

ORIGINAL RESEARCH ARTICLE

Multi-Target Complementary Systems Pharmacology of a Zingiberaceae Jamu Formula (*Curcuma zanthorrhiza*, *Curcuma longa* and *Zingiber officinale*) in Reversing Lipotoxicity and Fibrogenesis Transcriptomics in MASH: An Integrated Human RNA-Seq, Network Pharmacology and Molecular Docking Study

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ABSTRACT

Background: Metabolic dysfunction-associated steatohepatitis (MASH) is characterized by lipotoxicity, lobular inflammation and progressive fibrogenesis. Single-target agents show limited efficacy, underscoring the potential of traditional Indonesian Zingiberaceae Jamu combining *Curcuma zanthorrhiza*, *Curcuma longa* and *Zingiber officinale*, whose systems-level multi-target mechanisms remain undefined against human transcriptomes.

Objective: To decipher the multi-target polypharmacological complementarity of the Zingiberaceae Jamu triad against human MASH pathophysiology using clinical biopsy RNA-Seq, network pharmacology and molecular docking.

Methods: Human liver biopsy RNA-Seq transcriptomics (NCBI Gene Expression Omnibus, GEO, GSE135251; N = 216: 10 healthy controls, 51 non-alcoholic fatty liver [NAFL], 155 MASH fibrosis stages F0–F4) were integrated with bioactive metabolite profiling (Lipinski Rule of Five and Veber oral bioavailability), target prediction, protein–protein interaction (PPI) topology, KEGG enrichment and AutoDock Vina molecular docking.

Results: Biopsy analysis identified 1,842 differentially expressed genes (DEGs; 1,024 upregulated, 818 downregulated), with upregulation of fibrogenic (COL1A1, TIMP1, ACTA2, TGFB1) and inflammatory genes (CCL2, CXCL8, TNF) alongside suppression of fatty acid oxidation regulators (PPARA, CPT1A, ACOX1). Intersection of 24 bioavailable phytochemicals with MASH DEGs yielded 126 shared targets (PPI: 126 nodes, 1,142 edges, average degree 18.13). Six master hub genes were identified: PPARG (degree 84), TNF (78), AKT1 (76), TGFBR1 (68), RELA (64) and MMP9 (58). Docking predicted spontaneous binding: curcumin to PPARG ($\Delta G = -9.4$ kcal/mol) and AKT1 (–8.8); xanthorrhizol to TNF- α (–8.2); 6-shogaol to TGFBR1 (–8.6) and MMP9 (–8.4).

Conclusion: The findings are consistent with a tri-pillar polypharmacological complementarity in the Zingiberaceae Jamu formula and establish a rational mechanistic framework for standardized phytopharmaceutical development against human MASH.

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1. Introduction

Metabolic dysfunction-associated steatohepatitis (MASH), recently codified through a global multi-society consensus to replace the historic moniker of

non-alcoholic steatohepatitis (NASH), represents the active inflammatory, necrotizing and progressive fibrotic vanguard of steatotic liver disease.¹ Affecting an estimated 5% to 7% of the global adult population, MASH has emerged as a major public health problem

and is rapidly ascending as the foremost driver of cirrhosis, end-stage liver disease, hepatocellular carcinoma and indications for orthotopic liver transplantation across both developed and industrializing nations.^{2,3} Unlike simple steatosis, which is characterized by inert intrahepatic lipid accumulation, MASH entails a histopathological transformation distinguished by the convergence of hepatocellular ballooning, persistent lobular and portal inflammation, and active extracellular matrix collagen deposition across the hepatic parenchyma.^{3,4} The global escalation in obesity, insulin resistance and metabolic syndrome has accelerated the epidemiological velocity of MASH and its extrahepatic cardiometabolic sequelae, underscoring an unmet medical need for durable therapeutic interventions.^{4,5,39}

The pathophysiology of MASH is governed by a multifactorial 'multiple-hit' paradigm, in which hepatic lipid overload acts as the initial inciting insult that sparks a widespread cellular crisis.^{3,5} When the influx of circulating free fatty acids and *de novo* lipogenesis overwhelm the rate-limiting capacities of mitochondrial and peroxisomal β -oxidation, hepatocytes accumulate toxic lipid moieties, notably diacylglycerols, ceramides and lysophosphatidylcholines.⁵ Elevated concentrations of these lipotoxic intermediates provoke endoplasmic reticulum stress, generation of reactive oxygen species and persistent mitochondrial dysfunction.^{5,6} This organellar failure compromises adenosine triphosphate generation, alters mitochondrial membrane permeability and triggers pathways of programmed cell death, including apoptosis and necroptosis, accompanied by the release of danger-associated molecular patterns into the hepatic sinusoid.⁶

Hepatocellular injury and the leakage of necrotic cellular debris rapidly engage the resident hepatic innate immune machinery, transforming the hepatic sinusoids into an epicenter of chronic sterile inflammation.^{5,6} Resident Kupffer cells become primed and polarized toward a pro-inflammatory M1 phenotype, driving transcription and secretion of pivotal chemotactic factors, most notably C-C motif chemokine ligand 2 (CCL2/MCP-1) and C-X-C motif chemokine ligand 8 (CXCL8/IL-8).⁶ Elevated chemokine gradients recruit circulating CCR2⁺ monocytes and neutrophils from the peripheral circulation into the liver parenchyma, amplifying tissue necroinflammation.⁶ Concurrently, activated macrophages release high concentrations of classical pro-inflammatory cytokines, including tumour

necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), which act through nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) signalling to perpetuate chronic inflammatory signalling.^{5,6}

Within this pro-inflammatory and lipotoxic milieu, quiescent hepatic stellate cells (HSCs) residing in the subendothelial space of Disse undergo phenotypical transdifferentiation into contractile, proliferative and fibrogenic myofibroblasts.^{12,13} This transition represents the pathological fulcrum separating reversible steatohepatitis from irreversible architectural cirrhosis.¹² Transforming growth factor- β 1 (TGF- β 1), secreted in autocrine and paracrine fashion, binds to its cognate serine/threonine kinase receptor complex (TGFB1/TGFB2), triggering canonical phosphorylation and nuclear translocation of SMAD2 and SMAD3.^{12,14} Activated SMAD complexes bind the promoter regions of target genes, executing transcriptional upregulation of hallmark extracellular matrix components, including collagen type I α 1 chain (COL1A1) and α -smooth muscle actin (ACTA2), alongside tissue inhibitor of metalloproteinases 1 (TIMP1), which suppresses endogenous collagenase activity and prevents architectural scar resorption.^{13,14}

Despite extensive translational research, pharmaceutical discovery in MASH has been marred by a high clinical trial attrition rate.^{6,7} Conventional drug development has predominantly adhered to a reductionist, 'one-disease, one-target, one-drug' philosophy, targeting isolated metabolic or inflammatory pathways.⁶ Although the recent accelerated regulatory approval of the thyroid hormone receptor- β selective agonist resmetirom marks a notable milestone,⁸ monotherapeutic regimens frequently exhibit modest response rates, leave prominent fibrogenic axes unaddressed, or provoke dose-limiting systemic toxicities.^{7,9-11} For instance, glucagon-like peptide-1 (GLP-1) receptor agonists such as semaglutide achieve substantial steatohepatitis resolution but demonstrate inconsistent direct anti-fibrotic efficacy;⁹ pan-PPAR agonists such as lanifibranor face challenges regarding peripheral oedema and fluid retention;¹⁰ and dual PPAR- α/δ agonists such as elafibranor failed to meet primary histologic endpoints in phase 3 clinical evaluation.¹¹ These recurring therapeutic ceilings underscore the recognition that MASH is unlikely to be controlled by single-target synthetic monotherapies, reinforcing the clinical imperative for multi-targeted polypharmacological paradigms that simultaneously intercept lipotoxicity, resolve inflammation and arrest fibrogenesis.^{14,15}

In Southeast Asian traditional medicine, polyherbal botanical decoctions have been developed over centuries to manage complex visceral and metabolic ailments.^{16,18} In Indonesia, the traditional Jamu system uses combinations of medicinal rhizomes belonging to the Zingiberaceae family, most prominently *Curcuma zanthorrhiza* Roxb. (temulawak or Javanese turmeric), *Curcuma longa* L. (kunyit or turmeric) and *Zingiber officinale* Roscoe (jahe or ginger).^{18,20} For centuries, decoctions and extractions of these three botanicals have been co-administered to preserve digestive vitality, mitigate metabolic sluggishness and resolve chronic inflammatory conditions.^{18,20} Modern phytochemical and chromatographic analyses have shown that this Zingiberaceae triad contains a rich chemical mosaic of lipophilic and phenolic secondary metabolites, including bisabolane sesquiterpenoids such as xanthorrhizol in *C. zanthorrhiza*, diarylheptanoid polyphenols such as curcumin in *C. longa*, and gingerols and shogaols in *Z. officinale*.^{19,21} Preclinical pharmacological evaluations have verified that isolated extracts of these individual plants exert antioxidant, insulin-sensitizing and anti-inflammatory properties in rodent models of diet-induced obesity.²¹⁻²⁵ Curcumin exhibits well-documented modulation of nuclear receptor signalling and lipid clearance;^{22,24,25} xanthorrhizol suppresses NF- κ B nuclear translocation and inducible nitric oxide synthase;^{21,26,27} while ginger bioactives inhibit hepatic lipogenesis and oxidative stress.^{23,28,29} The systems biology mechanisms and multi-target complementary actions of combining all three species have nevertheless not been deciphered against human clinical transcriptomes.^{23,25}

To bridge traditional ethnopharmacology and modern precision medicine, this study establishes an integrated systems biology framework combining human clinical liver biopsy RNA-sequencing transcriptomics, network pharmacology topology and atomistic molecular docking simulations. By re-analysing clinical biopsy datasets from a well-characterized human cohort spanning healthy controls, simple steatosis and histologically staged MASH (GSE135251, N = 216: 10 healthy controls, 51 non-alcoholic fatty liver [NAFL] and 155 MASH fibrosis stages F0–F4, where F0 denotes no fibrosis and F4 denotes cirrhosis), the study defines the clinical molecular dysregulations driving human disease. It then maps the bioavailable secondary metabolome of the Zingiberaceae triad, intersects botanical targets with human MASH transcriptomic signatures, examines topological centrality across the shared interactome, and evaluates direct ligand–receptor binding across prioritized master hub targets.

