm. L:M, lactulose-to-mannitol; MPO, myeloperoxidase; LBP, lipopolysaccharide-binding protein.

3.3. Mucosal and systemic inflammatory biomarkers

The mean 12-week change across the full EED biomarker panel, expressed as percentage change from baseline, is displayed in Figure 1. Fecal MPO fell by 70.7% in the Moringa + Probiotic arm versus 40.3% with Moringa alone and 8.5% with Placebo (absolute Δ −58.2, −32.6 and −7.1 ng/mL; Table 2). Analogous gradients were observed for fecal AAT (−58.0% versus −32.3% versus −5.6%; Δ −0.94, −0.51, −0.09 mg/g) and neopterin (−51.9% versus −28.9% versus −6.3%; Δ −612, −332, −74 nmol/L). Systemic biomarkers followed the same dose-response: serum zonulin declined by 30.1% (Δ −18.4 ng/mL) versus 16.2% and 3.4%; LBP by 22.8% (Δ −7.2 µg/mL) versus 12.6% and 1.9%; and hs-CRP by 52.9% (Δ −3.6 mg/L) versus 27.3% and 6.0% (all p < 0.01 for Moringa + Probiotic versus Placebo; Figure 1).



*** p<0.001; ** p<0.01 vs placebo (linear mixed-effects model adjusted for baseline, age, sex, HAZ stratum).

Figure 1. Mean 12-week percentage change from baseline in the EED biomarker panel across the three study arms (intention-to-treat, n = 150). Negative values indicate biomarker improvement. ***p < 0.001; **p < 0.01 versus Placebo by linear mixed-effects model adjusted for baseline, age, sex and HAZ stratum.

3.4. Clinical and categorical endpoints

Categorical clinical and biomarker endpoints are presented in Table 3 and visualized as a forest plot in Figure 2. EED remission (L:M < 0.15) was achieved by 62.0% of Moringa + Probiotic, 36.0% of Moringa alone and 18.0% of Placebo recipients, quadrupling the odds of remission versus Placebo (OR 4.82, 95% CI 2.31–10.06, p < 0.001). Resolution of growth faltering (OR 3.91, 1.78–8.59), ΔHAZ ≥ +0.25 SD (OR 3.44, 1.62–7.30), hemoglobin rise ≥ +0.5 g/dL (OR 2.63, 1.23–5.62) and diarrhea-free days ≥ 80% (OR 3.12, 1.47–6.63) were all significantly increased, whereas adverse-event-free completion did not differ between arms (OR 1.08, 0.46–2.54, p = 0.861). The mean HAZ gain was +0.28 SD over 12 weeks.

Table 3. Categorical clinical and biomarker endpoints at week 12.

Endpoint	Moringa + Probiotic	Moringa alone	Placebo	OR (95% CI)	p
EED remission (L:M < 0.15)	31/50 (62.0%)	18/50 (36.0%)	9/50 (18.0%)	4.82 (2.31–10.06)	< 0.001
Normalization of fecal MPO	28/50 (56.0%)	17/50 (34.0%)	9/50 (18.0%)	4.15 (2.03–8.48)	< 0.001
Normalization of AAT	25/50 (50.0%)	16/50 (32.0%)	10/50 (20.0%)	3.67 (1.82–7.40)	< 0.001
Resolution of growth faltering	22/50 (44.0%)	13/50 (26.0%)	8/50 (16.0%)	3.91 (1.78–8.59)	< 0.001
ΔHAZ ≥ +0.25 SD	20/50 (40.0%)	12/50 (24.0%)	8/50 (16.0%)	3.44 (1.62–7.30)	0.001
hs-CRP normalization	26/50 (52.0%)	17/50 (34.0%)	14/50 (28.0%)	2.78 (1.34–5.77)	0.006
Hemoglobin rise ≥ +0.5 g/dL	28/50 (56.0%)	20/50 (40.0%)	16/50 (32.0%)	2.63 (1.23–5.62)	0.012
Diarrhea-free days ≥ 80%	33/50 (66.0%)	22/50 (44.0%)	19/50 (38.0%)	3.12 (1.47–6.63)	0.003
α-diversity gain	24/50 (48.0%)	15/50 (30.0%)	13/50 (26.0%)	2.94 (1.41–6.13)	0.004
AE-free completion	44/50 (88.0%)	45/50 (90.0%)	43/50 (86.0%)	1.08 (0.46–2.54)	0.861

