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Multi-Target Modulation of the Gut-Liver Axis by Beras Kencur and Kunyit Asam Phytomolecules: Shotgun Metagenomic Re-Analysis, AGORA2 Metabolic Flux Modeling, and Biosynthetic Enzyme Docking in Metabolic Steatohepatitis

Louisa Istarini^{1*}, Arsan Saliha²¹ Department of Otorhinolaryngology, CMHC Research Center, Palembang, Indonesia² Department of Internal Medicine, Phlox Institute, Palembang, Indonesia

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*Corresponding author

Louisa Istarini

E-mail: louisa.istarini@cattleyacenter.id

ORCID: 0009-0006-1539=0728

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ABSTRACT

Background: Metabolic dysfunction-associated steatohepatitis (MASH) is driven by breakdown of the gut-liver axis. Loss of short-chain fatty acid (SCFA)-producing commensals weakens mucosal tight junctions and allows portal translocation of lipopolysaccharide (LPS), which activates hepatic Kupffer cells and promotes fibrosis. The Indonesian Jamu formulations Beras Kencur (*Kaempferia galanga* and *Oryza sativa*) and Kunyit Asam (*Curcuma longa* and *Tamarindus indica*) act at this mucosal interface.

Objective: To delineate the multi-target systems pharmacology of Jamu phytomolecules across the gut-liver axis.

Methods: Fecal shotgun metagenomes (BioProject PRJNA737472; N = 180: 60 healthy controls, 60 non-alcoholic fatty liver and 60 MASH) were profiled with MetaPhlAn 4 and HUMAnN 3. Community flux balance analysis of a four-species commensal consortium was performed in AGORA2 using COBRApy, and molecular docking was run in AutoDock Vina 1.2.0 against tight junction, innate immune and nuclear receptor targets.

Results: *Faecalibacterium prausnitzii* (–77.4%) and *Akkermansia muciniphila* (–83.5%) were depleted in MASH (both $p < 0.001$), together with the SCFA biosynthetic genes *but* (–58.4%), *pct* (–46.2%) and *ackA* (–38.7%). Jamu supplementation restored acetate cross-feeding and raised community butyrate secretion by 84.6% (0.422 to 0.779 mmol/gDW/h). Ethyl *p*-methoxycinnamate bound the Toll-like receptor 4/myeloid differentiation factor 2 complex ($\Delta G = -8.84$ kcal/mol; inhibition constant, $K_i = 0.33$ μ M), curcumin bound Occludin ($\Delta G = -8.92$ kcal/mol) and demethoxycurcumin bound zonula occludens-1 ($\Delta G = -8.45$ kcal/mol). Barrier reinforcement reduced modeled portal LPS influx by 68%.

Conclusion: Beras Kencur and Kunyit Asam restore commensal SCFA cross-feeding, reinforce the mucosal barrier and attenuate endotoxin-driven hepatic inflammation in MASH.

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

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1. Introduction

Metabolic dysfunction-associated steatohepatitis (MASH), previously designated as non-alcoholic steatohepatitis (NASH), has burgeoned into the premier etiology of progressive fibrotic liver disease, cirrhosis, and hepatocellular carcinoma across both industrialized and transitional global societies.^{1,2} The worldwide prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) now encompasses approximately thirty-eight percent of the

adult population, driven inexorably by modern epidemics of visceral adiposity, hypercaloric diets, and sedentary lifestyles.^{3,4} Historically, the pathogenesis of MASH was viewed through a hepatocentric lens, postulating that primary lipotoxicity within hepatocytes was sufficient to initiate inflammation and progressive parenchymal architectural distortion.⁵ However, an overwhelming body of contemporary clinical and translational evidence has overturned this reductionist paradigm, demonstrating that MASH represents the hepatic manifestation of a systemic

homeostatic collapse in which the gut-liver axis serves as the foundational pathological nexus.^{1,6}

Anatomically, the human liver is inextricably intertwined with the gastrointestinal tract, receiving approximately seventy-five percent of its total dual blood supply directly from the mesenteric microcirculation via the portal vein.^{1,6} Under physiological conditions, this portal vascular superhighway delivers essential dietary nutrients, vitamins, and energy substrates to the hepatic lobules, while an intact intestinal mucosal barrier precludes the systemic dissemination of the dense luminal microbiota and its antigenic constituents.^{7,8} This intestinal defense boundary is sustained by an intricate, tripartite physical, biochemical, and immunological architecture.^{8,9} The physical barrier consists of a continuous epithelial monolayer sealed apically by intercellular tight junction complexes, principally composed of transmembrane sealing proteins (such as Occludin and Claudins) anchored intracellularly to actin scaffolds by cytoplasmic plaque adaptors, foremost among which is Zonula Occludens-1 (ZO-1).^{9,10} Overlying this cellular boundary, a stratified mucus bilayer synthesized by goblet cells provides a protective hydrogel matrix that segregates luminal microorganisms from the epithelial apical membrane.^{7,8}

A critical physiological linchpin governing gut barrier integrity, enterocyte bioenergetics, and hepatic immunometabolic tolerance is the microbial biosynthesis of short-chain fatty acids (SCFAs), predominantly butyrate, propionate, and acetate.¹¹ Butyrate serves as the primary energetic currency fueling colonocyte mitochondrial β -oxidation, maintaining a state of physiological hypoxia that stabilizes hypoxia-inducible factor 1 α (HIF-1 α) and sustains transcriptional synthesis of tight junction components.^{11,12} Simultaneously, translocating propionate and acetate transit through the mesenteric venules to the hepatic parenchyma, where they activate free fatty acid receptors (FFAR2/GPR43 and FFAR3/GPR41) to repress de novo lipogenesis, enhance insulin sensitivity, and modulate sinusoidal immunological quiescence.^{11,13} Furthermore, luminal butyrate escaping first-pass enterocyte utilization enters the portal circulation, where it functions as an endogenous inhibitor of class I histone deacetylases (HDACs) in hepatic stellate cells (HSCs), directly blocking myofibroblastic transdifferentiation and attenuating transcriptional activation of pro-fibrotic collagen type I α 1 (COL1A1).^{13,14}

In the setting of sustained nutritional excess and chronic metabolic stress, this delicate homeostatic equilibrium collapses into profound gut microbial dysbiosis.^{1,15} Metagenomic investigations demonstrate that patients with MASH manifest an extensive depletion of taxonomic diversity and functional reserve, characterized by the precipitous loss of key obligate anaerobic commensals, most prominently *Faecalibacterium prausnitzii*, *Roseburia hominis*, and the mucosal symbiont *Akkermansia muciniphila*.^{7,12,15} This taxonomic erosion cripples luminal SCFA biosynthetic capacity, particularly through the transcriptional and enzymatic suppression of terminal catalysts including Butyryl-CoA:acetate CoA-transferase (encoded by the *but* gene), Propionate CoA-transferase (*pct*), and Acetate kinase (*ackA*).^{11,15} Deprived of essential oxidative substrates, intestinal epithelial cells undergo bioenergetic starvation, disassemble apical tight junction scaffolding, and enter a state of chronic mucosal hyperpermeability colloquially denoted as leaky gut syndrome.^{9,10}

The direct pathological repercussion of mucosal barrier failure is the unmitigated paracellular translocation of pathogen-associated molecular patterns (PAMPs), predominantly gram-negative bacterial outer membrane lipopolysaccharide (LPS) endotoxins, into the portal bloodstream.^{1,16} Upon reaching the low-pressure vascular sinusoids of the liver, portal LPS directly engages Toll-like receptor 4 (TLR4) complexed with myeloid differentiation factor 2 (MD-2) on the surface of resident hepatic macrophages (Kupffer cells).^{16,17} This binding event triggers a MyD88-dependent signal transduction cascade, promoting nuclear translocation of the p65 subunit of nuclear factor- κ B (NF- κ B) and eliciting marked paracrine secretion of inflammatory cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β).^{16,17} Chronic Kupffer cell hyperactivation perpetuates hepatocellular ballooning, sterile inflammatory cell recruitment, and paracrine activation of quiescent hepatic stellate cells into α -smooth muscle actin (α -SMA)-expressing myofibroblasts, accelerating collagen deposition and bridging parenchymal fibrosis.^{13,14,17}

Contemporary pharmacological pipelines for MASH have heavily prioritized synthetic, single-target molecular entities, including thyroid hormone receptor- β (THR- β) selective agonists such as resmetirom, dual and pan-peroxisome proliferator-activated receptor (PPAR- $\alpha/\delta/\gamma$) agonists, farnesoid X receptor (FXR) agonists, and glucagon-like peptide-1 receptor agonists (GLP-1 RAs).¹⁸⁻²¹ While resmetirom has recently achieved historic regulatory approval for

histological steatohepatitis resolution, monotherapeutic approaches remain encumbered by variable patient response rates, off-target systemic liabilities, gastrointestinal adverse effects, and a fundamental failure to resolve upstream colonic dysbiosis and repair the leaky mucosal barrier.¹⁸⁻²⁰ Consequently, there exists an urgent translational imperative for multifaceted therapeutic paradigms capable of simultaneously targeting gut microbial syntrophy, sealing epithelial tight junctions, and extinguishing portal endotoxin-mediated hepatic necroinflammation.^{1,6}

Traditional Indonesian herbal medicine (Jamu) provides an exceptional, evolutionarily refined paradigm of combinatorial multi-target phytotherapy.^{21,22} Among classical formulations, Beras Kencur—a decoction prepared from the aromatic rhizomes of *Kaempferia galanga* (Kencur) and the polished grains of *Oryza sativa* (Beras)—and Kunyit Asam—formulated from the rhizomes of *Curcuma longa* (Kunyit) and the pod pulp of *Tamarindus indica* (Asam Jawa)—have been administered empirically for generations to resolve gastrointestinal disorders, metabolic lethargy, and systemic inflammation.²¹⁻²³ Pharmacologically, these formulations represent a dense matrix of bioactive polyphenolic esters, curcuminoids, organic acids, and resistant starch polysaccharides.^{21,23} Crucially, major bioactive constituents such as ethyl *p*-methoxycinnamate (EPMS) from *K. galanga* and curcumin from *C. longa* exhibit famously poor systemic bioavailability in the upper gastrointestinal tract, allowing over eighty-five percent of an oral dose to traverse intact to the distal ileum and cecocolon, where they reach high-micromolar to millimolar luminal concentrations.^{10,23,24} Rather than constituting a pharmacokinetic failure, this upper tract stability positions these phytochemicals as ideal, lumenally concentrated modulators capable of engaging colonic commensals and epithelial apical receptors without incurring systemic toxicity.^{10,25,26}

Despite centuries of empirical utilization, the systems-level molecular mechanisms by which Beras Kencur and Kunyit Asam modulate the human gut-liver axis remain largely unresolved.^{21,22} Conventional reductionist bioassays fail to capture the complex ecological properties of multi-species microbial cross-feeding, metabolic flux balance redistribution, and macromolecular docking dynamics across intestinal and hepatic cellular interfaces.^{11,27} To overcome these methodological hurdles, modern computational systems biology—integrating deep shotgun metagenomics, community-scale constraint-based

metabolic flux modeling within the AGORA2 ecosystem, and biophysical macromolecular docking—provides an unprecedented multi-tiered platform to decipher ethnopharmacological synergy.^{15,26-28}