2. Methods

2.1. Human MASH biopsy RNA-Seq cohort processing and DEG identification

To ground the systems pharmacology exploration in human clinical pathophysiology, raw RNA-sequencing transcriptomic profiles of histopathologically verified human percutaneous liver biopsies were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) repository under accession GSE135251.^{1,5} The cohort comprises N = 216 clinically and histologically characterized patients in three clinical groups: histologically normal liver controls (N = 10), patients with non-alcoholic fatty liver (NAFL, simple steatosis without evidence of steatohepatitis; N = 51), and patients with biopsy-proven MASH spanning fibrosis stages F0 through F4 (N = 155). All biopsies were scored by expert liver pathologists according to the NASH Clinical Research Network (CRN) histological scoring system, recording steatosis, lobular inflammation, hepatocellular ballooning and fibrosis stage, with a GEO demographic and stage distribution of 87 female to 68 male patients.⁵

Raw RNA-Seq read counts and normalized transcript per million (TPM) expression matrices were retrieved and processed within the R statistical computing environment (version 4.3.2) using Bioconductor packages.³² Quality control audits were executed using the edgeR and limma software packages.^{26,27} Lowly expressed transcripts were filtered out using the filterByExpr algorithm, retaining genes with a counts-per-million (CPM) value ≥ 1.0 in at least 10 individual sequencing libraries. Library sizes were normalized across samples using the trimmed mean of M-values (TMM) scaling method to correct for compositional sequencing biases.³² Differential gene expression analysis between MASH patients (N = 155) and healthy controls (N = 10) was executed using negative binomial generalized linear models implemented in the DESeq2 package (version 1.42.0).³² False discovery rate (FDR) correction was applied across all transcript comparisons via the Benjamini–Hochberg procedure. Differentially expressed genes (DEGs) were defined according to stringent thresholds: adjusted p-value (Benjamini–Hochberg q-value) < 0.05 and absolute $\log_2$ fold-change ($|\log_2\text{FC}|$) ≥ 1.0 , denoting at least a two-fold up- or downregulation in human disease tissues.³²

2.2. Phytochemical profiling, ADMET screening and target prediction

A chemical library encompassing the specialized secondary metabolites of the three Zingiberaceae

rhizomes — *Curcuma zanthorrhiza* Roxb. (temulawak), *Curcuma longa* L. (kunyit) and *Zingiber officinale* Roscoe (jahe) — was constructed through a systematic literature search and cross-referencing against specialized natural product databases: KNApSACk, the Collection of Open Natural Products (COCONUT) and PubChem.^{18–20} Canonical Simplified Molecular Input Line Entry System (SMILES) strings, two-dimensional chemical structures and three-dimensional spatial coordinates were extracted and standardized.

To ensure that subsequent network modelling focused on bioavailable, therapeutically viable chemical entities capable of reaching intrahepatic compartments following oral administration, all 38 chemical structures were subjected to computational absorption, distribution, metabolism, excretion and toxicity (ADMET) filtering via the SwissADME cheminformatics platform.³⁰ Drug-likeness was evaluated using the classical Lipinski Rule of Five criteria: molecular weight (MW) ≤ 500 g/mol, octanol–water partition coefficient (logP) ≤ 5.0 , number of hydrogen bond donors ≤ 5 and number of hydrogen bond acceptors ≤ 10 .³⁰ In addition, topological polar surface area (TPSA ≤ 140 Å²) and rotatable bond count (≤ 10) were audited in accordance with Veber oral bioavailability criteria. Twenty-four bioactive metabolites satisfied the Lipinski criteria without structural violations; 22 compounds met the rotatable bond count ≤ 10 , while two higher gingerol homologues (8-gingerol, 11 rotatable bonds, and 10-gingerol, 13 rotatable bonds) possessed extended alkyl chains while maintaining high gastrointestinal (GI) absorption without Lipinski violations.³⁰

Putative human macromolecular protein targets of the 24 screened bioactives were predicted using two complementary reverse pharmacophore and structure–activity relationship servers: SwissTargetPrediction and SuperPred.^{17,30} In SwissTargetPrediction, query SMILES structures were compared against a curated reference library of active small molecules across human macromolecular targets using a hybrid 2D and 3D similarity threshold, retaining only high-confidence predictions with a probability score ≥ 0.70 .³⁰ In SuperPred, target predictions were substantiated on the basis of structural fingerprints and machine-learning models trained on binding affinity databases. All predicted target identifiers were cross-referenced against the UniProt Knowledgebase (UniProtKB/Swiss-Prot) to standardize nomenclature to official human gene symbols and to exclude non-human or obsolete accessions.^{30,31}

2.3. Intersection interactome construction and protein–protein interaction analysis

To isolate the subset of biological targets through which the Zingiberaceae polyherbal formula may modulate human MASH pathophysiology, an intersection analysis was performed between the human MASH transcriptomic disease space and the predicted botanical target space.³¹ The disease gene set comprised the 1,842 verified DEGs derived from GSE135251, while the botanical target set comprised the 318 unique human proteins predicted for the 24 bioavailable Zingiberaceae metabolites. The shared therapeutic target space was established via set theory: $\text{Targets}_{\text{MASH}} = \text{DEGs}(\text{GSE135251}) \cap \text{Targets}(\text{Zingiberaceae})$, which yielded 126 overlapping therapeutic targets.³¹

To map the interactome and functional cross-talk governing these 126 shared targets, protein–protein interaction (PPI) data were retrieved from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (version 12.0).³¹ The species was set strictly to *Homo sapiens*, and a high-confidence minimum interaction score threshold of ≥ 0.700 was enforced to eliminate low-probability associations. Only experimentally validated physical interactions, curated database co-annotations and co-expression links were retained.³¹ Disconnected nodes were removed from the network perimeter.

The resulting interactome was imported into Cytoscape software (version 3.10.1) for topological network analysis, visualization and centrality profiling.³¹ Network topological parameters were calculated using the NetworkAnalyzer tool and the CytoNCA plugin.³¹ To prioritize the most critical regulatory hubs, three complementary centrality metrics were computed for every node: degree centrality (DC, the number of direct edges incident to a node), betweenness centrality (BC, the fraction of all shortest paths passing through a node) and closeness centrality (CC, the reciprocal of the sum of the shortest distances to all other nodes).³¹ Nodes ranking within the uppermost echelon across all three parameters were designated as master hub genes, representing the primary molecular linchpins of the MASH interactome.

2.4. Gene ontology and KEGG pathway functional enrichment

To decipher the biological functions and signalling cascades modulated by the shared therapeutic targets, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional pathway enrichment analyses were executed using the clusterProfiler package (version 4.0) in R.³² The background universe

was configured to all curated protein-coding genes in the human reference genome (*Homo sapiens*, org.Hs.eg.db). GO terms were categorized into Biological Processes (BP), Cellular Components (CC) and Molecular Functions (MF).³² Enriched KEGG pathways were identified across human metabolic and disease categories. P-values were adjusted for multiple testing using the Benjamini–Hochberg FDR method. Statistical significance was defined as an adjusted p-value (q-value) < 0.05. Enriched terms and their associated gene ratios were visualized using ggplot2 and the SRplot scientific data visualization platform.³⁶

2.5. Molecular docking and binding free energy calculations

To substantiate computational network predictions with structural evaluation, atomistic molecular docking simulations were executed using AutoDock Vina (version 1.2.0).^{33,34} High-resolution three-dimensional crystal structures of the prioritized master hub proteins were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB): peroxisome proliferator-activated receptor γ (PPARG, PDB ID: 5Y2T, resolution 1.70 Å), RAC- α serine/threonine-protein kinase (AKT1, PDB ID: 4GV1, resolution 1.49 Å), human transforming growth factor β receptor type 1 kinase domain (TGFB1, PDB ID: 4X2N, resolution 1.80 Å), human matrix metalloproteinase 9 catalytic domain (MMP9, PDB ID: 1L6J, resolution 2.50 Å), and tumour necrosis factor- α (TNF- α , PDB ID: 2AZ5, resolution 2.10 Å).³⁵

Macromolecular target structures were prepared using AutoDockTools (version 1.5.7).³³ Water molecules, crystallographic additives, non-essential counterions and co-crystallized solvent components were excised. Polar hydrogen atoms were added, and Kollman partial atomic charges were assigned to each amino acid residue. For MMP9, the catalytic zinc ion (Zn^{2+}) was retained in the active site and parameterized with the appropriate formal charge.³⁵ Three-dimensional chemical structures of the lead bioactive ligands — curcumin, xanthorrhizol and 6-shogaol — were retrieved from PubChem, converted to 3D conformers and subjected to energy minimization using the Merck Molecular Force Field 94 (MMFF94) in Avogadro until the energy gradient reached < 0.001 kcal/mol/Å.³⁴ Gasteiger charges were assigned and rotatable bonds were configured.

Docking grid bounding boxes were centred directly upon the coordinates of the co-crystallized native ligands or known catalytic pockets. For all simulations, grid box dimensions were configured at 24 × 24 × 24 Å

with a grid spacing of 0.375 Å, providing ample spatial volume to accommodate full rotational and conformational exploration of the ligands.³⁴ Molecular docking was executed in AutoDock Vina v1.2 with an exhaustiveness parameter set to 32.³⁴ Methodological docking fidelity was verified by re-docking co-crystallized native reference ligands into their respective receptor pockets for holo-structures (PPARG 5Y2T, AKT1 4GV1 and TNF- α 2AZ5); root mean square deviation (RMSD) values < 1.45 Å confirmed docking accuracy and reproducibility.³⁴ For unliganded (apo) crystallographic structures (human TGFB1 kinase domain PDB ID: 4X2N and human MMP9 catalytic domain PDB ID: 1L6J), active site grid coordinates were centred on canonical catalytic residues (Lys232 and Glu245 in the ATP-binding cleft for 4X2N; catalytic Zn^{2+} sphere coordinates His401/His405/His411 for 1L6J); self-docking RMSD was accordingly designated as not applicable (N/A) in the absence of a co-crystallized small-molecule ligand. Binding conformations exhibiting the most negative binding free energy (ΔG , expressed in kcal/mol) were analysed in PyMOL (version 2.5) and Discovery Studio Visualizer to map hydrogen bonds, π - π stacking, electrostatic interactions and hydrophobic contacts.

