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Emerging opportunities in network pharmacology mechanisms of *Saussurea costus* in regulating inflammation and intestinal barrier dysfunction in non-alcoholic fatty liver disease

Oportunidades emergentes en farmacología de redes: Mecanismos de *Saussurea costus* en la regulación de la inflamación y la disfunción de la barrera intestinal en la enfermedad del hígado graso no alcohólico

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SUMMARY

Background: *Non-alcoholic fatty liver disease (NAFLD) is a heterogeneous metabolic liver disorder involving excessive lipid deposition, redox imbalance, inflammatory activation, and disruption of gut-liver axis homeostasis. Loss of epithelial junction integrity and increased intestinal permeability are increasingly considered important contributors to disease progression. Saussurea costus contains several phytochemicals with reported antioxidant and anti-inflammatory potential, but its molecular relevance to NAFLD and intestinal barrier regulation remains incompletely defined.* **Objective**: *This study aimed to predict the molecular targets and biological pathways through which Saussurea costus may influence NAFLD, with particular emphasis on inflammation and intestinal junction-associated mechanisms.* **Methods**: *Phytochemicals reported from Saussurea costus were compiled from the literature and screened for drug-likeness using ADMETlab. Chemical information, including canonical SMILES and three-dimensional structures, was obtained from PubChem. Putative compound targets and NAFLD-related targets were retrieved from public resources and integrated to identify shared targets. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were then conducted, followed by network visualization to examine relationships among overlapping proteins, inflammatory pathways, and intestinal barrier-related processes.* **Results**: *The analysis identified 217 predicted Saussurea costus targets and 827 NAFLD-associated targets, producing 49 shared targets. Drug-likeness assessment indicated that 22 of 23 selected compounds met Lipinski's rule of five, with rutin excluded. Enrichment analysis linked the shared targets to inflammatory response, oxidative stress response, NF-kappaB signaling, protein kinase activity, extracellular exosome-related components, lipid and atherosclerosis pathway, MAPK signaling, TNF signaling, and adherens junction. Network analysis highlighted several biologically relevant nodes, including RELA, NFKB1, TNFRSF1A, PTGS2, JUN, NFE2L2, EGFR, MMP2, MMP9, SMAD3, and VEGFA, which may connect Saussurea costus with inflammatory regulation, redox control, extracellular matrix remodeling, and epithelial barrier-related pathways.* **Conclusion**: *These findings suggest that Saussurea*

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costus may affect NAFLD-related pathobiology through coordinated regulation of inflammatory, oxidative, lipid-associated, and junction-related pathways. The data support a plausible indirect role in preserving intestinal barrier function through upstream modulation rather than direct targeting of classical tight-junction proteins. Further compound-target network construction, molecular docking, and experimental validation are required to verify the predicted mechanisms.

Keywords: *Saussurea costus, NAFLD, network pharmacology, tight junction, intestinal barrier, inflammation*