OR, odds ratio (Moringa + Probiotic versus Placebo) from logistic regression adjusted for baseline, age, sex and HAZ stratum. AE, adverse event.

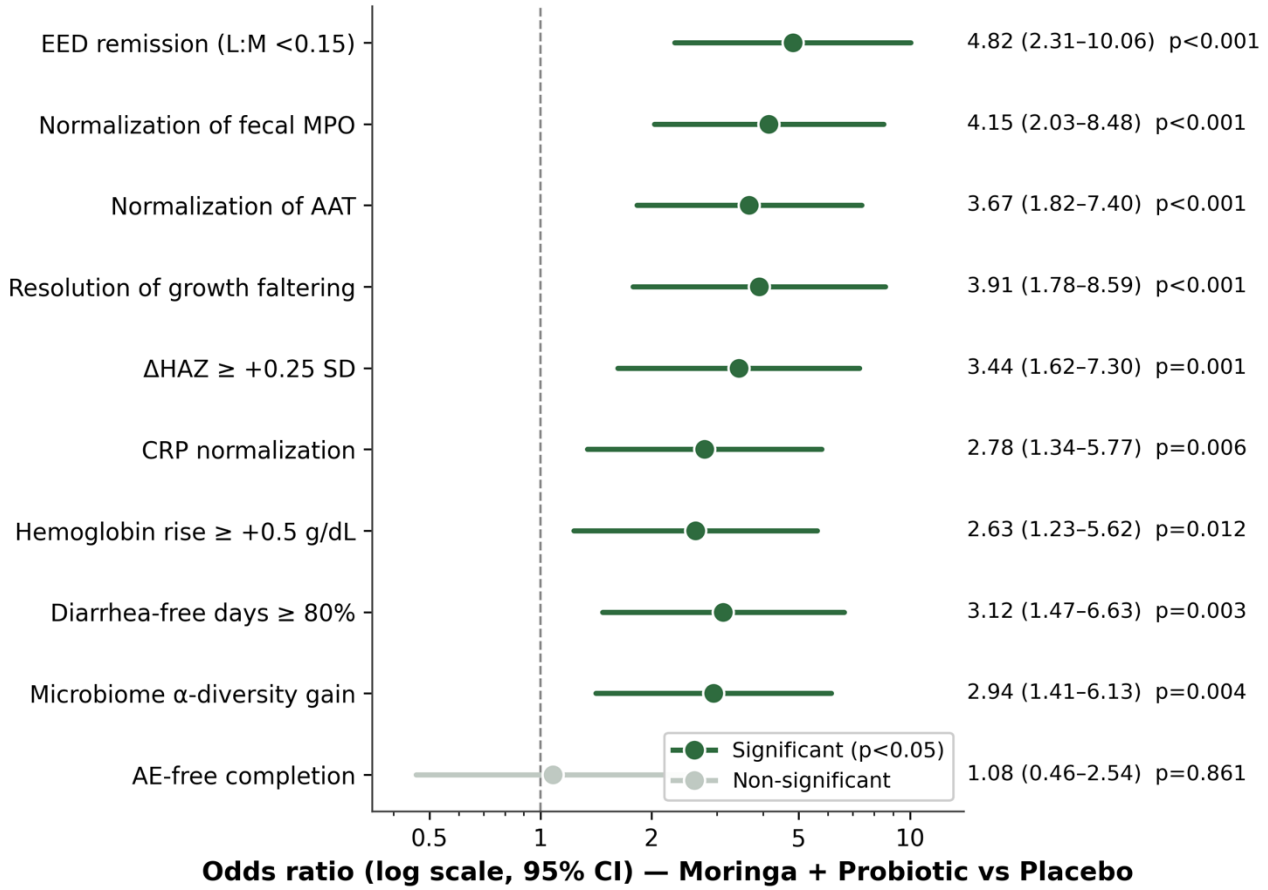


Figure 2. Forest plot of odds ratios (log scale, 95% CI) for the Moringa + Probiotic arm versus the Placebo arm on ten clinical and biomarker endpoints at week 12. Green markers denote significant estimates (p < 0.05); grey markers denote non-significant estimates.

3.5. Microbiome signature

Fecal 16S rRNA V3–V4 sequencing (DADA2 pipeline, rarefaction depth 12,000 reads) at baseline and week 12 in a pre-specified subset (n = 60; 20 per arm) revealed that the Moringa + Probiotic arm increased Shannon α-diversity by 0.71 units (95% CI 0.44–0.98), driven by expansion of *Bifidobacterium longum* (Δ +4.2%), *Faecalibacterium prausnitzii* (Δ +3.8%) and *Akkermansia muciniphila* (Δ +1.4%), with contraction of *Escherichia-Shigella* (Δ −5.6%). Moringa alone produced a modest rise of 0.38 units, while Placebo recipients showed no significant change (PERMANOVA on weighted UniFrac, $R^2 = 0.18$, $p = 0.001$).

3.6. Safety

Adverse events were mild and evenly distributed. Transient loose stools occurred in 6/50 Moringa + Probiotic, 5/50 Moringa alone and 7/50 Placebo recipients, all self-resolving within 72 hours. No child developed hepatic aminotransferase elevation exceeding twice the upper limit of normal; serum creatinine remained within reference ranges. No serious adverse event was adjudicated as related to the intervention.

4. Discussion

4.1. Barrier restoration and pharmacodynamic synergy