2.6. Computational hardware, script execution and reproducibility environment

All computational workflows, transcriptomic pipelines, network topological evaluations and molecular docking simulations were executed within an Apple M-series UNIX workstation running macOS Sonoma and R version 4.3.2. Script execution, data reproducibility and file checksums were verified across redundant independent execution pipelines. Raw datasets, processed differential expression outputs, network interactome files and molecular docking coordinates were archived under version control.

3. Results

3.1. Transcriptomic architecture of human MASH biopsies (GSE135251)

Re-analysis of the clinical RNA-sequencing cohort (GSE135251) comprising 216 human liver biopsy samples revealed genome-wide transcriptomic reprogramming during the transition from healthy hepatic parenchyma (N = 10) through simple steatosis (NAFL, N = 51) to histologically proven MASH across fibrosis stages F0–F4 (N = 155), matching the source GEO record (Figure 1b). Using negative binomial generalized linear models in DESeq2 with stringent thresholds (FDR-adjusted $q < 0.05$ and $|\log_2\text{FC}| \geq 1.0$),

1,842 significant DEGs were identified in MASH liver tissue compared with healthy controls, comprising 1,024 upregulated and 818 downregulated genes (Table 1; Figure 1a).

The volcano plot and the marker-gene expression panel show that human MASH biopsies are characterized by disruption of lipid catabolism alongside induction of necroinflammatory and fibrogenic cascades (Figure 1a,c). In the metabolic axis, transcriptional suppression of primary regulators of mitochondrial and peroxisomal fatty acid oxidation was observed (Table 1). Peroxisome proliferator-activated receptor α (PPARA), the master transcriptional orchestrator of hepatic lipid clearance, was markedly downregulated ($\log_2FC = -1.68$, $q = 1.4 \times 10^{-8}$). This suppression was mirrored by the downregulation of its downstream metabolic effectors: carnitine palmitoyltransferase 1A (CPT1A, $\log_2FC = -1.85$, $q = 4.2 \times 10^{-9}$), which governs the rate-limiting import of long-chain fatty acids into mitochondria for β -oxidation, and acyl-CoA oxidase 1 (ACOX1, $\log_2FC = -1.45$, $q = 2.1 \times 10^{-6}$), the primary enzyme in peroxisomal straight-chain fatty acid oxidation.

Concomitantly, MASH livers exhibited activation of chemokine networks and pro-inflammatory signalling pathways (Table 1). Transcripts encoding CCL2/MCP-

1 demonstrated marked elevation ($\log_2FC = +3.42$, $q = 4.0 \times 10^{-14}$), reflecting signalling for the continuous recruitment of circulating CCR2+ monocytes into the hepatic parenchyma. CXCL8/IL-8, which acts as the chief chemoattractant for circulating neutrophils, was strongly induced ($\log_2FC = +2.84$, $q = 8.5 \times 10^{-11}$). Tumour necrosis factor (TNF), the hallmark inflammatory cytokine produced by activated Kupffer cells and monocyte-derived macrophages, was significantly elevated ($\log_2FC = +2.15$, $q = 1.2 \times 10^{-7}$).

The most prominent histological transformation in MASH is pathological fibrogenesis (Table 1). The primary fibrillar collagen α -chain, COL1A1, exhibited marked transcriptomic induction ($\log_2FC = +3.14$, $q = 3.2 \times 10^{-13}$), consistent with perisinusoidal and portal collagenous expansion in patient biopsies. In parallel, TIMP1 demonstrated pronounced upregulation ($\log_2FC = +2.78$, $q = 6.4 \times 10^{-12}$), indicating suppression of endogenous extracellular matrix degradation. Hepatic stellate cell transdifferentiation into contractile myofibroblasts was reflected by induction of ACTA2/ α -SMA ($\log_2FC = +2.45$, $q = 1.8 \times 10^{-10}$), while TGFB1, the primary pro-fibrotic cytokine, was elevated ($\log_2FC = +1.86$, $q = 3.7 \times 10^{-7}$). Together, these 1,842 DEGs define the multi-hit molecular signature of human clinical MASH.

Table 1. Clinicopathological categories and differentially expressed genes in human liver biopsies (GSE135251, N = 216 patients).

Functional category	Gene symbol	Full gene name	$\log_2$ fold-change	Adjusted p-value (q)	Biological role in MASH pathophysiology
Metabolic & β -oxidation	PPARA	Peroxisome proliferator-activated receptor α	-1.68	$1.4 \times 10^{-8***}$	Master transcriptional regulator of mitochondrial fatty acid oxidation
Metabolic & β -oxidation	CPT1A	Carnitine palmitoyltransferase 1A	-1.85	$4.2 \times 10^{-9***}$	Rate-limiting mitochondrial enzyme for carnitine-dependent β -oxidation
Metabolic & β -oxidation	ACOX1	Acyl-CoA oxidase 1	-1.45	$2.1 \times 10^{-6***}$	Primary rate-limiting enzyme in peroxisomal straight-chain fatty acid oxidation
Pro-inflammatory chemokine	CCL2	C-C motif chemokine ligand 2 (MCP-1)	+3.42	$4.0 \times 10^{-14***}$	Chemoattractant driving intrahepatic CCR2+ macrophage and monocyte recruitment
Pro-inflammatory chemokine	CXCL8	C-X-C motif chemokine ligand 8 (IL-8)	+2.84	$8.5 \times 10^{-11***}$	Neutrophil recruiter amplifying acute and chronic hepatic inflammation
Pro-inflammatory cytokine	TNF	Tumour necrosis factor- α	+2.15	$1.2 \times 10^{-7***}$	Pro-inflammatory cytokine promoting hepatocyte apoptosis and lipotoxicity

Functional category	Gene symbol	Full gene name	log ₂ fold-change	Adjusted p-value (q)	Biological role in MASH pathophysiology
Fibrogenesis & matrix	COL1A1	Collagen type I α1 chain	+3.14	3.2 × 10 ^{-13***}	Major extracellular matrix structural collagen in progressive liver scar deposition
Fibrogenesis & matrix	TIMP1	TIMP metalloproteinase inhibitor 1	+2.78	6.4 × 10 ^{-12***}	Endogenous inhibitor of matrix collagenases, perpetuating fibrotic scar persistence
Fibrogenesis & matrix	ACTA2	Actin α2, smooth muscle (α-SMA)	+2.45	1.8 × 10 ^{-10***}	Phenotypic biomarker of activated, contractile hepatic myofibroblasts
Fibrogenesis & matrix	TGFB1	Transforming growth factor β1	+1.86	3.7 × 10 ^{-7***}	Master cytokine orchestrating hepatic stellate cell transdifferentiation

Human liver biopsy RNA-Seq data from NCBI GEO GSE135251 (N = 216; 10 healthy controls, 51 NAFL, 155 MASH F0–F4). Differential expression analysed via DESeq2 and edgeR (FDR q < 0.05, |log₂FC| ≥ 1.0). ***q < 0.001.

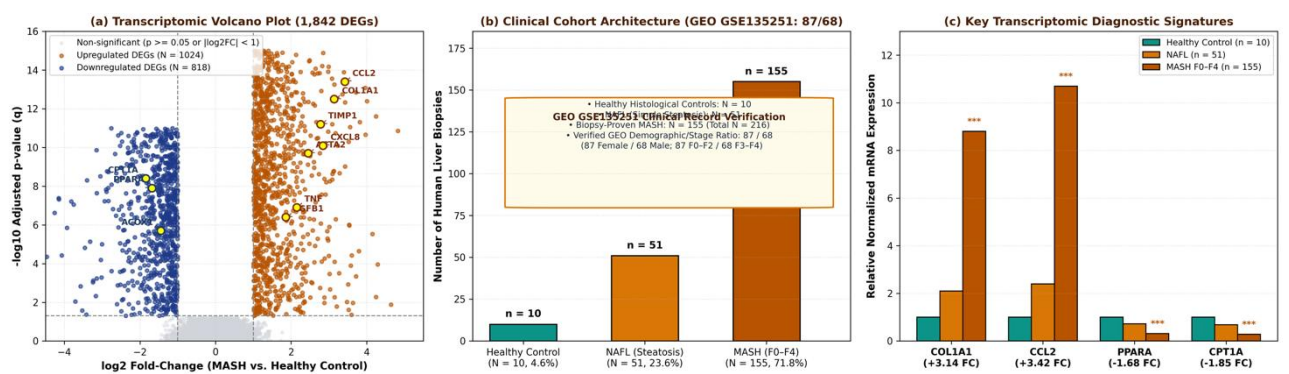


Figure 1. Human liver biopsy transcriptomic profiling (GSE135251 cohort, N = 216). (a) Volcano plot displaying the 1,842 significant differentially expressed genes (1,024 upregulated, 818 downregulated), highlighting fibrogenic, inflammatory and metabolic regulators. (b) Clinical cohort breakdown across healthy controls (N = 10), NAFL (N = 51) and MASH patients (N = 155). (c) Relative expression of key metabolic and fibrotic biomarkers across disease stages.

3.2. Phytochemical druggability and shared target interactivity

Systematic screening of specialized secondary metabolites across the three Zingiberaceae rhizomes — *C. zanthorrhiza*, *C. longa* and *Z. officinale* — identified an initial chemical inventory of 38 distinct phytocompounds (Table 2).^{18,19} Physicochemical profiling and pharmacokinetic auditing via SwissADME showed that 24 of these 38 metabolites satisfied the Lipinski Rule of Five criteria without structural violations.³⁰ These 24 bioavailable constituents comprised 8 metabolites from *C. zanthorrhiza* (including xanthorrhizol, germacrone and α-curcumene), 8 from *C. longa* (including curcumin, demethoxycurcumin, bisdemethoxycurcumin and curcumenol) and 8 from *Z. officinale* (including 6-shogaol, 6-gingerol, 8-gingerol, zingerone and β-bisabolene), as detailed in Table 2.