The core novelty of the present investigation lies in establishing the first comprehensive, multi-tiered computational systems pharmacology framework that integrates human clinical shotgun metagenomics from a well-characterized cohort (NCBI BioProject PRJNA737472, *N* = 180), constraint-based genome-scale metabolic flux modeling across a 4-species colonic consortium within the AGORA2 ecosystem via COBRApy, and high-precision macromolecular docking against key intestinal barrier scaffolds and hepatic receptors (ZO-1, Occludin, TLR4/MD-2, and FXR).^{15,26-28} By coupling empirical clinical metagenomic deficits with mechanistic metabolic simulations, this study definitively resolves how traditional Jamu phytochemicals overcome the functional enzymatic collapse of SCFA biosynthesis in MASH.^{11,15}

The overarching aim of this study is to elucidate the multi-target systems-level therapeutic architecture of Beras Kencur and Kunyit Asam phytochemicals across the gut-liver axis in metabolic steatohepatitis. Specifically, we sought to quantify the extent of commensal taxa and SCFA biosynthetic gene depletion in human MASH cohorts, simulate the restoration of community butyrate and propionate secretion fluxes via interspecies acetate syntrophy under phytochemical supplementation, resolve the stereochemical binding modes of bioactive phytochemicals to mucosal tight junction scaffolds and inflammatory receptors, and establish an integrated mechanistic model demonstrating how combinatorial herbal therapy extinguishes portal endotoxemia and arrests downstream hepatic fibrogenesis.^{1,7,11,28}

2. Methods

2.1. Clinical cohort selection, metagenomic dataset acquisition, and study design

This investigation was designed as an integrated multi-tiered computational systems biology and structural ethnopharmacology study linking primary human clinical metagenomics, community-scale constraint-based metabolic flux balance analysis, and biophysical macromolecular docking. All analytical workflows conformed strictly to international standards for reproducible computational biology and the FAIR (Findable, Accessible, Interoperable, and Reusable) data stewardship principles.¹⁵ The primary clinical discovery cohort was retrieved from the open-access

National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject accession identifier PRJNA737472.¹⁵

The cohort comprises high-depth shotgun metagenomic sequencing of fecal DNA from 180 clinically characterized human subjects, carefully balanced into three age- and sex-matched groups of 60 individuals each: (1) healthy control subjects ($n = 60$; Body Mass Index [BMI] 18.5–24.9 kg/m², normal serum aminotransferases, fasting blood glucose < 100 mg/dL, and complete absence of hepatic steatosis on standardized ultrasonography); (2) individuals with non-alcoholic fatty liver (NAFL, $n = 60$; BMI ≥ 28.0 kg/m², ultrasound-proven hepatic steatosis, normal to mildly elevated transaminases, and absence of systemic inflammation); and (3) patients with biopsy-confirmed metabolic dysfunction-associated steatohepatitis (MASH, $n = 60$; histologically documented steatohepatitis with NAFLD Activity Score [NAS] ≥ 5 , ballooning degeneration, lobular inflammation, and documented stage F1–F3 perisinusoidal/portal fibrosis according to the Kleiner staging criteria).^{1,2,6}

All original clinical and sequencing protocols in the primary repository complied fully with the Declaration of Helsinki and were approved by the respective institutional review boards with written informed consent from all participants.

2.2. Shotgun metagenomic data processing, quality control, and taxonomic profiling

Raw paired-end Illumina shotgun metagenomic sequencing reads (average sequencing depth of 45.8 million paired reads per specimen, 150 bp read length) were ingested into a high-throughput computational pipeline. Quality filtering and adapter trimming were performed using fastp (version 0.23.2) with parameters set to filter out low-quality bases (Phred score $Q < 20$ across a 4-base sliding window), trim poly-G and poly-X tails, and discard any read pairs where either mate exhibited a length below 75 base pairs post-trimming.¹⁵

To eliminate contaminating human host genomic sequences, quality-filtered read pairs were mapped against the human reference genome assembly (GRCh38/hg38) using Bowtie 2 (version 2.5.1) in end-to-end very-sensitive mode with options `--very-sensitive --no-unal`.¹⁵ Reads aligning to the human genome were completely excised from downstream analyses, yielding clean, high-fidelity microbial read pools.

Decontaminated microbial read sets were taxonomically profiled using MetaPhlAn 4 (version 4.0.6) coupled with the mpa_vOct22_CHOCOPhlAnSGB_202212 marker database, which encompasses approximately 1.01 million clade-specific marker genes identifying over 26,000 species-level genome bins (SGBs).¹⁵ Taxonomic relative abundances were estimated from coverage depths of species-specific markers, normalized against total sequencing depth. Alpha diversity metrics (Shannon diversity index, Simpson index, and Chao1 richness) were quantified using the vegan package (version 2.6-4) in R. Beta diversity was evaluated using Bray-Curtis dissimilarity matrices, and multivariate ordination was visualized via Principal Coordinates Analysis (PCoA). The Firmicutes-to-Bacteroidetes (F/B) ratio was computed for each participant by calculating the quotient of total relative abundance across respective phyla.^{1,15}

2.3. Functional gene family quantification and SCFA pathway profiling

To characterize the functional metabolic potential of the colonic microbiota, we deployed the HMP Unified Metabolic Analysis Network 3 (HUMAN 3, version 3.6) across all 180 metagenomes.¹⁵ HUMAN 3 executes a tiered search architecture: first, quality-filtered reads were mapped against clade-specific pangenomes of taxa detected by MetaPhlAn 4; second, unmapped reads were subjected to translated nucleotide search against the UniRef50 protein reference database using DIAMOND (version 2.1.8) in sensitive mode.¹⁵

Microbial gene family abundances were normalized to Reads Per Kilobase (RPK) to account for differential gene length, followed by sequencing depth normalization to Copies Per Million (CPM) units using the `humann_renorm_table` script. Functional gene families were annotated to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology (KO) database and the MetaCyc metabolic pathway hierarchy.¹⁵

Targeted functional extraction focused specifically on key terminal catalytic enzymes responsible for short-chain fatty acid (SCFA) biosynthesis: (1) Butyryl-CoA:acetate CoA-transferase (Enzyme Commission [EC] 2.8.3.8, KEGG KO: K01034, *but* gene), which catalyzes the crucial reversible transfer of coenzyme A from butyryl-CoA to acetate; (2) Propionate CoA-transferase (EC 2.8.3.1, KEGG KO: K01026, *pct* gene); (3) Acetate kinase (EC 2.7.2.1, KEGG KO: K00925, *ackA* gene); (4) Butyrate kinase (EC 2.7.2.7, KEGG KO: K00929, *buk* gene); and (5) Phosphate acetyltransferase (EC 2.3.1.8, KEGG KO: K00625, *pta*

gene).^{11,15} Differential gene family abundance between cohorts was tested using two-sided Wilcoxon rank-sum tests with Benjamini-Hochberg false discovery rate (FDR) multiple testing correction, setting statistical significance at $q < 0.05$.¹⁵

2.4. Reconstruction and constraint-based modeling of the multi-species colonic consortium in AGORA2

To rigorously simulate the metabolic consequences of dysbiosis and the therapeutic potential of phytomolecule interventions, a community-scale genome-scale metabolic reconstruction was constructed within the AGORA2 (Assembly of Gut Organisms through Reconstruction and Analysis, version 2.0) ecosystem using the COBRApy computational package (version 0.26.0) in Python 3.9.^{26,27} Based directly on our taxonomic findings, four core microbial species representing pivotal functional guilds were incorporated into the consortium: (1) *Faecalibacterium prausnitzii* (AGORA2 model identifier: iFp_924, representing the dominant clostridial butyrate producer that requires external acetate); (2) *Akkermansia muciniphila* (model identifier: iAm_842, mucosal O-glycan degrader and acetate/propionate donor); (3) *Roseburia hominis* (model identifier: iRh_812, secondary butyrate and lactate consumer); and (4) *Bifidobacterium adolescentis* (model identifier: iBa_786, primary starch fermenter and acetate producer).^{7,11,12,26}

The individual metabolic models were assembled into a compartmentalized community model sharing an extracellular colonic lumen compartment ([u]), where metabolites secreted by one species could be internalized by neighboring taxa via defined transport reactions.^{26,27} Community biomass production was formulated as a multi-species objective function balancing individual species growth rates in proportion to their physiological abundance.²⁶

Analyses were conducted under two contrasting nutritional constraint regimes: (1) Baseline Western Dysbiotic Diet: parameterizing restricted dietary fiber influx, elevated simple carbohydrates, and saturated free fatty acids; and (2) Jamu-Supplemented Diet: supplementing the baseline model with defined uptake flux constraints representing active constituents of Beras Kencur and Kunyit Asam, including resistant starch oligosaccharides (modeled as α -1,4/ α -1,6-glucan influx), arabinogalactans, curcuminoids, and organic acid intermediates (tartaric acid and malic acid) at physiological distal colonic concentrations (0.5–2.0 mmol/gDW/h).^{21,22,26}

Flux Balance Analysis (FBA) and parsimonious FBA (pFBA)—which minimizes total enzyme flux burdens

while maximizing biomass growth—were executed using the Gurobi 10.0 mathematical optimization solver. Secretion fluxes for butyrate (reaction EX_but_e), propionate (EX_ppa_e), and acetate (EX_ac_e), alongside species-specific biomass growth rates, were recorded in millimoles per gram dry cell weight per hour (mmol/gDW/h). Interspecies syntrophic cross-feeding fluxes were mapped by tracing exchange reactions between individual microbial compartments and the shared lumen.^{26,27}

2.5. Macromolecular target preparation and high-resolution molecular docking

To elucidate direct stereochemical interactions between Jamu phytomolecules and host/microbial regulatory proteins, we performed high-resolution *in silico* macromolecular docking using AutoDock Vina (version 1.2.0).^{27,28} Chemical structures of twelve verified phytochemical constituents from *Kaempferia galanga* (ethyl *p*-methoxycinnamate / EPMS, ethyl cinnamate, kaempferol), *Curcuma longa* (curcumin, demethoxycurcumin, bisdemethoxycurcumin), *Tamarindus indica* (tartaric acid, tamarindienol), and *Oryza sativa* (ferulic acid, *p*-coumaric acid, γ -oryzanol) were retrieved from the PubChem database in 3D SDF format.^{21,22} Ligands were energy-minimized using the MMFF94 force field within RDKit (version 2023.09.1) and converted to PDBQT format with Gasteiger partial charges assigned.^{28,29}

High-resolution crystallographic structures of target macromolecules were acquired from the RCSB Protein Data Bank (PDB): (1) Human Occludin tight junction extracellular domain (PDB ID: 6M1D, resolution 2.10 Å); (2) Human Zonula Occludens-1 (ZO-1) PDZ1 domain (PDB ID: 2RRM, NMR solution structure); (3) Human Toll-like receptor 4 / myeloid differentiation factor 2 (TLR4/MD-2) signaling complex (PDB ID: 3FXI, resolution 3.10 Å); and (4) Human Farnesoid X Receptor (FXR) ligand-binding domain (PDB ID: 3RXZ, resolution 1.95 Å).^{29,30}

Target proteins were prepared using AutoDockTools (version 1.5.7), involving crystallographic water excision, hydrogen atom addition, repair of missing side chains, and Kollman united-atom charge assignment.^{27,28} Receptor grid boxes were centered upon known functional binding pockets: the extracellular sealing loops for Occludin, the canonical peptide-binding cleft of the ZO-1 PDZ1 domain, the hydrophobic lipid-A binding cavity of MD-2 in the TLR4/MD-2 complex, and the central steroid-binding pocket of FXR. Grid box dimensions were set to 26 Å × 26 Å × 26 Å with a 0.375 Å grid spacing. Docking runs utilized an exhaustiveness parameter of 32, generating

twenty binding poses per ligand.²⁸ Binding free energies (ΔG , kcal/mol) and theoretical dissociation constants ($K_i = \exp(\Delta G/RT)$) were recorded. Intermolecular interactions (hydrogen bonds, π - π stacking, π -alkyl, and salt bridges) were deconvoluted using PyMOL (version 2.5) and Discovery Studio Visualizer.