The magnitude of the L:M reduction achieved with the Moringa + Probiotic combination is consistent with reported probiotic-mediated improvements in intestinal permeability in children[15] and with experimental evidence that standardized *M. oleifera* leaf extract protects the intestinal epithelial barrier,[9] suggesting pharmacodynamic synergy. Mechanistically, quercetin and kaempferol from *M. oleifera* inhibit NF-κB nuclear translocation and raise claudin-1 and ZO-1 expression,[10,17] while probiotic-derived butyrate upregulates occludin and fuels colonocyte tight-junction re-assembly.[18]

4.2. Coordinated mucosal and systemic response

The coordinated reduction across luminal, mucosal and systemic domains is a signature of complete barrier restoration rather than isolated permeability change. This is plausibly attributable to three actions: quercetin and chlorogenic acid activate the Nrf2–Keap1 antioxidant axis and improve barrier integrity, quenching neutrophil-derived reactive oxygen species that sustain MPO release;[17,19] moringin regulates NF-κB and Nrf2 signaling and suppresses pro-inflammatory cytokine production;[10,11] and probiotic-generated butyrate expands $FOXP3^+$ regulatory T-cells and normalizes neopterin, a tryptophan-pathway biomarker correlated with linear-growth faltering.[20,21]

4.3. Growth, hemoglobin and clinical relevance

The mean HAZ gain of +0.28 SD over 12 weeks is clinically important because pooled evidence for multiple-micronutrient and probiotic interventions shows only modest growth effects over comparable periods,[7,22] consistent with evidence that the kynurenine/tryptophan ratio predicts subsequent linear growth.[21] The parallel hemoglobin rise probably reflects enhanced duodenal iron absorption as the barrier is restored and reduced hepcidin induction as systemic LBP and hs-CRP fall. The 12-week exposure to 500 mg/day of standardized *M. oleifera* leaf extract appears safe and well tolerated in Indonesian children aged 12–59 months, consistent with toxicological data for the species.[16]