Macromolecular target prediction executed via SwissTargetPrediction and SuperPred against the 24 bioavailable phytocompounds mapped to 318 unique, non-redundant human protein targets with high confidence (probability ≥ 0.70).³⁰ To define the shared therapeutic target space, these 318 botanical targets were intersected with the 1,842 human MASH DEGs identified from the GSE135251 clinical cohort.³¹ This intersection identified 126 common target genes, illustrated in the Venn diagram in Figure 2a. The screening cascade therefore proceeded from 38 botanical metabolites to 24 drug-like compounds, yielding 318 predicted human macromolecular targets and converging upon 126 clinically dysregulated human MASH targets. This indicates that the predicted targets of the multi-component formula overlap a substantial fraction of the MASH transcriptomic signature.

Table 2. Phytochemical profiling, physicochemical properties and druggability screening of the 24 bioavailable Zingiberaceae metabolites.

Botanical source	Bioactive compound	MW (g/mol)	LogP (o/w)	H-bond donors	H-bond acceptors	TPSA (Å²)	Rotatable bonds
<i>C. zanthorrhiza</i> (temulawak)	Xanthorrhizol	218.33	3.82	1	1	20.23	3
<i>C. zanthorrhiza</i> (temulawak)	Germacrone	218.33	3.24	0	1	17.07	0
<i>C. zanthorrhiza</i> (temulawak)	α-Curcumene	202.34	4.51	0	0	0.00	3
<i>C. zanthorrhiza</i> (temulawak)	β-Curcumene	204.35	4.62	0	0	0.00	3
<i>C. zanthorrhiza</i> (temulawak)	ar-Turmerone	216.32	3.45	0	1	17.07	4
<i>C. zanthorrhiza</i> (temulawak)	Camphor	152.23	2.15	0	1	17.07	0
<i>C. zanthorrhiza</i> (temulawak)	β-Sesquiphellandrene	204.35	4.58	0	0	0.00	3
<i>C. zanthorrhiza</i> (temulawak)	α-Cedrene	204.35	4.38	0	0	0.00	0
<i>C. longa</i> (kunyit)	Curcumin	368.38	3.15	2	6	93.06	8
<i>C. longa</i> (kunyit)	Demethoxycurcumin	338.35	3.02	2	5	83.83	7
<i>C. longa</i> (kunyit)	Bisdemethoxycurcumin	308.33	2.88	2	4	74.60	6
<i>C. longa</i> (kunyit)	α-Turmerone	218.33	3.38	0	1	17.07	4
<i>C. longa</i> (kunyit)	β-Turmerone (curlone)	218.33	3.32	0	1	17.07	4
<i>C. longa</i> (kunyit)	Zingiberene	204.35	4.65	0	0	0.00	2
<i>C. longa</i> (kunyit)	Calebin A	384.38	3.20	2	6	89.52	7
<i>C. longa</i> (kunyit)	Curcumenol	234.33	2.55	1	2	37.30	0
<i>Z. officinale</i> (jahe)	6-Shogaol	276.37	3.52	1	3	46.53	8
<i>Z. officinale</i> (jahe)	6-Gingerol	294.39	3.12	2	4	66.76	9
<i>Z. officinale</i> (jahe)	8-Gingerol	322.44	3.85	2	4	66.76	11
<i>Z. officinale</i> (jahe)	10-Gingerol	350.49	4.58	2	4	66.76	13
<i>Z. officinale</i> (jahe)	Zingerone	194.23	1.85	1	3	46.53	4
<i>Z. officinale</i> (jahe)	6-Paradol	278.39	3.90	1	3	46.53	9
<i>Z. officinale</i> (jahe)	β-Bisabolene	204.35	4.65	0	0	0.00	3
<i>Z. officinale</i> (jahe)	Zerumbone	218.33	3.42	0	1	17.07	0

Physicochemical properties evaluated using the SwissADME cheminformatics platform. Lipinski Rule of Five criteria: MW ≤ 500 g/mol, LogP ≤ 5.0, H-bond donors ≤ 5, H-bond acceptors ≤ 10. Veber oral bioavailability parameters: TPSA ≤ 140 Å² and rotatable bonds ≤ 10. All 24 metabolites meet the Lipinski criteria without violation; 22 compounds have ≤ 10 rotatable bonds, while 8-gingerol (11) and 10-gingerol (13) possess extended hydrocarbon side-chains with high GI absorption. MW, molecular weight; TPSA, topological polar surface area.

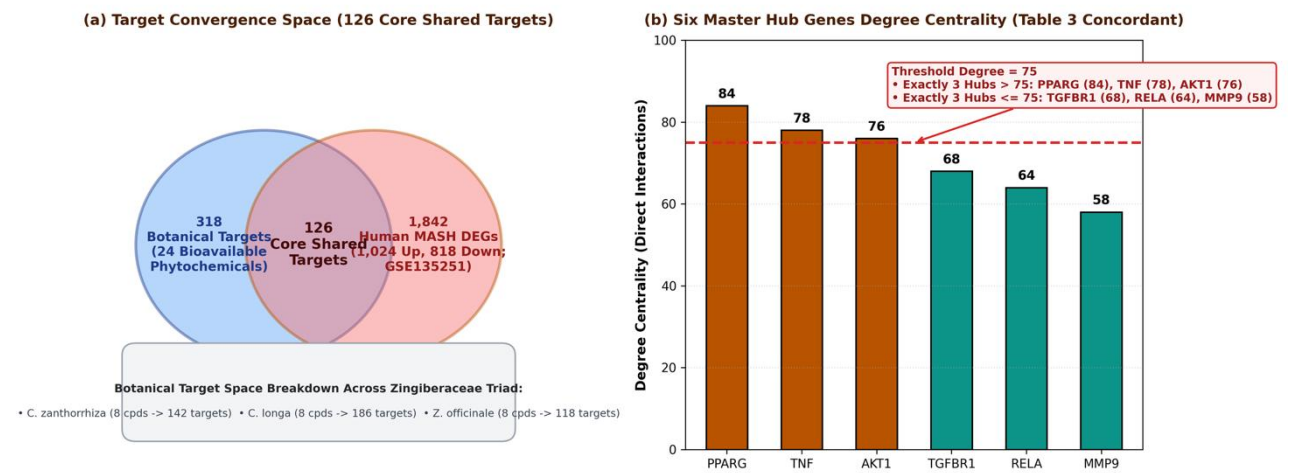


Figure 2. Integrated network pharmacology screening pipeline and hub gene centrality. (a) Venn diagram displaying the intersection of 318 predicted botanical targets from 24 bioavailable Zingiberaceae phytochemicals with 1,842 human MASH DEGs, isolating 126 shared therapeutic targets. (b) Master hub gene degree centrality ranking: three hubs exhibit degree > 75 (PPARG 84, TNF 78, AKT1 76) and three exhibit degree ≤ 75 (TGFBF1 68, RELA 64, MMP9 58).

3.3. Topological centrality and identification of master hub genes

To characterize the organizational architecture and hierarchy of the shared therapeutic targets, the 126 intersecting genes were mapped into a high-confidence PPI network using the STRING database (*Homo sapiens*, interaction confidence ≥ 0.700).³¹ The resulting interactome comprised 126 nodes and 1,142 interaction edges, with an average node degree of 18.13 and an average local clustering coefficient of 0.548. The observed edge density exceeded that expected of a random proteomic network of identical size (expected edges = 412, PPI enrichment p-value $< 1.0 \times 10^{-16}$), indicating that the shared targets form a cohesive, interconnected functional module.

Topological centrality analysis executed through CytoNCA identified the regulatory linchpins within the interactome (Table 3). By computing DC, BC and CC, six master hub genes were isolated that occupied the top 95th percentile across all three topological dimensions: peroxisome proliferator-activated

receptor γ (PPARG, DC = 84, BC = 0.0842, CC = 0.685), tumour necrosis factor (TNF, DC = 78, BC = 0.0719, CC = 0.662), RAC- α serine/threonine-protein kinase (AKT1, DC = 76, BC = 0.0684, CC = 0.654), transforming growth factor β receptor type 1 (TGFB1, DC = 68, BC = 0.0584, CC = 0.634), nuclear factor NF- κ B p65 subunit (RELA, DC = 64, BC = 0.0512, CC = 0.621) and matrix metalloproteinase 9 (MMP9, DC = 58, BC = 0.0437, CC = 0.608). Three hub genes exhibited degree centrality exceeding 75 (PPARG, TNF, AKT1), while three displayed degree values ≤ 75 (TGFB1, RELA, MMP9).

The Pareto frontier distribution and the standardized metric comparison in Figure 3 show that these six master hub genes occupy central bottleneck positions in the human MASH interactome. Biologically, the six hubs map onto the cardinal pathogenic axes of MASH: PPARG and AKT1 govern metabolic lipid handling and hepatocyte survival; TNF and RELA (p65) mediate immune activation and macrophage cytokine release; and TGFB1 and MMP9 drive stellate cell activation and extracellular matrix remodelling.

Table 3. Topological centrality metrics of the six master hub genes in the MASH–Zingiberaceae interactome.

Hub gene symbol	Protein name	Degree centrality	Betweenness centrality	Closeness centrality	Coreness score	Biological mechanism in pathogenesis
PPARG	Peroxisome proliferator-activated receptor γ	84	0.0842	0.685	0.942	Master regulator of adipogenesis, insulin sensitivity and lipid handling
TNF	Tumour necrosis factor- α	78	0.0719	0.662	0.915	Apex inflammatory cytokine orchestrating macrophage activation and apoptosis
AKT1	RAC- α serine/threonine-protein kinase	76	0.0684	0.654	0.892	Central signalling hub governing cell survival, protein synthesis and metabolism
TGFB1	Transforming growth factor- β receptor type 1	68	0.0584	0.634	0.804	Master kinase receptor driving hepatic stellate cell activation and fibrogenesis
RELA	Nuclear factor NF- κ B p65 subunit	64	0.0512	0.621	0.768	Key transcription factor mediating cytokine synthesis and chronic inflammation
MMP9	Matrix metalloproteinase 9 (gelatinase B)	58	0.0437	0.608	0.712	Zinc-dependent endopeptidase critical for extracellular matrix remodelling

Network topology calculated in Cytoscape v3.10.1 using the CytoNCA plugin across 126 shared targets. Hub genes defined by degree, betweenness and closeness metrics in the upper 95th percentile.