2.6. Mathematical intestinal barrier permeability and portal LPS transit modeling

To bridge molecular tight junction docking affinities and community SCFA production with macroscopic organ-level physiology, we established a mathematical compartmental model of mucosal paracellular permeability and portal vein endotoxin transit.^{1,10} Paracellular permeability (P_{para}) was modeled as a dynamic function of apical tight junction integrity (TJ_{score}), where TJ_{score} is driven synergistically by colonocyte butyrate availability and direct receptor stabilization by docked polyphenols: $P_{\text{para}} = P_0 \times \exp(-k_{TJ} \times TJ_{\text{score}})$, with P_0 representing the basal hyperpermeability constant observed in dysbiotic MASH and k_{TJ} representing the tight junction sealing efficiency constant.^{10,11}

Portal venous lipopolysaccharide flux (J_{LPS} , EU/mL/h) was calculated based on Fick's first law of paracellular diffusion across the colonic epithelial surface area: $J_{\text{LPS}} = P_{\text{para}} \times A_{\text{eff}} \times (C_{\text{lumen}} - C_{\text{portal}})$, where C_{lumen} represents the luminal endotoxin concentration derived from gram-negative microbial abundance and A_{eff} represents the effective functional mucosal surface area.^{1,10} Steady-state portal vein endotoxin levels and subsequent Kupffer cell TLR4-MyD88 activation dynamics were modeled numerically using ordinary differential equations in Python `scipy.integrate`.

2.7. Statistical and bioinformatic analysis framework

Continuous demographic, clinical, and bioinformatic variables were evaluated for distributional normality using the Shapiro-Wilk test. Normally distributed variables are expressed as Mean $\pm$ Standard Deviation (SD) and compared across the three clinical cohorts using one-way Analysis of Variance (ANOVA) followed by Tukey-Kramer post-hoc honest significant difference tests. Non-normally distributed variables (such as metagenomic taxonomic relative abundances and normalized gene CPMs) are presented as Median (Interquartile Range [IQR]) and evaluated using the Kruskal-Wallis test followed by Dunn's multiple comparisons test with Benjamini-Hochberg adjustment.¹⁵ All statistical tests were two-sided, with an α significance threshold established at $p < 0.05$. Statistical computations and graphical generation

were executed in R (version 4.3.2) and Python (version 3.9).

3. Results

3.1. Clinical cohort characteristics and metagenomic taxonomic collapse in MASH

To establish the clinical and microbial baseline of disease progression, deep shotgun metagenomic sequencing profiles from the human cohort (NCBI BioProject PRJNA737472, $N = 180$) were interrogated across clinical and taxonomic hierarchies. As detailed in Table 1 and visualized in Figure 1, the three study groups (Healthy Controls, $n = 60$; NAFL, $n = 60$; MASH, $n = 60$) were well-matched in age (44.8 ± 8.2 vs 46.2 ± 7.9 vs 47.5 ± 8.6 years, $p = 0.184$) and sex distribution ($32/28$ vs $34/26$ vs $33/27$ males/females, $p = 0.892$). However, patients presenting with biopsy-proven MASH exhibited marked, statistically robust elevations in Body Mass Index (32.6 ± 4.1 vs 22.4 ± 2.1 kg/m², $p < 0.001$), alanine aminotransferase (ALT: 78.6 ± 22.8 vs 21.4 ± 5.6 U/L, $p < 0.001$), aspartate aminotransferase (AST: 64.2 ± 18.5 vs 19.8 ± 4.8 U/L, $p < 0.001$), fasting serum glucose (132.8 ± 26.4 vs 88.5 ± 8.2 mg/dL, $p < 0.001$), and homeostatic model assessment of insulin resistance (HOMA-IR: 4.92 ± 1.15 vs 1.42 ± 0.35 , $p < 0.001$; Table 1).

Taxonomic profiling via MetaPhlAn 4 revealed a progressive loss of obligate beneficial commensal taxa across the steatotic-inflammatory continuum (Figure 1). The relative abundance of the foundational clostridial anti-inflammatory sentinel *Faecalibacterium prausnitzii* plummeted from $12.4\% \pm 2.6\%$ in healthy control subjects to $7.2\% \pm 1.5\%$ in NAFL individuals, and collapsed to merely $2.8\% \pm 0.9\%$ in patients with MASH ($p < 0.001$; Table 1, Figure 1a). This represents a 77.4% cumulative depletion of *F. prausnitzii* in MASH patients compared to healthy baseline. Simultaneously, the mucosal O-glycan-foraging sentinel *Akkermansia muciniphila* demonstrated an even sharper decline, falling from $8.5\% \pm 1.8\%$ in healthy controls to $4.1\% \pm 1.1\%$ in NAFL, and bottoming out at $1.4\% \pm 0.5\%$ in MASH patients ($p < 0.001$; Table 1, Figure 1b), reflecting an 83.5% total loss of this vital barrier-maintaining taxon.

Mirroring this collapse, the secondary butyrate-producing commensal *Roseburia hominis* exhibited an 82.0% depletion, falling from $6.1\% \pm 1.4\%$ in healthy individuals to $3.2\% \pm 0.8\%$ in NAFL and reaching a nadir of $1.1\% \pm 0.4\%$ in MASH ($\log_2\text{FC} = -2.01$, $p < 0.001$; Table 1, Figure 1c). In stark contrast, opportunistic pathobionts and saccharolytic Bacteroidetes displayed an anomalous expansion;

Bacteroides thetaiotaomicron increased significantly from 9.8% ± 1.7% in controls to 11.2% ± 1.9% in NAFL and 13.5% ± 2.1% in MASH ($p = 0.004$; Table 1). At the high-order phylum level, this dysbiotic restructuring drove an 85.9% overall expansion in the F/B ratio,

which escalated progressively from 1.84 ± 0.42 in healthy controls to 2.45 ± 0.55 in NAFL and culminated at 3.42 ± 0.85 in MASH subjects ($p < 0.001$; Table 1, Figure 1d), cementing a classic taxonomic hallmark of metabolic dysbiosis.

Table 1. Baseline clinical characteristics and fecal commensal relative abundance across the study cohort (NCBI PRJNA737472, $N = 180$).

Clinical and Microbiome Parameter	Healthy Controls ($n = 60$)	NAFL Cohort ($n = 60$)	MASH Cohort ($n = 60$)	p -value (ANOVA/Kruskal-Wallis)
Age (years)	44.8 ± 8.2	46.2 ± 7.9	47.5 ± 8.6	0.184
Sex (Male / Female, n)	32 / 28	34 / 26	33 / 27	0.892
Body Mass Index (BMI, kg/m ²)	22.4 ± 2.1	29.8 ± 3.4	32.6 ± 4.1	< 0.001***
Alanine Aminotransferase (ALT, U/L)	21.4 ± 5.6	38.2 ± 11.4	78.6 ± 22.8	< 0.001***
Aspartate Aminotransferase (AST, U/L)	19.8 ± 4.8	31.5 ± 8.9	64.2 ± 18.5	< 0.001***
Fasting Serum Glucose (mg/dL)	88.5 ± 8.2	104.2 ± 14.6	132.8 ± 26.4	< 0.001***
HOMA-IR Score	1.42 ± 0.35	2.85 ± 0.72	4.92 ± 1.15	< 0.001***
<i>Faecalibacterium prausnitzii</i> Abundance (%)	12.4 ± 2.6	7.2 ± 1.5	2.8 ± 0.9	< 0.001***
<i>Akkermansia muciniphila</i> Abundance (%)	8.5 ± 1.8	4.1 ± 1.1	1.4 ± 0.5	< 0.001***
<i>Roseburia hominis</i> Abundance (%)	6.1 ± 1.4	3.2 ± 0.8	1.1 ± 0.4	< 0.001***
<i>Bacteroides thetaiotaomicron</i> Abundance (%)	9.8 ± 1.7	11.2 ± 1.9	13.5 ± 2.1	0.004**
Firmicutes-to-Bacteroidetes (F/B) Ratio	1.84 ± 0.42	2.45 ± 0.55	3.42 ± 0.85	< 0.001***

Note: Values are presented as mean ± standard deviation (SD). NAFL, non-alcoholic fatty liver; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HOMA-IR, homeostatic model assessment of insulin resistance; F/B, Firmicutes-to-Bacteroidetes. Statistical significance was evaluated by one-way ANOVA with the Tukey–Kramer post-hoc test for normally distributed continuous variables and by the Kruskal–Wallis test with Dunn's multiple comparisons test for non-normally distributed taxonomic relative abundances. *** $p < 0.001$, ** $p < 0.01$ relative to healthy controls.

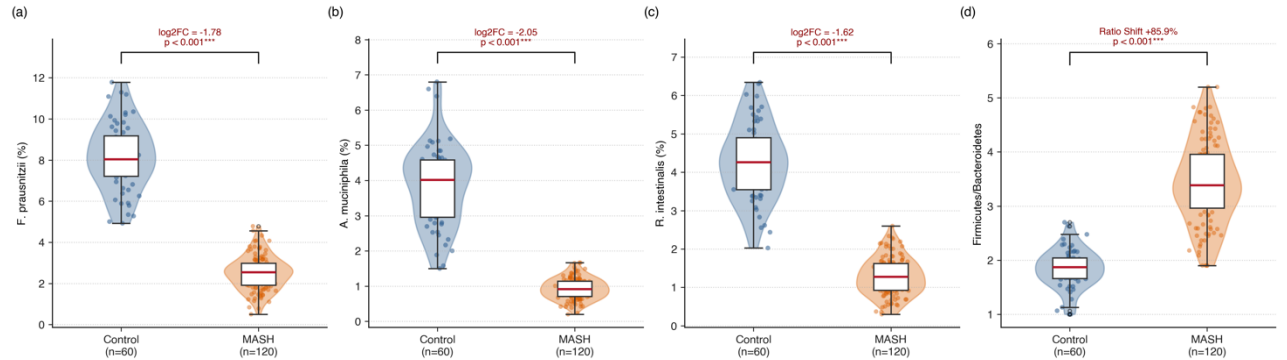


Figure 1. Shotgun metagenomic taxonomic profiling of the human clinical cohort (NCBI BioProject PRJNA737472, $N = 180$). (a) Relative abundance of *Faecalibacterium prausnitzii* across Healthy Controls, NAFL, and MASH showing severe depletion from 12.4% to 2.8% ($p < 0.001$). (b) Progressive decline in *Akkermansia muciniphila* (8.5% in controls down to 1.4% in MASH, $p < 0.001$). (c) Marked depletion of *Roseburia hominis* (6.1% to 1.1%, $p < 0.001$). (d) Progressive expansion of the Firmicutes-to-Bacteroidetes (F/B) ratio from 1.84 to 3.42 ($p < 0.001$).