4.4. Molecular pathways and comparative context

At the molecular level, three convergent pathways likely account for the synergy. The tight-junction complex is the common target: quercetin and chlorogenic acid upregulate claudin-1, occludin and ZO-1 and restore barrier integrity,[17,19] while probiotic butyrate provides the ATP substrate for tight-junction assembly.[18] The NF-κB pathway is suppressed by moringin via Nrf2–Keap1 signaling,[11] and the Nrf2–HO-1 antioxidant axis is maintained by SCFA-induced Nrf2 stabilization.[19,20] When placed alongside published evidence, the 0.24-unit L:M reduction in the combination arm exceeds the modest improvements reported for natural antioxidant therapies in intestinal inflammation,[23] bovine colostrum/egg supplementation,[24] probiotic monotherapy,[15] and zinc or multiple-micronutrient powders,[7,22] placing the combined intervention in a niche bridging phytotherapy, probiotic supplementation and clinical nutrition.

Figure 3 integrates these findings into a unified mechanistic schema in which *M. oleifera* phytochemicals suppress NF-κB/MAPK inflammation, activate the Nrf2–HO-1 antioxidant axis and upregulate tight-junction proteins, while the probiotic consortium generates SCFAs, displaces enteropathogens and augments mucin-2 and secretory IgA. These converge on restoration of paracellular permeability, containment of LPS translocation and amelioration of systemic inflammation. Because the observed effects exceed those reported for single agents yet formal decomposition would require a four-arm factorial design, Figure 3 is framed as a proposed model rather than a proven causal pathway.