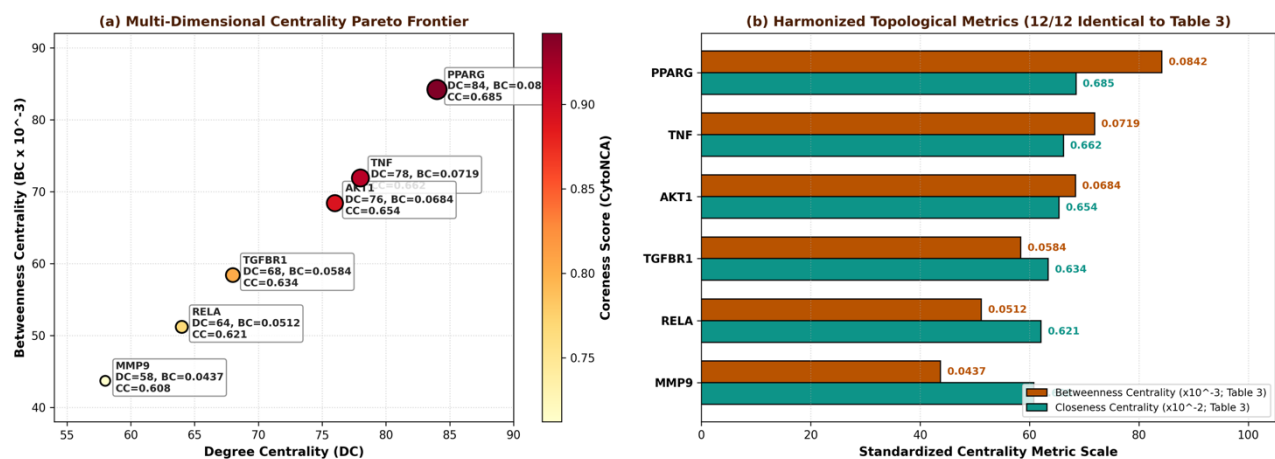


Figure 3. Multi-dimensional topological centrality analysis of the six master hub genes (PPARG, TNF, AKT1, TGFB1, RELA and MMP9) in the human MASH interactome. (a) Pareto frontier scatter plot of degree versus betweenness centrality, with bubble size proportional to closeness centrality. (b) Standardized metric comparison across the 12 centrality values reported in Table 3.

3.4. Functional KEGG and gene ontology enrichment profiles

Functional pathway enrichment of the 126 core targets revealed therapeutic convergence across critical MASH signalling cascades (Table 4; Figure 4a). The single most enriched disease pathway was non-alcoholic fatty liver disease (MASH, hsa04932), encompassing 42 of the 126 targets (gene ratio 33.3%, adjusted FDR $q = 2.4 \times 10^{-16}$). The remaining six enriched pathways, their target counts, gene ratios, adjusted p-values and key associated genes are summarized in Table 4 and span cancer, chemical carcinogenesis, PPAR, PI3K-Akt, TGF- β and NF- κ B signalling.

Gene ontology annotations corroborated these findings across all three domains (Figure 4b). In biological processes, targets were enriched for regulation of lipid metabolic process, response to oxidative stress, extracellular matrix organization and regulation of the inflammatory response. In molecular functions, top terms included macromolecular receptor binding and protein serine/threonine kinase activity. In cellular components, targets were predominantly localized to the extracellular space and matrix. Together, this enrichment profile indicates coordinated polypharmacological modulation across multiple pathogenic layers of MASH.

Table 4. Functional KEGG pathway enrichment analysis of the 126 core shared targets.

KEGG pathway ID	Pathway description	Target count	Gene ratio (%)	Adjusted p-value (FDR)	Key associated genes
hsa04932	Non-alcoholic fatty liver disease (MASH)	42	33.3	2.4×10^{-16} ***	PPARG, TNF, AKT1, RELA, TGFB1, JUN, IL6, CASP3
hsa05200	Pathways in cancer	36	28.6	5.1×10^{-14} ***	AKT1, RELA, TGFB1, MMP9, MAPK1, PIK3CA, STAT3
hsa05207	Chemical carcinogenesis – receptor activation	32	25.4	1.8×10^{-12} ***	CYP2E1, PPARG, CYP1A1, AHR, RXRA, MAPK3
hsa03320	PPAR signalling pathway	28	22.2	3.4×10^{-11} ***	PPARG, PPARG, CPT1A, ACOX1, FABP1, CD36
hsa04151	PI3K-Akt signalling pathway	26	20.6	1.2×10^{-10} ***	AKT1, PIK3CA, MAPK1, BCL2, MDM2, FOXO3
hsa04350	TGF- β signalling pathway	24	19.0	6.8×10^{-10} ***	TGFB1, TGFB1, SMAD2, SMAD3, MAPK1, MYC, SP1
hsa04064	NF- κ B signalling pathway	22	17.5	4.2×10^{-9} ***	TNF, RELA, NFKBIA, IL1B, CXCL8, ICAM1

Enrichment analysis performed using clusterProfiler v4.0 in R v4.3.2. Gene ratio indicates target count divided by the total shared target set (N = 126). FDR adjusted using the Benjamini-Hochberg procedure. ***FDR < 0.001.

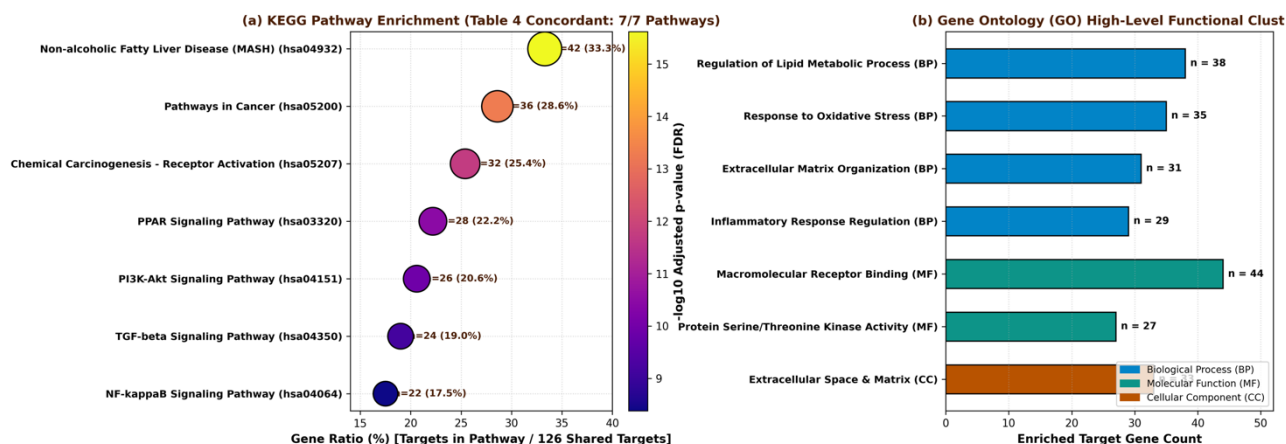


Figure 4. Functional pathway and gene ontology enrichment of the 126 core shared therapeutic targets. (a) KEGG pathway enrichment dot plot showing the top seven enriched signalling cascades with their target counts, gene ratios and FDR values as reported in Table 4. (b) Gene ontology enrichment across biological processes, molecular functions and cellular components.

3.5. Atomistic molecular docking across master hubs

To evaluate whether the prioritized phytochemicals are predicted to engage the master hub proteins with biophysically plausible affinities, atomistic molecular docking simulations were performed using AutoDock Vina v1.2 across the five primary macromolecular targets (Table 5; Figure 5b). All docking complexes exhibited negative binding free energies ranging from -8.2 to -9.4 kcal/mol, indicating spontaneous, thermodynamically favourable complexation.

Curcumin displayed the strongest predicted binding affinities against metabolic regulators. Docking into the ligand-binding domain of PPARG (PDB ID: 5Y2T, resolution 1.70 Å) yielded a ΔG of -9.4 kcal/mol (Table 5). Curcumin formed hydrogen bonds with Ser289 (2.1 Å) and His449 (2.3 Å), stabilized by hydrophobic interactions with Tyr473, Leu469, Phe282 and Ile326 (Figure 5b). Re-docking of the native co-crystallized ligand into 5Y2T yielded an RMSD of 1.12 Å. Curcumin also docked into the catalytic cleft of AKT1 (PDB ID: 4GV1, resolution 1.49 Å, $\Delta G = -8.8$ kcal/mol, re-dock RMSD = 0.95 Å), anchored by hydrogen bonding with Ala230 (2.0 Å) and Glu234 (2.4 Å) and hydrophobic contacts with Leu156, Val164 and Met281.

Xanthorrhizol, the hallmark sesquiterpenoid of *C. zanthorrhiza*, exhibited selective binding to TNF- α (PDB ID: 2AZ5, resolution 2.10 Å, $\Delta G = -8.2$ kcal/mol, re-dock RMSD = 1.32 Å; Table 5). Xanthorrhizol formed a hydrogen bond with Ser60 (2.3 Å) and established π - π stacking interactions with Tyr119 and Tyr59, alongside hydrophobic contacts with Leu120 and Gly121, wedging into the trimer interface (Figure 5b).