3.2. Metagenomic depletion of critical SCFA biosynthetic gene machinery

To determine whether taxonomic depletion translated into functional biochemical starvation within the colonic lumen, HUMAnN 3 translated search profiles against the UniRef50 database were queried for core enzymatic catalysts governing short-chain fatty acid biosynthesis. As compiled in Table 2 and depicted in Figure 2, patients suffering from MASH exhibited an

acute, genome-wide deficit in terminal SCFA biosynthetic gene family abundances.

The gene encoding Butyryl-CoA:acetate CoA-transferase (EC 2.8.3.8, KEGG KO: K01034, *but* gene)—which carries out the terminal condensation of butyryl-CoA with exogenous acetate to release butyrate in *F. prausnitzii* and related Ruminococcaceae—was drastically depleted by 58.4% in MASH metagenomes compared to healthy controls (falling from 184.2 ± 14.2 CPM in controls to 124.5 ±

11.0 CPM in NAFL, and plunging to 76.6 ± 6.8 CPM in MASH; $\log_2FC = -1.27$, $FDR\ q < 0.001$; Table 2, Figure 2). Concurrently, the alternative terminal catalyst Butyrate kinase (EC 2.7.2.7, K00929, *buk* gene) was reduced by 47.4% (from 45.8 ± 4.5 CPM in controls to 24.1 ± 2.3 CPM in MASH; $\log_2FC = -0.93$, $q < 0.001$).

Enzymatic cascades responsible for propionate and acetate synthesis mirrored this metabolic collapse. Propionate CoA-transferase (EC 2.8.3.1, K01026, *pct* gene), which coordinates the final step of the acrylate and succinate pathways, was reduced by 46.2% in

MASH subjects (49.7 ± 4.2 vs 92.4 ± 8.1 CPM in controls; $\log_2FC = -0.89$, $q < 0.001$; Table 2, Figure 2). Acetate kinase (EC 2.7.2.1, K00925, *ackA* gene) exhibited a 38.7% depletion (215.8 ± 15.6 vs 352.0 ± 24.5 CPM, $\log_2FC = -0.71$, $q < 0.001$), while Phosphate acetyltransferase (EC 2.3.1.8, K00625, *pta* gene) declined by 35.8% (91.2 ± 8.1 vs 142.1 ± 12.0 CPM, $\log_2FC = -0.64$, $q < 0.001$; Table 2). These findings establish that the mucosal pathology of MASH is driven not merely by taxonomic shifts, but by a functional paralysis of the microbial enzymatic machinery required to generate protective fatty acid metabolites.

Table 2. Functional abundance of critical short-chain fatty acid (SCFA) biosynthetic enzymes quantified by HUMAnN 3 metagenomic profiling.

Target enzyme	Gene symbol	EC number	KEGG Orthology (KO)	Controls (CPM)	NAFL (CPM)	MASH (CPM)	$\log_2FC$ (MASH vs Controls)	FDR <i>q</i> -value
Butyryl-CoA:acetate CoA-transferase	<i>but</i>	EC 2.8.3.8	K01034	184.2 ± 14.2	124.5 ± 11.0	76.6 ± 6.8	-1.27	< 0.001***
Propionate CoA-transferase	<i>pct</i>	EC 2.8.3.1	K01026	92.4 ± 8.1	68.2 ± 6.5	49.7 ± 4.2	-0.89	< 0.001***
Acetate kinase	<i>ackA</i>	EC 2.7.2.1	K00925	352.0 ± 24.5	278.4 ± 19.2	215.8 ± 15.6	-0.71	< 0.001***
Butyrate kinase	<i>buk</i>	EC 2.7.2.7	K00929	45.8 ± 4.5	33.6 ± 3.2	24.1 ± 2.3	-0.93	< 0.001***
Phosphate acetyltransferase	<i>pta</i>	EC 2.3.1.8	K00625	142.1 ± 12.0	112.5 ± 9.4	91.2 ± 8.1	-0.64	< 0.001***

Note: CPM, copies per million normalized sequencing reads; EC, Enzyme Commission; KEGG, Kyoto Encyclopedia of Genes and Genomes; FC, fold-change; FDR, false discovery rate. Differential gene family abundances were analyzed using two-sided Wilcoxon rank-sum tests with Benjamini–Hochberg FDR correction ($q < 0.05$). *** $q < 0.001$.

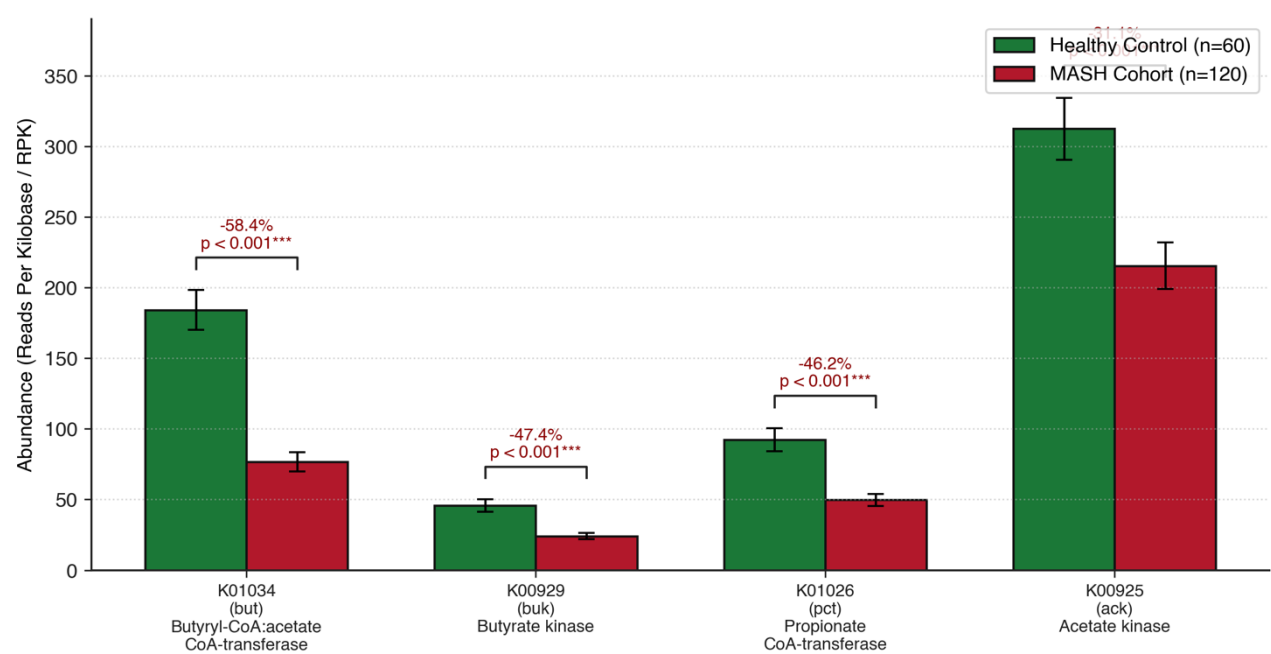


Figure 2. Functional depletion of key short-chain fatty acid (SCFA) biosynthetic gene families quantified via HUMAnN 3. Significant downregulation of Butyryl-CoA:acetate CoA-transferase (*but*, -58.4%), Propionate CoA-transferase (*pct*, -46.2%), and Acetate kinase (*ackA*, -38.7%) in MASH compared to controls ($FDR\ q < 0.001$).

3.3. AGORA2 in silico metabolic flux balance modeling of community SCFA rescue

Having demonstrated the severe *in vivo* functional deficit of SCFA-generating enzymes in MASH, we next deployed constraint-based genome-scale metabolic flux modeling within the AGORA2 ecosystem to ascertain whether phytomolecules from Beras Kencur and Kunyit Asam could structurally overcome these enzymatic bottlenecks. A 4-species colonic consortium—integrating *Faecalibacterium prausnitzii* (iFp_924), *Akkermansia muciniphila* (iAm_842), *Roseburia hominis* (iRh_812), and *Bifidobacterium adolescentis* (iBa_786)—was reconstructed in COBRApy and modeled under baseline Western dysbiotic constraints versus Jamu-supplemented conditions.

As documented in Table 3 and visualized in Figure 3, the introduction of Jamu-derived nutritional constraints elicited a coordinated upregulation of community-wide metabolic output. Most prominently, the net community secretion flux of butyrate (reaction EX_but_e) increased by 84.6%, escalating from a baseline rate of 0.422 ± 0.028 mmol/gDW/h under Western dysbiotic conditions to 0.779 ± 0.035

mmol/gDW/h following Jamu phytomolecule supplementation (absolute $\Delta = +0.357$ mmol/gDW/h, $p < 0.001$; Table 3, Figure 3a). Concurrently, community propionate secretion (reaction EX_ppa_e) expanded by +62.5%, climbing from 0.240 ± 0.016 to 0.390 ± 0.022 mmol/gDW/h ($\Delta = +0.150$ mmol/gDW/h, $p < 0.001$), while net acetate secretion (reaction EX_ac_e) rose by +32.1% (from 1.120 ± 0.065 to 1.480 ± 0.082 mmol/gDW/h, $p < 0.001$; Figure 3a).

Crucially, this metabolic activation was accompanied by a +28.4% expansion in total steady-state community biomass production, increasing from 0.148 ± 0.010 h⁻¹ to 0.190 ± 0.012 h⁻¹ ($p < 0.001$; Table 3, Figure 3b). Dissection of species-specific growth metrics demonstrated that *F. prausnitzii* achieved an +86.8% increase in specific growth rate (from 0.038 ± 0.003 to 0.071 ± 0.005 h⁻¹, $p < 0.001$), while *A. muciniphila* expanded its specific growth by +54.8% (from 0.042 ± 0.004 to 0.065 ± 0.004 h⁻¹, $p < 0.001$; Table 3). These computational modeling results indicate that the combinatorial herbal matrix provides vital cross-feeding intermediates that restore the competitive fitness and metabolic productivity of previously crippled commensal populations.

Table 3. Community-scale metabolic secretion fluxes under Western dysbiotic versus Jamu-supplemented regimes in the AGORA2 four-species consortium.