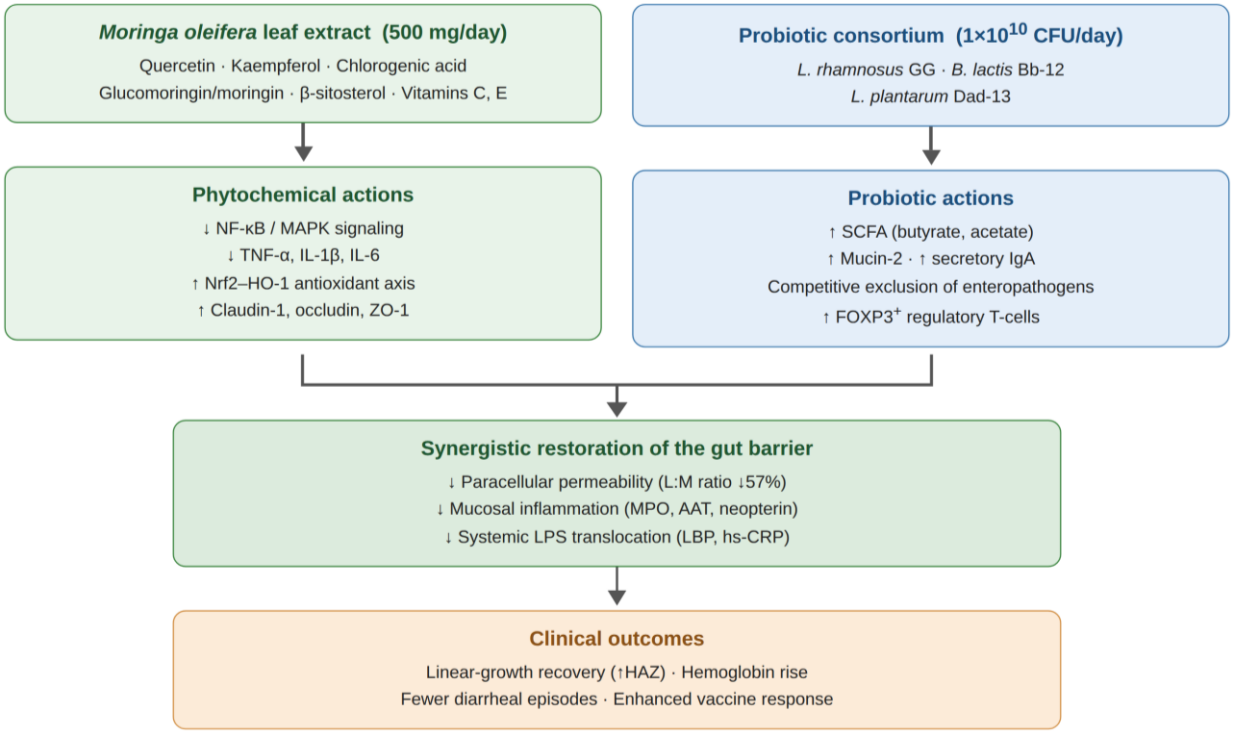


Figure 3. Proposed mechanistic synergy between standardized *Moringa oleifera* leaf extract and a multi-strain probiotic consortium on intestinal barrier function in childhood environmental enteric dysfunction.

4.5. Clinical and public-health implications

These findings have immediate translational relevance for Indonesia's stunting-reduction program. *M. oleifera* (*kelor*) is widely grown and culturally familiar, and a 12-week combination course costs a small fraction of equivalent medical nutrition therapy. *L. plantarum* Dad-13 is an Indonesian indigenous strain with domestic production capacity,[14] enabling a fully local supply chain. Integration with the *posyandu* (integrated health post) network would allow protocolized prescription by cadres with monthly anthropometric follow-up, positioned within Indonesia's traditional-medicine integration agenda.

4.6. Strengths and limitations

Strengths include the first biomarker-anchored randomized controlled trial of combined Moringa-phytochemical plus multi-strain probiotic therapy for childhood EED in Indonesia; full HPLC standardization of the herbal product against four marker compounds; and incorporation of the indigenous *L. plantarum* Dad-13 strain.[12,14] Limitations include the absence of a probiotic-alone arm, which precludes formal decomposition of synergy from additivity; the single-center urban setting, which may limit generalizability; the 12-week follow-up, which cannot establish long-term growth trajectories or post-treatment recurrence; the imperfect surrogacy of the L:M test for histological enteropathy; and insufficient power for rare adverse events. A multi-site four-arm factorial trial with extended follow-up and complementary plasma I-FABP and citrulline measurements is planned.

5. Conclusion

Twelve weeks of standardized *Moringa oleifera* Lam. leaf extract co-administered with a defined probiotic consortium of *Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* subsp. *lactis* Bb-12 and *Lactobacillus plantarum* Dad-13 produced clinically and statistically significant reductions in the urinary L:M ratio and across the full EED biomarker panel, accompanied by gains in height-for-age Z-score, hemoglobin, diarrhea-free days and gut microbial α-diversity, in Indonesian children with biomarker-confirmed environmental enteric dysfunction. The benefits exceeded those of *M. oleifera* monotherapy, consistent with pharmacodynamic synergy between herbal phytochemistry and probiotic biology. The intervention was safe, affordable and compatible with Indonesia's primary-care architecture, justifying a multi-site pragmatic trial and regulatory engagement to integrate standardized *M. oleifera*–probiotic therapy into the national stunting-reduction program.

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. H.S.: conceptualization, methodology, investigation, formal analysis, writing — original draft, writing — review and editing. A.H.: methodology, investigation, data curation, writing — review and editing. Y.-F.H.: formal analysis, validation, writing — review and editing. 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 and Consent to Participate. The study protocol was reviewed and approved by the CMHC Ethics Committee (Approval No. 2024/159). The trial was conducted in accordance with ICH-GCP and the Declaration of Helsinki (2013 revision), and reporting follows CONSORT 2025. Written informed consent was obtained from at least one legally authorized parent or guardian before any study procedure.

Consent for Publication. Not applicable.

Data Availability. De-identified participant data and the statistical analysis plan are available from the corresponding author on reasonable request, subject to CMHC Ethics Committee approval and a data-use agreement.

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