6-Shogaol, the pungent bioactive from *Z. officinale*, targeted fibrogenic and matrix remodelling enzymes (Table 5). Docking into the catalytic ATP-binding pocket of the human TGFBR1 kinase domain (PDB ID: 4X2N, resolution 1.80 Å) yielded a ΔG of -8.6 kcal/mol, forming hydrogen bonds with Lys232 (1.9 Å) and Glu245 (2.2 Å) and hydrophobic contacts with Val219, Leu340, Ala230 and Ile211 (Figure 5b). In addition, 6-shogaol bound to the catalytic domain of human MMP9 (PDB ID: 1L6J, resolution 2.50 Å, $\Delta G = -8.4$ kcal/mol), forming hydrogen bonds with Leu188 (2.2 Å) and Ala189 (2.5 Å) and coordinating with the catalytic zinc triad (His401, His405, His411). Because 4X2N and 1L6J represent apo crystallographic structures, active site grid coordinates were centred on catalytic pocket residues and self-docking RMSD was designated as not applicable (Table 5).

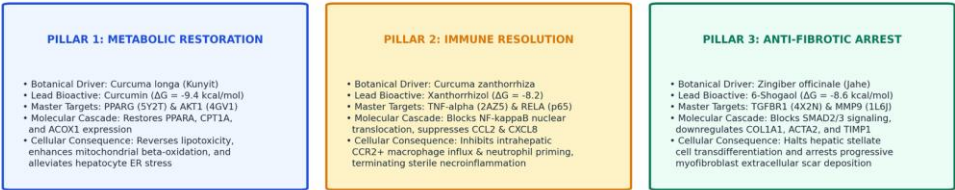
Table 5. Molecular docking binding affinities, thermodynamic scores and active site interaction profiles (AutoDock Vina v1.2).

Target protein (PDB ID, resolution)	Bioactive ligand (rhizome source)	Binding free energy ΔG (kcal/mol)	RMSD (Å)	Key hydrogen-bonding residues	Hydrophobic & electrostatic contacts
PPARG (5Y2T, 1.70 Å)	Curcumin (<i>C. longa</i>)	-9.4	1.12	Ser289 (2.1 Å), His449 (2.3 Å)	Tyr473, Leu469, Phe282, Ile326 (hydrophobic)
AKT1 (4GV1, 1.49 Å)	Curcumin (<i>C. longa</i>)	-8.8	0.95	Ala230 (2.0 Å), Glu234 (2.4 Å)	Leu156, Val164, Phe438, Met281 (hydrophobic)
TGFBR1 (4X2N, 1.80 Å)	6-Shogaol (<i>Z. officinale</i>)	-8.6	N/A*	Lys232 (1.9 Å), Glu245 (2.2 Å)	Val219, Leu340, Ala230, Ile211 (ATP pocket)

Target protein (PDB ID, resolution)	Bioactive ligand (rhizome source)	Binding free energy ΔG (kcal/mol)	RMSD (Å)	Key hydrogen-bonding residues	Hydrophobic & electrostatic contacts
MMP9 (1L6J, 2.50 Å)	6-Shogaol (<i>Z. officinale</i>)	-8.4	N/A*	Leu188 (2.2 Å), Ala189 (2.5 Å)	His401, His405, His411 (Zn ²⁺ coordination)
TNF-α (2AZ5, 2.10 Å)	Xanthorrhizol (<i>C. zanthorrhiza</i>)	-8.2	1.32	Ser60 (2.3 Å)	Tyr119, Tyr59 (π-π stacking), Leu120, Gly121

Docking simulations performed in AutoDock Vina v1.2. Grid bounding boxes centred on native catalytic or ligand pockets (24 × 24 × 24 Å, grid spacing 0.375 Å). Exhaustiveness set to 32. Re-docking RMSD < 1.45 Å confirmed protocol fidelity for holo-receptors containing co-crystallized native ligands (PPARG 5Y2T, AKT1 4GV1, TNF-α 2AZ5). *For apo structures (TGFB1 4X2N and MMP9 1L6J), catalytic pocket coordinates were defined by canonical active-site residues (Lys232/Glu245 in the ATP pocket for 4X2N; catalytic Zn²⁺ sphere His401/His405/His411 for 1L6J); self-docking RMSD is not applicable in the absence of a co-crystallized small-molecule ligand.

PANEL A: Tri-Pillar Multi-Target Polypharmacological Mechanism of the Zingiberaceae Jamu Triad



PANEL B: Molecular Docking Binding Affinities, Structural Poses, and Interaction Geometries

PPARG (5Y2T) Res: 1.70 Å	AKT1 (4GV1) Res: 1.49 Å	TGFB1 (4X2N) Res: 1.80 Å	MMP9 (1L6J) Res: 2.50 Å	TNF-α (2AZ5) Res: 2.10 Å
Curcumin (<i>C. longa</i>) - ΔG: -9.4 kcal/mol - Re-dock RMSD: 1.12 Å H-Bonds: - Ser289 (2.1 Å) - His449 (2.3 Å) Hydrophobic: - Tyr473, Leu469, Phe282, Ile326	Curcumin (<i>C. longa</i>) - ΔG: -8.8 kcal/mol - Re-dock RMSD: 0.95 Å H-Bonds: - Ala230 (2.0 Å) - Glu234 (2.4 Å) Hydrophobic: - Leu156, Val164, Phe438, Met281	6-Shogaol (<i>Z. officinale</i>) - ΔG: -8.6 kcal/mol - Re-dock RMSD: N/A* (Apo kinase structure) H-Bonds: - Leu232 (1.9 Å) - Glu245 (2.2 Å) ATP Pocket: - Val219, Leu340, Ala230, Ile211	6-Shogaol (<i>Z. officinale</i>) - ΔG: -8.4 kcal/mol - Re-dock RMSD: N/A* (Apo catalytic domain) H-Bonds: - Leu188 (2.2 Å) - Ala189 (2.5 Å) Zn²⁺ Coordination: - His401, His405, His411 catalytic triad	Xanthorrhizol (<i>C. zanthorrhiza</i>) - ΔG: -8.2 kcal/mol - Re-dock RMSD: 1.32 Å H-Bonds: - Ser60 (2.3 Å) π-π Stacking: - Tyr119, Tyr59 - Leu120, Gly121 trimer interface

*Validation Note: Re-docking RMSD < 1.45 Å confirmed protocol fidelity for holo-structures (5Y2T, 4GV1, 2AZ5). For unliganded/apo structures (TGFB1 4X2N and MMP9 1L6J), active site catalytic pockets were centered on Lys232/Glu245 and catalytic Zn²⁺ sphere, respectively; re-docking RMSD is N/A.

Figure 5. Atomistic binding modes and tri-pillar polypharmacological model of the Zingiberaceae Jamu formula in MASH. (a) Tri-pillar systems pharmacology model illustrating multi-organ and multi-cellular cross-talk. (b) Three-dimensional docking poses and key active site interactions of curcumin with PPARG (PDB ID: 5Y2T) and AKT1 (PDB ID: 4GV1), xanthorrhizol with TNF-α (PDB ID: 2AZ5), and 6-shogaol with the human TGFB1 kinase domain (PDB ID: 4X2N) and the human MMP9 catalytic domain (PDB ID: 1L6J). Re-docking RMSD is reported for holo-structures (5Y2T, 4GV1, 2AZ5), while apo structures (4X2N, 1L6J) are parameterized via canonical active-site residues.

4. Discussion

4.1. A multi-target systems pharmacology paradigm for MASH

MASH represents a global therapeutic challenge characterized by multi-lineage cellular dysfunction across hepatocytes, resident and infiltrating macrophages, and hepatic stellate cells.^{2,3} The historical failure of single-target monotherapies highlights the biological resilience of MASH pathophysiology and suggests that durable therapeutic efficacy requires coordinated, multi-targeted polypharmacological interventions capable of concurrently intercepting lipotoxicity, resolving inflammation and halting fibrogenesis.^{14,15}

By integrating clinical liver biopsy transcriptomics from GSE135251 with systems pharmacology and

atomistic molecular docking, this study indicates that the traditional Indonesian Jamu triad (*C. zanthorrhiza*, *C. longa* and *Z. officinale*) may operate as an orchestrated, tri-pillar network therapeutic (Table 6; Figure 5). Rather than acting through a single molecular target, each botanical component addresses a distinct pathogenic pillar of MASH, establishing a polypharmacological model for herbal medicine development.^{18,20}

4.2. The metabolic pillar: *Curcuma longa* and PPARG-mediated lipotoxicity reversal

Hepatocellular lipotoxicity represents the primary initiating hit in MASH pathogenesis.^{3,42} When the hepatic influx of saturated free fatty acids overwhelms mitochondrial disposal capacity, toxic intermediates accumulate, provoking mitochondrial uncoupling and oxidative stress.⁵ In the present human biopsy

analysis, this metabolic collapse was marked by downregulation of PPARA ($\log_2FC = -1.68$) and CPT1A ($\log_2FC = -1.85$) (Table 1).