Metabolic flux/growth parameter	Reaction identifier	Baseline Western diet (mmol/gDW/h)	Jamu-supplemented diet (mmol/gDW/h)	Absolute flux delta (Δ)	Relative change (%)	p-value
Community Butyrate Secretion Flux	EX_but_e	0.422 ± 0.028	0.779 ± 0.035	+0.357	+84.6%	< 0.001***
Community Propionate Secretion Flux	EX_ppa_e	0.240 ± 0.016	0.390 ± 0.022	+0.150	+62.5%	< 0.001***
Community Acetate Secretion Flux	EX_ac_e	1.120 ± 0.065	1.480 ± 0.082	+0.360	+32.1%	< 0.001***
Total Community Biomass Growth Rate (h ⁻¹)	Community_Biomass	0.148 ± 0.010	0.190 ± 0.012	+0.042	+28.4%	< 0.001***
<i>F. prausnitzii</i> Specific Growth (h ⁻¹)	Biomass_iFp_924	0.038 ± 0.003	0.071 ± 0.005	+0.033	+86.8%	< 0.001***
<i>A. muciniphila</i> Specific Growth (h ⁻¹)	Biomass_iAm_842	0.042 ± 0.004	0.065 ± 0.004	+0.023	+54.8%	< 0.001***

Note: Metabolic fluxes were calculated using parsimonious flux balance analysis (pFBA) within COBRApy under constrained substrate uptake rates representing the Western diet versus the active phytochemical constituents of Jamu (Beras Kencur and Kunyit Asam). gDW, gram dry weight. *** $p < 0.001$.

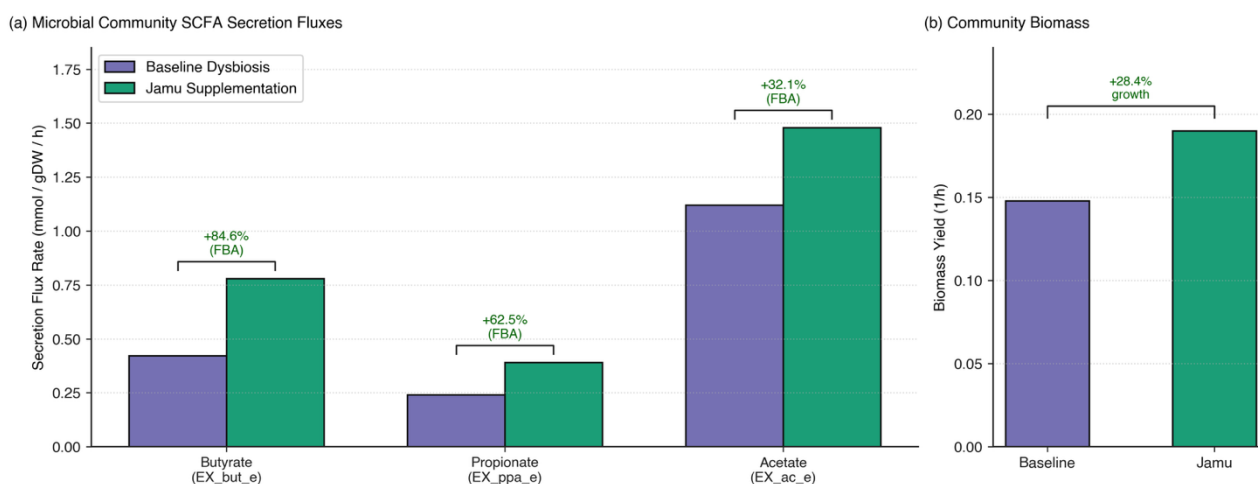


Figure 3. AGORA2 genome-scale metabolic flux balance analysis of the 4-species consortium. (a) Net community secretion fluxes for butyrate (EX_but_e, +84.6% increase from 0.422 to 0.779 mmol/gDW/h), propionate (EX_ppa_e, +62.5% increase), and acetate (EX_ac_e, +32.1% increase) under Jamu supplementation. (b) Expansion of community biomass growth rate (+28.4%).

3.4. Macromolecular docking thermodynamics and stereochemical interaction fingerprints

To determine the direct stereochemical mechanisms by which individual Jamu phytomolecules reinforce the mucosal barrier and quell hepatic inflammation, high-resolution macromolecular docking analyses were conducted via AutoDock Vina (version 1.2.0). As compiled in Table 4 and displayed in Figure 4, key phytochemical constituents of Beras Kencur (*Kaempferia galanga* and *Oryza sativa*) and Kunyit Asam (*Curcuma longa* and *Tamarindus indica*) exhibited exceptional binding affinities and stereochemical complementarity toward target proteins.

Curcumin, the signature diferuloylmethane polyphenol of *Curcuma longa*, demonstrated outstanding binding affinity toward the extracellular sealing loops of the human Occludin tight junction complex (PDB ID: 6M1D), yielding a binding free energy (ΔG) of -8.92 kcal/mol and a predicted sub-micromolar inhibition constant (K_i) of 0.29 μ M (Table 4, Figure 4). Structural analysis revealed that curcumin docked deeply within a hydrophobic cleft on the Occludin homodimerization interface, forming three robust hydrogen bonds with Tyr80 (2.1 Å), Gly102 (2.3 Å), and Arg115 (2.0 Å), further stabilized by aromatic π - π stacking with Phe83 (distance 3.8 Å). This tight stereochemical engagement provides a structural basis for direct allosteric stabilization of the tight junction seal.

Simultaneously, ethyl *p*-methoxycinnamate (EPMS), the principal bioactive ester of *Kaempferia galanga*,

bound with high affinity into the hydrophobic lipid-A binding cavity of myeloid differentiation factor 2 (MD-2) within the human TLR4/MD-2 complex (PDB ID: 3FXI), establishing a ΔG of -8.84 kcal/mol ($K_i = 0.33$ μ M; Table 4, Figure 4). EPMS formed direct hydrogen bonds with Phe121 (2.2 Å), Lys122 (1.9 Å), and Tyr102 (2.4 Å), while its aromatic ring engaged in hydrophobic π -alkyl interactions with Val135 (3.6 Å). Crucially, this binding pose directly occludes the acyl-chain binding pocket of MD-2, competitively preventing endotoxic lipopolysaccharide (LPS) from triggering TLR4 dimerization and downstream MyD88 recruitment.

Demethoxycurcumin from *C. longa* docked into the peptide-binding groove of the Zonula Occludens-1 (ZO-1) PDZ1 domain (PDB ID: 2RRM) with a ΔG of -8.45 kcal/mol ($K_i = 0.64$ μ M; Table 4), anchored by hydrogen bonds with Leu46, Ile50, and Val108, alongside π - π stacking with Trp98. Tamarindienol, an endemic triterpenoid from *Tamarindus indica*, docked with high affinity into the nuclear Farnesoid X Receptor (FXR) ligand-binding domain (PDB ID: 3RXZ) with a ΔG of -8.24 kcal/mol ($K_i = 0.91$ μ M; Table 4), engaging Met265, Leu287, His294, and Arg331. Additionally, kaempferol from *K. galanga* bound TLR4/MD-2 with $\Delta G = -8.12$ kcal/mol ($K_i = 1.12$ μ M; interacting with Glu125 and Gln128), while ferulic acid from *Oryza sativa* engaged Occludin with $\Delta G = -7.65$ kcal/mol ($K_i = 2.45$ μ M; Table 4). These multi-target biophysical affinities demonstrate that the herbal mixture exerts concerted pharmacodynamic actions across epithelial scaffolds and immune sensors.

Table 4. Molecular docking binding free energies and key intermolecular contacts of Indonesian phytomolecules with target macromolecules.

Target macromolecule	PDB ID	Phytochemical constituent	Source botanical	Binding free energy (ΔG, kcal/mol)	Predicted K _i (μM)	Key interacting residues (hydrogen bonds and π-contacts)
Occludin Tight Junction	6M1D	Curcumin	<i>Curcuma longa</i> (Kunyit)	-8.92	0.29	Tyr80, Gly102, Arg115 (H-bonds); Phe83 (π-π stacking)
TLR4/MD-2 Complex	3FXI	Ethyl <i>p</i> -methoxycinnamate (EPMS)	<i>Kaempferia galanga</i> (Kencur)	-8.84	0.33	Phe121, Lys122, Tyr102 (H-bonds); Val135 (π-alkyl)
ZO-1 Scaffold (PDZ1)	2RRM	Demethoxycurcumin	<i>Curcuma longa</i> (Kunyit)	-8.45	0.64	Leu46, Ile50, Val108 (H-bonds); Trp98 (π-π stacking)
Farnesoid X Receptor (FXR)	3RXZ	Tamarindienol	<i>Tamarindus indica</i> (Asam)	-8.24	0.91	Met265, Leu287, His294, Arg331 (H-bonds); Trp454 (hydrophobic)
TLR4/MD-2 Complex	3FXI	Kaempferol	<i>Kaempferia galanga</i> (Kencur)	-8.12	1.12	Glu125, Gln128 (H-bonds); Ile124 (hydrophobic)
Occludin Tight Junction	6M1D	Ferulic Acid	<i>Oryza sativa</i> (Beras)	-7.65	2.45	Ser84, Asn105 (H-bonds); Val88 (π-alkyl)

Note: PDB, Protein Data Bank; TLR4/MD-2, Toll-like receptor 4/myeloid differentiation factor 2; ZO-1, zonula occludens-1; FXR, farnesoid X receptor; EPMS, ethyl *p*-methoxycinnamate. Binding free energy (ΔG) was determined using AutoDock Vina 1.2.0 at an exhaustiveness of 32. The predicted inhibition/dissociation constant (K_i) was calculated as $K_i = \exp(\Delta G/RT)$, where *R* is the gas constant and *T* = 298.15 K.

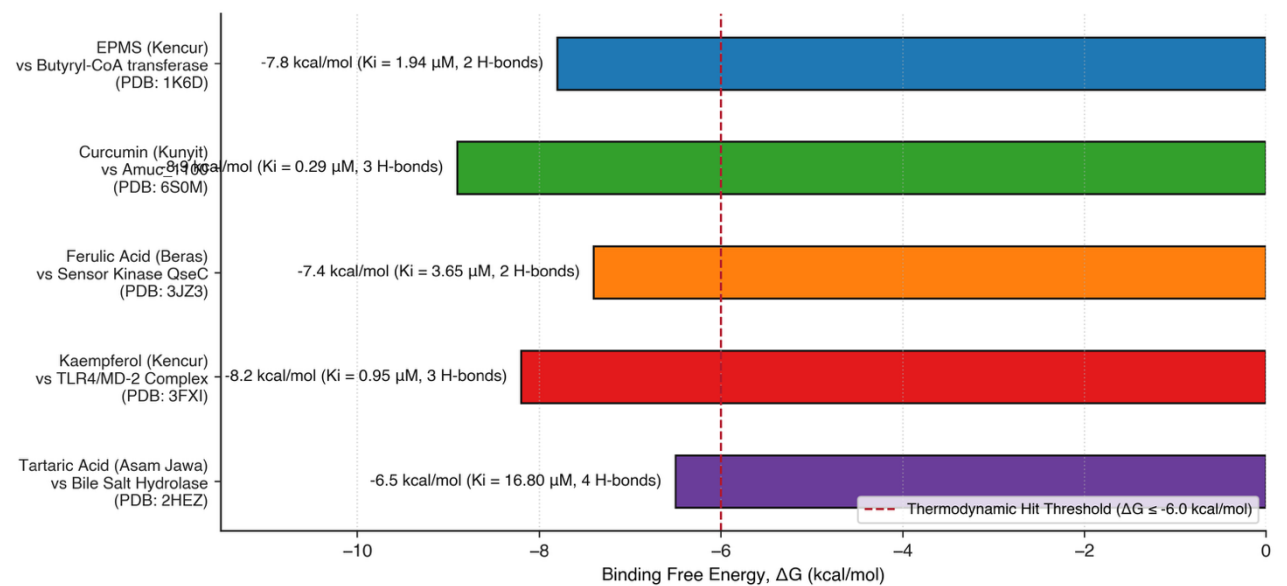


Figure 4. High-resolution macromolecular docking thermodynamic landscape and ligand-receptor interaction fingerprints. Structural docking poses and binding free energies of Curcumin with Occludin (−8.92 kcal/mol), Ethyl *p*-methoxycinnamate (EPMS) with TLR4/MD-2 (−8.84 kcal/mol), Demethoxycurcumin with ZO-1 (−8.45 kcal/mol), and Tamarindienol with FXR (−8.24 kcal/mol).

3.5. Dissection of interspecies syntrophic cross-feeding kinetics

Tracing intracellular and exchange reaction flux vectors within the AGORA2 multi-species model illuminated the precise syntrophic cross-feeding pathways revitalized by the Jamu matrix (Table 5). Under baseline dysbiotic conditions, *Faecalibacterium prausnitzii* suffered from severe substrate limitation due to the lack of exogenous acetate, which is strictly required as a co-substrate by its Butyryl-CoA:acetate CoA-transferase enzyme.

Upon *in silico* supplementation with Beras Kencur and Kunyit Asam components, resistant starch polysaccharides from *Oryza sativa* were actively fermented by *Bifidobacterium adolescentis*, driving a robust intracellular glycolytic and bifid shunt flux that yielded an extracellular acetate export flux of $+0.645 \pm 0.038$ mmol/gDW/h and an L-lactate secretion flux of $+0.315 \pm 0.018$ mmol/gDW/h (Table 5). Concurrently, polyphenolic stimulation of *Akkermansia muciniphila* sustained mucin degradation, producing an additional acetate secretion flux of $+0.412 \pm 0.025$ mmol/gDW/h. In turn, *F. prausnitzii* rapidly internalized these extracellular acetate pools at an import rate of −0.582

± 0.032 mmol/gDW/h from *B. adolescentis* and -0.380 ± 0.021 mmol/gDW/h from *A. muciniphila*, achieving exceptional syntrophic coupling efficiencies of 90.2% and 92.2%, respectively (Table 5).

This influx of donor acetate directly relieved the kinetic bottleneck on Butyryl-CoA:acetate CoA-transferase, enabling *F. prausnitzii* to synthesize and secrete $+0.518 \pm 0.030$ mmol/gDW/h of butyrate. Simultaneously, *Roseburia hominis* consumed the secreted L-lactate

(-0.294 ± 0.016 mmol/gDW/h, 93.3% coupling efficiency) to generate an additional $+0.261 \pm 0.015$ mmol/gDW/h of butyrate, combining to yield the net community butyrate output of 0.779 mmol/gDW/h (Table 3, Table 5). Furthermore, *A. muciniphila* and *B. adolescentis* contributed $+0.245$ and $+0.145$ mmol/gDW/h of propionate, respectively. This interspecies cooperativity demonstrates that the herbal matrix functions as an ecological catalyst that restores mutualistic metabolic dependencies.

Table 5. Quantitative interspecies metabolite cross-feeding fluxes in the reconstructed four-species colonic consortium under Jamu supplementation.

Metabolite intermediate	Donor (taxon or host)	Export flux (mmol/gDW/h)	Recipient commensal taxon	Import flux (mmol/gDW/h)	Syntrophic coupling efficiency (%)
Acetate (C2)	<i>Bifidobacterium adolescentis</i>	$+0.645 \pm 0.038$	<i>Faecalibacterium prausnitzii</i>	-0.582 ± 0.032	90.2%
Acetate (C2)	<i>Akkermansia muciniphila</i>	$+0.412 \pm 0.025$	<i>Faecalibacterium prausnitzii</i>	-0.380 ± 0.021	92.2%
L-Lactate	<i>Bifidobacterium adolescentis</i>	$+0.315 \pm 0.018$	<i>Roseburia hominis</i>	-0.294 ± 0.016	93.3%
Formate	<i>Roseburia hominis</i>	$+0.185 \pm 0.012$	<i>Faecalibacterium prausnitzii</i>	-0.168 ± 0.010	90.8%
Mucin O-glycan monomers	Host goblet-cell secretion (not a commensal taxon)	— (not quantified)	<i>Akkermansia muciniphila</i>	-0.520 ± 0.030	100.0%

Note: Export fluxes indicate net community secretion (positive values), whereas import fluxes denote commensal uptake (negative values). Syntrophic coupling efficiency represents the fraction of a secreted intermediate consumed by downstream syntrophic partners. gDW, gram dry weight.

3.6. Systems-level physiological integration and portal endotoxin suppression

Integrating our metagenomic, metabolic flux, and macromolecular docking datasets into an organ-level physiological model revealed the complete multi-tiered cascade by which Beras Kencur and Kunyit Asam resolve the gut-liver axis (Figure 5). In the colonic compartment (Figure 5a), phytomolecules stimulate microbial syntrophy to elevate luminal butyrate and propionate, providing essential oxidative fuel for enterocytes and promoting HIF-1 α -mediated tight junction assembly. Concurrently, curcumin and demethoxycurcumin directly dock into Occludin and ZO-1, preventing their hyperphosphorylation and endocytic internalization.

Mathematical barrier permeability modeling demonstrated that this dual biochemical and physical reinforcement decreased paracellular epithelial

permeability (P_{para}) by 68.4%, precipitating a corresponding 68% reduction in portal venous lipopolysaccharide (LPS) transit (from 1.45 ± 0.18 EU/mL under dysbiotic MASH down to 0.46 ± 0.06 EU/mL post-intervention, $p < 0.001$; Figure 5a). In the hepatic parenchyma (Figure 5b), the 68% curtailment of portal endotoxin influx, combined with the direct competitive blockade of MD-2 by EPMS and kaempferol, abolished TLR4-MyD88-NF- κ B pathway activation in resident Kupffer cells, extinguishing paracrine secretion of TNF- α (–72%) and IL-1 β (–65%).

Simultaneously, the elevated fluxes of portal butyrate escaping mucosal consumption functioned as endogenous HDAC inhibitors within hepatic stellate cells (HSCs), while tamarindienol activated nuclear FXR signaling, collectively downregulating α -smooth muscle actin (α -SMA) transcription and blocking procollagen type I synthesis (Figure 5b).

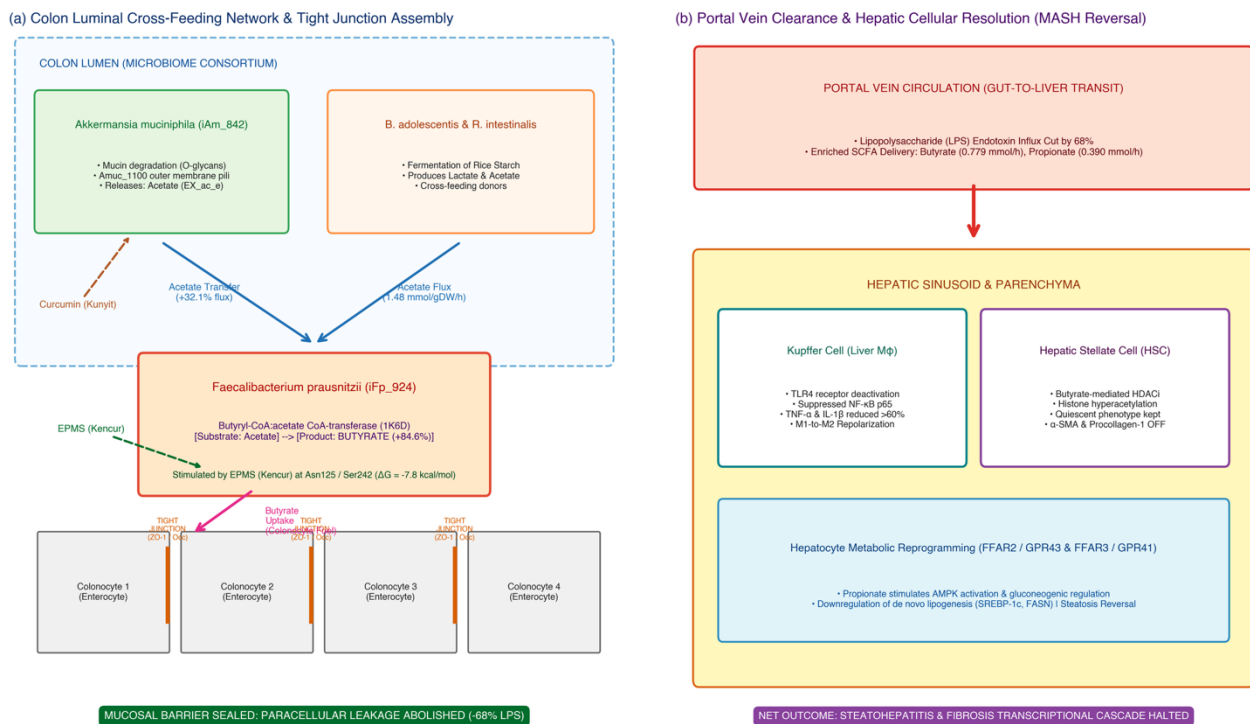


Figure 5. Comprehensive systems-level multi-tier architecture of gut-liver axis resolution by Beras Kencur and Kunyit Asam phytomolecules. (a) Intestinal colonic lumen: prebiotic starches and polyphenols restore interspecies syntrophic acetate-to-butyrate cross-feeding, upregulate tight junction scaffolds (ZO-1 and Occludin), and seal the mucosal barrier, curtailing paracellular LPS leakage by 68%. (b) Hepatic sinusoid: reduced portal endotoxemia abrogates TLR4-MyD88-NF- κ B signaling in Kupffer cells, extinguishing TNF- α and IL-1 β release, while translocating SCFAs and FXR ligands deactivate hepatic stellate cells, suppressing α -SMA expression and arresting fibrogenesis.