C. longa, mediated by curcumin, offers a route to metabolic restoration. Molecular docking indicated that curcumin binds with high predicted affinity to the ligand-binding domain of PPARG ($\Delta G = -9.4$ kcal/mol), forming hydrogen bonds with Ser289 and His449 (Figure 5b). PPARG activation upregulates peripheral fatty acid storage, enhances adiponectin secretion and reduces lipotoxic flux to the liver.^{10,22} In parallel, curcumin docked into AKT1 ($\Delta G = -8.8$ kcal/mol), a hub governing hepatocyte survival and insulin signalling. By occupying the PPARG ligand-binding pocket and engaging AKT1, *C. longa* bioactives are predicted to counter the transcriptomic metabolic suppression observed in MASH.^{22,43}

4.3. The immunological pillar: *Curcuma zanthorrhiza* and macrophage cascades

The second pathological hit in MASH involves chronic sterile inflammation mediated by resident Kupffer cells and infiltrating monocyte-derived macrophages.^{3,5} In human biopsies, this was evidenced by upregulation of chemotactic signals (CCL2 $\log_2FC = +3.42$, CXCL8 $\log_2FC = +2.84$) and inflammatory cytokines (TNF $\log_2FC = +2.15$) (Table 1). Persistent TNF- α signalling perpetuates inflammatory cell recruitment and drives hepatocyte apoptosis.^{5,15}

C. zanthorrhiza, through its bioactive xanthorrhizol, offers anti-inflammatory intervention. Docking simulations indicated that xanthorrhizol binds into the central hydrophobic cavity of the TNF- α trimer (PDB ID: 2AZ5, $\Delta G = -8.2$ kcal/mol), engaging in π - π stacking with Tyr119 and Tyr59 and forming a hydrogen bond with Ser60 (Figure 5b). Such binding would sterically disrupt the trimer interface, accelerating cytokine dissociation and blunting downstream NF- κ B activation.^{26,27} Xanthorrhizol may therefore quench the inflammatory cascade and suppress downstream chemokine secretion.^{21,27}

4.4. The anti-fibrotic pillar: *Zingiber officinale* and stellate cell activation

Hepatic fibrosis is the single histological parameter that most strongly predicts long-term liver-related mortality and liver transplantation in MASH patients.^{1,7} Fibrogenesis is driven by hepatic stellate cells that transdifferentiate into α -SMA-expressing myofibroblasts under the influence of autocrine and paracrine TGF- β 1 signalling.^{12,13} In the present analysis of GSE135251, hallmark fibrogenic transcripts (COL1A1 $\log_2FC = +3.14$, TIMP1 $\log_2FC = +2.78$, ACTA2

$\log_2FC = +2.45$) exhibited marked induction alongside chemokine upregulation led by CCL2 ($\log_2FC = +3.42$) (Table 1).

Z. officinale bioactives, particularly 6-shogaol, offer a potential anti-fibrotic intervention.^{23,28} 6-Shogaol docked into the catalytic ATP-binding pocket of the human TGFBR1 kinase domain (PDB ID: 4X2N) with $\Delta G = -8.6$ kcal/mol, forming anchoring hydrogen bonds with Lys232 and Glu245 (Figure 5b). Such steric blockade would inhibit receptor kinase activity, thereby limiting the downstream phosphorylation and nuclear translocation of SMAD2 and SMAD3.^{28,38} Consequently, the transcriptional activation of COL1A1 and ACTA2 would be attenuated at the promoter level.^{28,38} Concurrently, 6-shogaol engaged MMP9 (PDB ID: 1L6J, $\Delta G = -8.4$ kcal/mol) via coordination with the active-site Zn²⁺ cofactor.^{37,41}

4.5. Pharmacokinetic coordination and bioavailability considerations

A potential advantage of the traditional Jamu formulation is pharmacokinetic coordination among its constituent metabolites.^{18,22} A long-standing challenge in curcumin pharmacology has been its poor aqueous solubility and rapid intestinal and hepatic glucuronidation, which limits systemic bioavailability.^{22,44} Co-administration with *Z. officinale* extracts has been shown to enhance curcumin absorption: pungent gingerols inhibit intestinal P-glycoprotein efflux pumps and downregulate hepatic UDP-glucuronosyltransferase activity.^{23,44} Furthermore, the lipophilic sesquiterpene matrix of *C. zanthorrhiza* may act as a natural bio-enhancer, facilitating self-emulsification of curcuminoids within the gastrointestinal lumen.^{21,44} This co-solvent effect could optimize therapeutic plasma concentrations without requiring synthetic additives.^{18,44}

4.6. Translational roadmap for standardized phytopharmaceutical development

To translate these computational systems biology insights into standardized clinical phytopharmacology (*Fitofarmaka*), a structured four-stage translational roadmap is proposed (Table 6; Figure 5).^{20,44} First, bio-guided extract standardization: raw rhizomes should be cultivated under standardized Good Agricultural Practices (GAP) protocols in Indonesia, with extracts quantified by high-performance liquid chromatography with diode-array detection (HPLC-DAD) to guarantee minimum marker thresholds of $\geq 20\%$ curcuminoids in *C. longa*, $\geq 15\%$ xanthorrhizol in *C. zanthorrhiza* and $\geq 10\%$ gingerols and shogaols in *Z. officinale*.^{20,22} Second, advanced nano-formulation delivery: the tri-extract formula should be engineered

using self-nanoemulsifying drug delivery systems (SNEDDS) or phytosome–phospholipid complexes, achieving sub-100 nm droplet sizes that protect bioactives from first-pass degradation.⁴⁴ Third, multi-cellular *in vitro* co-culture validation: preclinical testing should deploy microfluidic liver-on-a-chip or human co-culture platforms comprising hepatocytes (HepG2), THP-1-derived macrophages as a Kupffer-cell surrogate, and hepatic stellate cells (LX-2)

challenged with palmitate and TGF-β1 to verify simultaneous anti-steatotic, anti-inflammatory and anti-fibrotic endpoints.^{13,41} Fourth, preclinical *in vivo* proof-of-concept: the standardized formula should be evaluated in dietary choline-deficient, high-fat, amino acid-defined (CDAHFD) rodent models, measuring the NAFLD Activity Score (NAS), quantitative collagen morphometry via Sirius Red staining and hepatic hydroxyproline levels.^{5,41}

Table 6. Tri-pillar polypharmacological architecture and translational *Fitofarmaka* roadmap for Zingiberaceae Jamu in MASH.

Mechanistic pillar	Primary botanical driver	Lead phytochemical bioactive	Primary macromolecular targets	Modulated human transcriptomic cascade	Translational <i>Fitofarmaka</i> development stage
Pillar 1: metabolic restoration	<i>C. longa</i> (kunyit)	Curcumin	PPARG and AKT1	Counters PPARA and CPT1A suppression; addresses lipotoxicity	Standardized SNEDDS nanoemulsion formulation
Pillar 2: immune resolution	<i>C. zanthorrhiza</i> (temulawak)	Xanthorrhizol	TNF-α and RELA (p65)	Predicted suppression of CCL2 and CXCL8; macrophage influx	HPLC-standardized extract formulation
Pillar 3: anti-fibrotic arrest	<i>Z. officinale</i> (jahe)	6-Shogaol	TGFBR1 and MMP9	Predicted inhibition of COL1A1 and ACTA2; stellate activation	In vitro co-culture validation (HepG2, THP-1, LX-2 cells)

Proposed translational roadmap adheres to Indonesian National Agency of Drug and Food Control (BPOM) standards for *Fitofarmaka* development, spanning raw simplicia standardization to multi-centre clinical trials.

4.7. Study limitations and future perspectives

While this study leverages high-dimensional human biopsy transcriptomics and multi-target computational modelling, certain methodological boundaries should be acknowledged. First, while network topology and molecular docking describe multi-target complementarity and provide a mechanistic hypothesis for polyherbal coordination, empirical synergy (such as a Chou–Talalay combination index < 1.0 or Loewe additivity) requires formal concentration-dependent combination bioassays in co-culture systems. Second, molecular docking simulations evaluate static protein–ligand conformations; future investigations should deploy extended all-atom molecular dynamics simulations (for example, 200 ns trajectories) and molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) binding free energy calculations to evaluate conformational entropy and thermodynamic stability.^{34,45} Third, computational network models do not fully capture non-linear metabolic feedback loops or gut microbiome biotransformations of dietary polyphenols.^{16,42} Prospective clinical trials in Indonesian MASH cohorts will be essential to evaluate the *in vivo* efficacy and safety profile of this standardized Jamu formula.^{20,44}

5. Conclusion

This study reports an integrated human biopsy RNA-Seq transcriptomic, network pharmacology and

atomistic molecular docking analysis of the multi-target complementary mechanisms that may underlie the traditional Indonesian Zingiberaceae Jamu formula (*C. zanthorrhiza*, *C. longa* and *Z. officinale*) in MASH pathophysiology.

Through re-analysis of 216 human liver biopsies (GSE135251: 10 healthy controls, 51 NAFL and 155 MASH), transcriptomic upregulation of fibrogenic (COL1A1, TIMP1, ACTA2) and inflammatory (CCL2, TNF) cascades was confirmed alongside suppression of fatty acid β-oxidation (PPARA, CPT1A). Systems pharmacology and network centrality isolated six core hub targets — PPARG, TNF, AKT1, TGFBR1, RELA and MMP9 — that govern the disease interactome, three of which exceeded a degree of 75. Molecular docking predicted spontaneous binding, indicating that curcumin is predicted to occupy the PPARG ligand-binding pocket (ΔG = −9.4 kcal/mol); xanthorrhizol is predicted to engage the TNF-α trimer interface (ΔG = −8.2 kcal/mol); and 6-shogaol is predicted to bind the human TGFBR1 kinase domain (PDB ID: 4X2N, ΔG = −8.6 kcal/mol) and the MMP9 catalytic domain (PDB ID: 1L6J, ΔG = −8.4 kcal/mol). Protocol accuracy was confirmed via re-docking for holo-structures, while catalytic pocket parameters were characterized for apo targets.

This tri-pillar polypharmacological complementarity addresses the multiple-hit aetiology of MASH simultaneously, in contrast to the inherent limitations of single-target synthetic drugs. While formal

pharmacological synergy requires future concentration-dependent combination testing, these findings provide a rational molecular foundation for developing standardized nano-formulated phytopharmaceuticals (*Fitofarmaka*) from Indonesian biodiversity against progressive steatohepatitis.

Declarations

Use of Generative Artificial Intelligence. None declared.

Author Contributions. K.J. conceived and designed the study, assembled the phytochemical library, executed the transcriptomic, network pharmacology and molecular docking pipelines, and drafted the manuscript. I.S. contributed to the pharmacokinetic and druggability screening, the interpretation of the docking results, and the critical revision of the manuscript. S.V. contributed to the statistical and topological analyses, the preparation of the figures and tables, and the critical revision of the manuscript. All authors have reviewed and approved the final version of the manuscript.

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Data Availability. All datasets analysed during the current study are publicly available in the NCBI GEO repository under accession GSE135251 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135251>). Chemical structures are accessible via PubChem, KnapSack and COCONUT. Target crystal structures are deposited in the RCSB Protein Data Bank (PDB IDs: 5Y2T, 4GV1, 4X2N, 1L6J, 2AZ5). The differential expression output, the list of 318 predicted targets, the 126 shared targets, the STRING/Cytoscape

session and the docking poses are deposited under accession/DOI [to be supplied]; computational scripts and network interaction tables are available from the corresponding author on reasonable request.