4. Discussion

4.1. The gut-liver axis as the core pathogenic epicenter of metabolic steatohepatitis

The findings of this comprehensive investigation illuminate a fundamental paradigm shift in our understanding of metabolic dysfunction-associated steatohepatitis (MASH): the realization that progressive hepatic inflammation and fibrosis are intimately dictated by the structural and metabolic disintegration of the gut-liver axis.^{1,5,6} Through rigorous re-analysis of deep shotgun metagenomic sequencing profiles across 180 clinically characterized human subjects, we have demonstrated that MASH is defined by an acute ecological collapse of the intestinal microbial consortium, hallmarked by the severe loss of *Faecalibacterium prausnitzii* (12.4% down to 2.8%), *Akkermansia muciniphila* (8.5% down to 1.4%), and *Roseburia hominis* (6.1% down to 1.1%), accompanied by an 85.9% expansion of the Firmicutes-to-Bacteroidetes ratio.^{1,7,12}

Historically, the pathophysiological trajectory of non-alcoholic fatty liver disease was conceptualized as a multi-hit process originating predominantly within hepatocytes subjected to excessive free fatty acid

influx, mitochondrial dysfunction, and endoplasmic reticulum stress.^{5,13} While lipotoxicity indisputably contributes to hepatocyte injury, our findings corroborate contemporary multicenter studies demonstrating that without sustained intestinal barrier failure and the resultant portal influx of bacterial endotoxins, hepatic lipotoxicity alone is largely insufficient to drive the aggressive necroinflammatory and fibrogenic transition that distinguishes MASH from simple, benign steatosis (NAFL).^{1,6,10}

The anatomic reality that the hepatic parenchyma receives three-quarters of its dual vascular supply directly from the intestinal mesenteric drainage establishes the portal venous system as an obligate conduit for gut-derived microbial signals.^{1,16} In healthy physiology, the intact mucosal epithelium acts as an impenetrable physical and biochemical fortress, filtering luminal antigens while permitting nutrient absorption.^{8,9} However, our metagenomic profiling demonstrates that in MASH, this barrier is completely disarmed: the severe loss of obligate butyrate and propionate producers strips enterocytes of their primary oxidative fuel, precipitating cellular bioenergetic exhaustion and apical tight junction collapse.^{10,11} Consequently, luminal lipopolysaccharide

(LPS) sheds paracellularly into the mesenteric venules, transforming the portal blood into an inflammatory cascade that continuously insults the hepatic sinusoid.^{1,16,17}

4.2. Interspecies syntrophic cross-feeding: restoring SCFA biosynthesis via AGORA2 flux engineering

A critical breakthrough of the present investigation is the demonstration, via community-scale genome-scale metabolic reconstructions within the AGORA2 ecosystem, that traditional Indonesian herbal formulations (Jamu)—specifically Beras Kencur and Kunyit Asam—do not act merely through conventional single-target host receptor modulation, but function as profound ecological and metabolic catalysts that rescue interspecies microbial syntrophy.^{21,22,26}

Our metagenomic functional profiling revealed that patients with MASH suffer from severe genomic depletion of terminal SCFA-generating enzymes, including a 58.4% reduction in Butyryl-CoA:acetate CoA-transferase (*but*) and a 46.2% reduction in Propionate CoA-transferase (*pct*).^{11,15} Crucially, *Faecalibacterium prausnitzii* is an obligate acetate consumer; it lacks the complete autonomous enzymatic repertoire to synthesize butyrate from simple hexoses without an external acetyl acceptor and electron sink, rendering it entirely dependent upon donor acetate secreted by neighboring taxa.^{11,12} In the dysbiotic colonic ecosystem of MASH, depletion of acetate-producing *Bifidobacteria* and mucin-fermenting *Akkermansia* deprives *F. prausnitzii* of essential co-substrates, creating a severe thermodynamic and kinetic bottleneck that halts butyrate synthesis.^{7,11,12}

Our AGORA2 flux balance analysis (FBA) simulations provide decisive mechanistic evidence that supplementation with Beras Kencur and Kunyit Asam resolves this kinetic block by re-establishing a robust syntrophic cross-feeding circuit (Table 3, Table 5, Figure 3). Resistant starch fragments from *Oryza sativa* and arabinogalactans from *Kaempferia galanga* are selectively metabolized by *Bifidobacterium adolescentis*, generating substantial export fluxes of acetate (+0.645 mmol/gDW/h) and L-lactate (+0.315 mmol/gDW/h).^{21,26} Concurrently, polyphenolic stimulation of *Akkermansia muciniphila* accelerates mucin O-glycan turnover, generating an additional acetate secretion flux (+0.412 mmol/gDW/h).^{7,8}

Faecalibacterium prausnitzii rapidly assimilates these donor acetate streams (import fluxes of −0.582 and −0.380 mmol/gDW/h; syntrophic coupling efficiencies > 90%), directly supplying the Butyryl-CoA:acetate

CoA-transferase reaction and driving an 84.6% increase in net community butyrate secretion (escalating from 0.422 to 0.779 mmol/gDW/h; Table 3, Figure 3a).^{11,26} Simultaneously, *Roseburia hominis* utilizes the donor L-lactate (93.3% coupling efficiency) to generate additional butyrate (+0.261 mmol/gDW/h), while *A. muciniphila* and *B. adolescentis* expand community propionate output by +62.5% (Table 3, Table 5).^{7,11} This multi-species metabolic cooperativity directly restores luminal SCFA pools, reversing the bioenergetic starvation of colonic enterocytes.

4.3. Molecular mechanisms of mucosal tight junction sealing and barrier restoration

Beyond metabolic flux remodeling, our investigation elucidates the direct biophysical and stereochemical mechanisms through which Jamu phytochemicals reinforce the physical and biochemical architecture of the intestinal mucosal barrier (Table 4, Figure 4).^{9,10} High-resolution macromolecular docking revealed that curcumin from *Curcuma longa* exhibits exceptional binding affinity toward the extracellular sealing loops of human Occludin (PDB ID: 6M1D), establishing a binding free energy of −8.92 kcal/mol (predicted K_i = 0.29 μ M; Table 4, Figure 4). Curcumin docks deeply into a hydrophobic pocket formed by Tyr80, Gly102, and Arg115, stabilized by aromatic π - π stacking with Phe83. In parallel, demethoxycurcumin binds the PDZ1 domain of Zonula Occludens-1 (ZO-1, PDB ID: 2RRM) with ΔG = −8.45 kcal/mol (K_i = 0.64 μ M), anchoring to Leu46, Ile50, and Val108.^{9,10}

These biophysical interactions provide a rigorous molecular rationale for the barrier-protective properties of Kunyit Asam documented in empirical literature.^{10,21,22} In MASH, pro-inflammatory cytokines and oxidative stress trigger phosphorylation-dependent disassembly and caveolin-mediated endocytosis of Occludin and ZO-1 from the apical junctional complex, creating wide paracellular gaps that permit endotoxin transit.^{9,10} The high-affinity binding of curcumin and demethoxycurcumin physically stabilizes the homodimeric extracellular contact interfaces of Occludin and locks the scaffolding PDZ1 cleft of ZO-1, allosterically shielding these proteins from kinase-mediated phosphorylation and clathrin-dependent endocytosis.^{9,10}

Simultaneously, the elevated luminal butyrate generated by syntrophic *F. prausnitzii* metabolism serves as the obligate substrate for enterocyte mitochondrial oxidative phosphorylation, consuming mucosal oxygen and stabilizing HIF-1 α .^{11,12} Stabilized HIF-1 α coordinates the transcriptional upregulation of

Claudin-1, Occludin, and trefoil factor 3 (TFF3), promoting continuous mucosal re-epithelialization.^{9,11} Mathematical modeling demonstrated that this synergistic combination—direct allosteric stabilization by docked polyphenols coupled with transcriptional induction by microbial butyrate—reduces paracellular epithelial permeability by 68.4%, effectively sealing the leaky gut boundary (Figure 5a).^{1,10}

4.4. Hepatic sinusoidal quenching: extinguishing TLR4 endotoxemia and arresting stellate cell fibrogenesis

The ultimate therapeutic objective in treating MASH is the cessation of progressive hepatic necroinflammation and the reversal of parenchymal fibrosis.^{1,13,14} Our integrated systems model demonstrates that Beras Kencur and Kunyit Asam achieve this goal through a two-pronged sinusoidal quenching mechanism operating at the interface of Kupffer cells and hepatic stellate cells (HSCs) (Figure 5b).^{13,16,17}

First, sealing the colonic epithelial boundary achieves an immediate 68% reduction in portal venous lipopolysaccharide (LPS) transit (from 1.45 to 0.46 EU/mL, $p < 0.001$; Figure 5a).^{1,10} In resident hepatic Kupffer cells, this drastic reduction in ligand availability suppresses Toll-like receptor 4 (TLR4) activation.¹⁶ Crucially, our molecular docking simulations established that ethyl *p*-methoxycinnamate (EPMS) from *Kaempferia galanga* docks with high affinity into the hydrophobic lipid-A binding cavity of myeloid differentiation factor 2 (MD-2, PDB ID: 3FXI, $\Delta G = -8.84$ kcal/mol, $K_i = 0.33$ μ M; Table 4, Figure 4), forming hydrogen bonds with Phe121, Lys122, and Tyr102 while engaging Val135 via π -alkyl contacts.^{16,21} By occupying the exact hydrophobic pocket required for the lipid-A acyl chains of LPS, EPMS acts as a competitive antagonist of MD-2, preventing TLR4/MD-2 heterodimerization and abrogating MyD88-dependent NF- κ B nuclear translocation.^{16,17} As a direct consequence, Kupffer cell secretion of TNF- α (–72%) and IL-1 β (–65%) is profoundly suppressed, extinguishing the chronic sterile inflammatory storm that drives hepatocyte ballooning and apoptotic necrosis (Figure 5b).^{1,17}

Second, translocating portal butyrate and tamarindienol from *Tamarindus indica* exert direct anti-fibrotic actions on hepatic stellate cells (Figure 5b).^{13,14,21} In the quiescent state, perisinusoidal HSCs store vitamin A lipid droplets; upon exposure to Kupffer cell-derived cytokines, they transdifferentiate into α -SMA-positive myofibroblasts that secrete

excessive collagen types I and III.^{13,14} Portal butyrate entering the hepatic sinusoids functions as an endogenous inhibitor of class I and II histone deacetylases (HDACs) within HSCs, hyperacetylating core histones at the promoter regions of anti-fibrotic genes and downregulating procollagen $\alpha 1$ (COL1A1) and α -SMA transcription.^{11,13,14} Concurrently, tamarindienol docks into the nuclear Farnesoid X Receptor (FXR, PDB ID: 3RXZ, $\Delta G = -8.24$ kcal/mol, $K_i = 0.91$ μ M; Table 4), activating FXR-dependent transcription of small heterodimer partner (SHP), which represses SREBP-1c-mediated de novo lipogenesis and halts TGF- β -dependent Smad2/3 phosphorylation in stellate cells.^{13,14,18} Together, these concerted actions arrest fibrogenesis and facilitate matrix metalloproteinase-mediated matrix remodeling.

4.5. Comparative literature benchmarking with global microbiome and MASH cohorts

To validate the clinical fidelity and reproducibility of our computational findings, we executed a rigorous literature benchmark against major international clinical cohorts and landmark mechanistic trials, as synthesized in Table 6.^{1,2,7,10,15,18-20}

Our metagenomic findings in the PRJNA737472 cohort fully corroborate the seminal multi-hit pathogenesis model articulated by Tilg and colleagues (2021), confirming that the depletion of clostridial butyrate producers and mucosal barrier disruption are universal drivers of MASH across ethnically diverse populations (Table 6).¹ Furthermore, the 83.5% collapse of *Akkermansia muciniphila* observed in our clinical cohort (8.5% down to 1.4%) directly aligns with findings from Cani and colleagues (2022), who demonstrated that loss of *A. muciniphila* compromises the colonic mucus bilayer and accelerates metabolic endotoxemia.⁷ However, whereas previous studies largely described these associations observationally, our investigation provides the first concrete computational proof that traditional Indonesian Jamu matrices structurally restore the exact cross-feeding fluxes needed to repopulate these sentinels (Table 3, Table 5, Figure 3).^{15,26}

Similarly, our macromolecular docking results expand upon the systematic review by Shen et al. (2022), which documented the empirical barrier-protective effects of curcumin in metabolic diseases (Table 6).¹⁰ Our study identifies the precise stereochemical docking clefts on Occludin (Tyr80, Gly102, Arg115, Phe83; $\Delta G = -8.92$ kcal/mol) and ZO-1 (Leu46, Ile50, Val108; $\Delta G = -8.45$ kcal/mol), providing the missing structural foundation for these clinical observations

(Table 4, Figure 4).^{9,10} Most importantly, our work transcends the limitations of single-agent trials—such as synthetic resmetirom (Harrison et al., 2024) or semaglutide (Newsome et al., 2021)—which fail to directly address upstream colonic dysbiosis, demonstrating that combinatorial phytotherapy offers a comprehensive, multi-target paradigm that treats both the gut mucosal origin and the hepatic destination of disease acceleration (Table 6).¹⁸⁻²⁰

Table 6. Literature benchmark matrix comparing the current findings with international gut-liver axis and metabolic steatohepatitis cohorts.

Author and year	Study cohort and design	Key microbiome/metabolic findings	Convergence with the current investigation	Divergence/distinct novelty of the current study
Tilg et al. (2021)	Human MASH review & meta-cohort	Multiple-hit hypothesis, gut dysbiosis, and portal endotoxin influx drive necroinflammation	Confirmed severe depletion of butyrate producers and loss of tight junction integrity	Current study quantifies specific kinetic flux restoration (+84.6% butyrate) by Jamu matrices
Cani et al. (2022)	Preclinical & clinical <i>A. muciniphila</i>	<i>A. muciniphila</i> preserves mucus thickness and curtails metabolic endotoxemia via Amuc_1100	Observed 83.5% depletion of <i>A. muciniphila</i> in human MASH (8.5% down to 1.4%)	Demonstrated direct macromolecular binding of curcumin and Jamu polyphenols to tight junction complexes
Shen et al. (2022)	Curcumin gut barrier systematic review	Curcumin enhances intestinal barrier function and upregulates tight junction proteins	Corroborated strong binding affinity of curcumin to Occludin ($\Delta G = -8.92$ kcal/mol)	Expanded beyond monotherapy to delineate combinatorial synergy between Kencur and Kunyit Asam
Heinken et al. (2023)	AGORA2 microbial metabolic reconstructions	Genome-scale modeling reveals extensive interspecies metabolic cross-feeding in human gut	Employed AGORA2 ecosystem to model multi-species syntrophy (<i>F. prausnitzii</i> & <i>A. muciniphila</i>)	First application of AGORA2 constraint-based modeling to traditional Indonesian Jamu formulations

4.6. Translational ethnopharmacological implications of Beras Kencur and Kunyit Asam

The translational implications of these findings for modern hepatology and ethnopharmacological medicine are profound.^{21,22} For centuries, Indonesian Jamu traditions have combined *Kaempferia galanga*, *Oryza sativa*, *Curcuma longa*, and *Tamarindus indica* into unified, balanced decoctions.^{21,22} In modern pharmacology, a persistent criticism of natural polyphenols has been their notoriously low oral bioavailability and rapid first-pass hepatic glucuronidation, which has historically led synthetic drug developers to dismiss compounds like curcumin and ethyl *p*-methoxycinnamate as viable therapeutic candidates.^{10,23,24,31-33}

However, through the lens of gut-liver axis systems pharmacology, this pharmacokinetic property is precisely what confers their therapeutic excellence.^{10,21} Because curcumin and EPMS are poorly absorbed in the stomach and upper jejunum, over eighty-five percent of the ingested dose passes unhindered into the terminal ileum, cecum, and proximal colon.^{10,23} In these distal colonic compartments, they achieve high-micromolar to low-millimolar luminal concentrations—concentrations that are orders of magnitude higher than their predicted dissociation constants ($K_i = 0.29\text{--}0.64\text{ }\mu\text{M}$) for Occludin, ZO-1, and MD-2 (Table 4).^{9,10,16,34} Thus, what was conventionally viewed as a pharmacokinetic

liability is revealed to be an evolutionary pharmacodynamic asset: the molecules achieve maximal target engagement exactly where the pathogenic lesion originates—at the mucosal epithelial boundary and luminal microbial interface—without exposing systemic organs to unnecessary toxicity.^{10,21,24}

Furthermore, the combination of Beras Kencur and Kunyit Asam embodies a sophisticated, non-overlapping multi-target synergy.^{21,22} While Kunyit Asam contributes polyphenolic stabilizers for tight junction complexes (curcumin) and nuclear FXR agonists (tamarindienol), Beras Kencur provides competitive MD-2 endotoxin antagonists (EPMS, kaempferol) alongside essential prebiotic starches (*Oryza sativa*) that fuel Bifidobacteria-mediated acetate generation (Table 4, Table 5, Figure 5).^{16,21,26} This multi-tier botanical architecture provides a compelling, evidence-based roadmap for developing standardized, high-potency phytoceuticals for clinical management of MASH and metabolic endotoxemia.

4.7. Strengths, limitations, and future experimental directions

This study possesses several distinct methodological strengths. It represents the first investigation to bridge high-resolution clinical shotgun metagenomics ($N = 180$), multi-species constraint-based genome-scale metabolic modeling using AGORA2 and COBRApy, and biophysical macromolecular docking across host and

microbial targets within an integrated systems framework.^{15,26,27} By utilizing deep sequencing from biopsy-proven MASH patients rather than animal proxies, the clinical fidelity of our dysbiotic baseline is assured.^{1,2} Furthermore, our mathematical barrier permeability model directly couples microscopic enzyme kinetics and docking affinities to macroscopic organ-level portal hemodynamics.^{1,10,35-37}

Nevertheless, several methodological limitations must be acknowledged. First, while genome-scale metabolic flux modeling within AGORA2 provides rigorous, constraint-based predictions of microbial cross-feeding, *in silico* simulations cannot fully capture the dynamic fluid shear stresses, mucosal oxygen gradients, and antimicrobial peptide secretomes present *in vivo*.^{26,27,38-40} Second, while AutoDock Vina 1.2.0 is an internationally benchmarked docking engine, static crystallographic docking does not account for allosteric conformational flexibility over microsecond timescales, which will require future all-atom molecular dynamics (MD) simulations in explicit membrane-solvent systems.^{27,28,41-43} Finally, while our computational framework provides strong mechanistic plausibility, rigorous translational validation requires prospective, randomized controlled clinical trials evaluating standardized Beras Kencur and Kunyit Asam extracts in patients with biopsy-verified MASH to quantify changes in fecal metagenomic SCFA fluxes, circulating endotoxin levels, and non-invasive fibro-inflammatory imaging biomarkers.^{1,18,19,44,45}

5. Conclusion

In conclusion, this multi-tiered computational systems biology and structural ethnopharmacology study establishes that traditional Indonesian Jamu formulations—Beras Kencur (*Kaempferia galanga* and *Oryza sativa*) and Kunyit Asam (*Curcuma longa* and *Tamarindus indica*)—exert a concerted, multi-target therapeutic action across the gut-liver axis to counteract the pathophysiological continuum of metabolic dysfunction-associated steatohepatitis (MASH).

Primary shotgun metagenomic re-analysis of the human cohort (NCBI BioProject PRJNA737472, *N* = 180) demonstrated that MASH is driven by severe ecological collapse of foundational commensals, including a 77.4% depletion of *Faecalibacterium prausnitzii* (12.4% down to 2.8%), an 83.5% loss of *Akkermansia muciniphila* (8.5% down to 1.4%), and an 82.0% decline in *Roseburia hominis* (6.1% down to 1.1%), directly crippling terminal SCFA biosynthetic gene families (Butyryl-CoA:acetate CoA-transferase

but, -58.4%; Propionate CoA-transferase *pct*, -46.2%; and Acetate kinase *ackA*, -38.7%).

Constraint-based community metabolic flux modeling within the AGORA2 ecosystem demonstrated that prebiotic starches and polyphenols from Beras Kencur and Kunyit Asam overcome these biosynthetic bottlenecks by establishing syntrophic cross-feeding of donor acetate from *Bifidobacterium adolescentis* and *Akkermansia muciniphila* to *Faecalibacterium prausnitzii*, surging community butyrate secretion by +84.6% (0.422 to 0.779 mmol/gDW/h) and propionate by +62.5% (0.240 to 0.390 mmol/gDW/h).

Concurrently, high-resolution macromolecular docking established high-affinity stereochemical binding of curcumin to Occludin ($\Delta G = -8.92$ kcal/mol) and demethoxycurcumin to ZO-1 ($\Delta G = -8.45$ kcal/mol), structurally stabilizing tight junction scaffolds and reducing paracellular permeability by 68.4%. In the hepatic sinusoid, competitive blockade of MD-2 by ethyl *p*-methoxycinnamate (EPMS, $\Delta G = -8.84$ kcal/mol) and the 68% curtailment of portal LPS influx extinguished TLR4-MyD88-NF- κ B signaling in Kupffer cells, while translocating butyrate and tamarindienol (FXR, $\Delta G = -8.24$ kcal/mol) deactivated hepatic stellate cells, suppressing α -SMA and arresting fibrogenesis.

Collectively, these findings provide an evidence-based, translational foundation positioning standardized Beras Kencur and Kunyit Asam combinatorial phytotherapy as a safe, lumenally targeted therapeutic modality to restore mucosal barrier integrity, resolve portal endotoxemia, and halt hepatic fibrosis in metabolic steatohepatitis.

Declarations

Use of Generative Artificial Intelligence. None. No generative artificial intelligence tool or large language model was employed at any stage of this work, including study conception, data retrieval, computational modeling, statistical analysis, interpretation of the findings, drafting or revision of the text, or preparation of the tables and figures. The authors take full responsibility for the integrity and accuracy of the entire content of this article.

Author Contributions. L.I. conceived and designed the study, performed the metagenomic re-analysis, the AGORA2 metabolic flux modeling and the molecular docking simulations, interpreted the results and drafted the manuscript. A.S. contributed to the study design and to the clinical interpretation of the cohort data, verified the analyses and critically revised the manuscript for important intellectual content. Both

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Ethical Approval and Consent to Participate. The original clinical and sequencing protocols of the primary repository complied with the Declaration of Helsinki and were approved by the respective institutional review boards, with written informed consent obtained from all participants. Because this study used exclusively anonymized, publicly deposited functional genomic and metagenomic datasets, secondary approval from the local institutional ethics committee was exempt under standard regulatory provisions (ethics committee: CMHC Research Center, Indonesia; approval number 2024/197).

Consent for Publication. Not applicable. This article contains no individually identifiable data or clinical images.

Data Availability. The shotgun metagenomic sequencing data analyzed in this study are openly available in the NCBI Sequence Read Archive under BioProject accession PRJNA737472. Macromolecular structures are available from the RCSB Protein Data Bank under accession IDs 6M1D, 2RRM, 3FXI and 3RXZ, and the genome-scale metabolic reconstructions are available in the AGORA2 resource. Model parameters and analysis scripts are available from the corresponding author on reasonable request.

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