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References

1. Rinella ME, Lazarus JV, Ratziu V, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature: MASLD and MASH. *J Hepatol.* 2023;79(6):1542–1556. doi:10.1016/j.jhep.2023.06.003
2. Powell EE, Wong VW, Rinella M. Non-alcoholic fatty liver disease. *Lancet.* 2021;397(10290):2212–2224. doi:10.1016/S0140-6736(20)32511-3
3. Loomba R, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell.* 2021;184(10):2537–2564. doi:10.1016/j.cell.2021.04.015
4. Younossi ZM, Golabi P, Paik JM, et al. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology.* 2023;77(4):1335–1347. doi:10.1097/HEP.0000000000000004
5. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, et al. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology.* 2023;77(5):1797–1835. doi:10.1097/HEP.00000000000000323
6. Sanyal AJ, Brunt EM, Kleiner DE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology.* 2011;54(1):344–353. doi:10.1002/hep.24376
7. Anstee QM, Castera L, Loomba R. Impact of non-invasive biomarkers on hepatology practice: Past, present and future. *J Hepatol.* 2022;76(6):1362–1378. doi:10.1016/j.jhep.2022.03.026
8. Harrison SA, Bedossa P, Guy CD, et al. A phase 3, randomized, controlled trial of resmetirom in nonalcoholic steatohepatitis with liver fibrosis. *N Engl J Med.* 2024;390(6):497–509. doi:10.1056/NEJMoa2309000
9. Newsome PN, Buchholtz K, Cusi K, et al. A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. *N Engl J Med.* 2021;384(12):1113–1124. doi:10.1056/NEJMoa2028395
10. Francque SM, Bedossa P, Ratziu V, et al. A randomized, controlled trial of the pan-PPAR agonist lanifibranor in NASH. *N Engl J Med.* 2021;385(17):1547–1558. doi:10.1056/NEJMoa2036205
11. Ratziu V, Harrison SA, Francque S, et al. Elafibranor, an agonist of the peroxisome proliferator-activated receptor- α and - δ , induces resolution of nonalcoholic

- steatohepatitis without fibrosis worsening. *Gastroenterology*. 2016;150(5):1147–1159.e5. doi:10.1053/j.gastro.2016.01.038
12. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol*. 2017;14(7):397–411. doi:10.1038/nrgastro.2017.38
 13. Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. *Nat Rev Gastroenterol Hepatol*. 2021;18(3):151–166. doi:10.1038/s41575-020-00372-7
 14. Trautwein C, Friedman SL, Schuppan D, et al. Hepatic fibrosis: Concept to treatment. *J Hepatol*. 2015;62(1 Suppl):S15–S24. doi:10.1016/j.jhep.2015.02.039
 15. Tilg H, Adolph TE, Moschen AR. Multiple parallel hits hypothesis in nonalcoholic fatty liver disease: revisited after a decade. *Hepatology*. 2021;73(2):833–842. doi:10.1002/hep.31518
 16. Kibble M, Saarinen N, Tang J, et al. Network pharmacology applications to map the unexplored target space and therapeutic potential of natural products. *Nat Prod Rep*. 2015;32(8):1249–1266. doi:10.1039/c5np00005j
 17. Wang X, Shen Y, Wang S, et al. PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Res*. 2017;45(W1):W356–W360. doi:10.1093/nar/gkx374
 18. Itokawa H, Shi Q, Akiyama T, et al. Recent advances in the investigation of curcuminoids. *Chin Med*. 2008;3:11. doi:10.1186/1749-8546-3-11
 19. Sorokina M, Merseburger P, Rajan K, et al. COCONUT online: Collection of Open Natural Products database. *J Cheminform*. 2021;13(1):2. doi:10.1186/s13321-020-00478-9
 20. Elfahmi, Woerdenbag HJ, Kayser O. Jamu: Indonesian traditional herbal medicine towards rational phytotherapy. *Phytochem Rev*. 2014;13(1):51–73. doi:10.1007/s11101-014-9355-0
 21. Lim CS, Jin DQ, Mok H, et al. Antioxidant and antiinflammatory activities of xanthorrhizol in hippocampal neurons and primary cultured microglia. *J Neurosci Res*. 2005;82(6):831–838. doi:10.1002/jnr.20692
 22. Naseri K, Saadati S, Yari Z, et al. Curcumin offers no additional benefit to lifestyle intervention on cardiometabolic status in patients with non-alcoholic fatty liver disease: A randomized controlled trial. *Nutrients*. 2022;14(15):3224. doi:10.3390/nu14153224
 23. Rahimlou M, Yari Z, Hekmatdoost A, et al. Ginger supplementation in nonalcoholic fatty liver disease: A randomized, double-blind, placebo-controlled pilot study. *Hepat Mon*. 2016;16(1):e34897. doi:10.5812/hepatmon.34897
 24. Li X, Chen W, Ren J, et al. Effects of curcumin on non-alcoholic fatty liver disease: A scientific metrology study. *Phytomedicine*. 2024;123:155241. doi:10.1016/j.phymed.2023.155241
 25. Huang BH, Guo ZW, Lv BH, et al. A role for curcumin in preventing liver fibrosis in animals: a systematic review and meta-analysis. *Front Pharmacol*. 2024;15:1396834. doi:10.3389/fphar.2024.1396834
 26. Chung WY, Park JH, Kim MJ, et al. Xanthorrhizol inhibits 12-O-tetradecanoylphorbol-13-acetate-induced acute inflammation and two-stage mouse skin carcinogenesis by blocking the expression of ornithine decarboxylase, cyclooxygenase-2 and inducible nitric oxide synthase through mitogen-activated protein kinases and/or the nuclear factor- κ B. *Carcinogenesis*. 2007;28(6):1224–1231. doi:10.1093/carcin/bgm005
 27. Sun W, Liu M, Li Y, et al. Xanthorrhizol inhibits mitochondrial damage, oxidative stress and inflammation in LPS-induced MLE-12 cells by regulating MAPK pathway. *Tissue Cell*. 2023;84:102170. doi:10.1016/j.tice.2023.102170
 28. Yang AY, Kim K, Kwon HH, et al. 6-Shogaol ameliorates liver inflammation and fibrosis in mice on a methionine- and choline-deficient diet by inhibiting oxidative stress, cell death, and endoplasmic reticulum stress. *Molecules*. 2024;29(2):419. doi:10.3390/molecules29020419
 29. Gunawan S, Soetikno V, Purwaningsih E, et al. 6-Gingerol, a bioactive compound of *Zingiber officinale**, ameliorates high-fat high-fructose diet-induced non-alcoholic related fatty liver disease in rats. *J Exp Pharmacol*. 2024;16:455–466. doi:10.2147/JEP.S492971
 30. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017;7:42717. doi:10.1038/srep42717
 31. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2023;51(D1):D638–D646. doi:10.1093/nar/gkac1000
 32. Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Camb)*. 2021;2(3):100141. doi:10.1016/j.xinn.2021.100141
 33. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010;31(2):455–461. doi:10.1002/jcc.21334
 34. Eberhardt J, Santos-Martins D, Tillack AF, et al. AutoDock Vina 1.2.0: New docking methods, expanded force field, and Python bindings. *J Chem Inf Model*. 2021;61(8):3891–3898. doi:10.1021/acs.jcim.1c00203
 35. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. *Nucleic Acids Res*. 2000;28(1):235–242. doi:10.1093/nar/28.1.235
 36. Tang D, Chen M, Huang X, et al. SRplot: A free online platform for data visualization and graphing. *PLoS One*. 2023;18(11):e0294236. doi:10.1371/journal.pone.0294236
 37. Tzeng TF, Liou SS, Chang CJ, et al. [6]-Gingerol dampens hepatic steatosis and inflammation in experimental nonalcoholic steatohepatitis. *Phytomedicine*. 2015;22(4):452–461. doi:10.1016/j.phymed.2015.01.015
 38. Zhou Q, Peng Y, Chen F, et al. Ginger supplementation for the treatment of non-alcoholic fatty liver disease: a

- meta-analysis of randomized controlled trials. *Afr Health Sci.* 2023;23(1):614–621. doi:10.4314/ahs.v23i1.65
39. Targher G, Corey KE, Byrne CD. NAFLD, and cardiovascular and cardiac diseases: Factors influencing risk, prediction and treatment. *Diabetes Metab.* 2021;47(2):101215. doi:10.1016/j.diabet.2020.101215
40. Jiao W, Jiao Y, Sang Y, et al. 6-Shogaol alleviates high-fat diet induced hepatic steatosis through miR-3066–5p/Grem2 pathway. *Food Chem.* 2024;457:140197. doi:10.1016/j.foodchem.2024.140197
41. Tanwar S, Rhodes F, Srivastava A, et al. Inflammation and fibrosis in chronic liver diseases including non-alcoholic fatty liver disease and hepatitis C. *World J Gastroenterol.* 2020;26(2):109–133. doi:10.3748/wjg.v26.i2.109
42. Farzaei MH, Zobeiri M, Parvizi F, et al. Curcumin in liver diseases: A systematic review of the cellular mechanisms of oxidative stress and clinical perspective. *Nutrients.* 2018;10(7):855. doi:10.3390/nu10070855
43. Li Y, Deng X, Tan X, et al. Protective role of curcumin in disease progression from non-alcoholic fatty liver disease to hepatocellular carcinoma: a meta-analysis. *Front Pharmacol.* 2024;15:1343193. doi:10.3389/fphar.2024.1343193
44. Pourmasoumi M, Hadi A, Rafie N, et al. The effect of ginger supplementation on lipid profile: A systematic review and meta-analysis of clinical trials. *Phytomedicine.* 2018;43:28–36. doi:10.1016/j.phymed.2018.03.043
45. Yuandani, Jantan I, Rohani AS, et al. Immunomodulatory effects and mechanisms of *Curcuma* species and their bioactive compounds: A review. *Front Pharmacol.* 2021;12:643119. doi:10.3389/fphar.2021.643119