RESUMEN

Antecedentes: *La enfermedad del hígado graso no alcohólico (EHGNA) es un trastorno metabólico hepático heterogéneo que implica una acumulación excesiva de lípidos, desequilibrio redox, activación de la respuesta inflamatoria y alteración de la homeostasis del eje intestino-hígado. La pérdida de la integridad de las uniones epiteliales y el aumento de la permeabilidad intestinal se consideran cada vez más factores importantes en la progresión de la enfermedad. Saussurea costus contiene varios fitoquímicos con potencial antioxidante y antiinflamatorio, pero su relevancia molecular para la EHGNA y la regulación de la barrera intestinal aún no se ha definido por completo.* **Objetivo**: *Este estudio tuvo como objetivo predecir las dianas moleculares y las vías biológicas a través de las cuales Saussurea costus podría influir en la EHGNA, con especial énfasis en la inflamación y en los mecanismos asociados a las uniones intestinales.* **Métodos**: *Se recopilaron los compuestos fitoquímicos de Saussurea costus de la literatura y se evaluó su potencial farmacológico mediante ADMETlab. La información química, incluidas las estructuras SMILES canónicas y tridimensionales, se obtuvo de PubChem. Se recuperaron posibles dianas de compuestos y dianas relacionadas con la EHGNA de recursos públicos y se integraron para identificar dianas comunes. Posteriormente, se realizaron análisis de enriquecimiento de Gene Ontology y de Kyoto Encyclopedia of Genes and Genomes, seguidos de una visualización de la red para examinar las relaciones entre proteínas superpuestas, vías inflamatorias y procesos relacionados con la barrera intestinal.* **Resultados**: *El análisis identificó 217 dianas predichas de Saussurea costus y 827 dianas asociadas a la EHGNA, lo que dio como resultado 49 dianas compartidas. La evaluación de la similitud con fármacos indicó que 22 de los 23 compuestos seleccionados cumplían la regla de los cinco de Lipinski, mientras que la rutina fue excluida. El análisis de enriquecimiento vinculó las dianas compartidas con la respuesta inflamatoria, la respuesta al estrés oxidativo, la señalización de NF-κB, la actividad de la proteína quinasa, los componentes relacionados con los exosomas extracelulares, la vía de los lípidos y la aterosclerosis, la señalización de MAPK, la señalización de TNF y las uniones adherentes. El análisis de redes destacó varios nodos biológicamente relevantes, incluidos RELA, NFKB1, TNFRSF1A, PTGS2, JUN, NFE2L2, EGFR, MMP2, MMP9, SMAD3 y VEGFA, que podrían conectar Saussurea costus con la regulación inflamatoria, el control redox, la remodelación de la matriz extracelular y las vías relacionadas con la barrera epitelial.* **Conclusión**: *Estos hallazgos sugieren que Saussurea costus podría afectar la patobiología de la EHGNA mediante la regulación coordinada de vías inflamatorias, oxidativas, asociadas a lípidos y relacionadas con las uniones intercelulares. Los datos respaldan un posible papel indirecto en la preservación de la función de la barrera intestinal mediante la modulación ascendente, en lugar de una acción directa sobre las proteínas clásicas de las uniones estrechas. Se requieren la construcción de redes adicionales de compuestos y dianas, el acoplamiento molecular y la validación experimental para verificar los mecanismos predichos.*

Palabras clave: *Saussurea costus, EHGNA, farmacología de redes, unión estrecha, barrera intestinal, inflamación*

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), now frequently discussed alongside the newer term metabolic dysfunction-associated steatotic liver disease (MASLD), represents one of the most prevalent chronic liver conditions and is strongly linked with obesity, insulin resistance, type 2 diabetes mellitus, and broader cardiometabolic dysfunction (1,2). The recent nomenclature update defines MASLD as hepatic steatosis accompanied by at least one cardiometabolic risk factor, while retaining steatohepatitis as a clinically meaningful inflammatory phenotype (1). Despite this updated nomenclature, the term NAFLD remains widely used in prior experimental and computational studies; therefore, it remains relevant for interpreting existing evidence and database-derived targets (3,4).

NAFLD develops through the interaction of metabolic and inflammatory disturbances rather than through a single pathogenic event. Hepatic lipid accumulation, mitochondrial dysfunction, oxidative

injury, insulin resistance, inflammatory signaling, and progressive hepatocyte damage collectively contribute to the transition from simple steatosis to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (5,6). Signaling pathways such as nuclear factor kappa B (NF-kappaB), tumor necrosis factor (TNF), and mitogen-activated protein kinase (MAPK) pathways promote cytokine expression, immune cell recruitment, and liver injury. At the same time, impaired antioxidant defense, including dysregulation of nuclear factor erythroid 2-related factor 2 (NFE2L2/NRF2), can intensify reactive oxygen species-mediated hepatocellular damage (6,7).

Current evidence also indicates that NAFLD should be viewed not only as a liver-centered disorder, but as a systemic metabolic disease influenced by bidirectional communication between the intestine and the liver. Within this gut-liver axis, intestinal dysbiosis, enhanced permeability, impaired epithelial barrier function, and portal delivery of microbial products such as lipopolysaccharide can activate innate immune responses and amplify hepatic inflammation (8,9). Disruption of epithelial junctional complexes, including tight and adherens junctions, has been implicated in the development of gut barrier dysfunction, linking intestinal permeability to hepatic inflammatory responses in NAFLD (10,11). These findings indicate the potential of treatment approaches to modulate NAFLD development by controlling inflammation, oxidative stress, and intestinal barrier dysfunction (12).

Natural products remain a crucial source of bioactive molecules for diseases driven by complex and overlapping biological pathways. *Saussurea costus* (Falc.) Lipsch, a traditional medicinal plant widely used across several medical systems, contains a diverse array of chemical constituents, including sesquiterpene lactones, flavonoids, phenolic acids, and other secondary metabolites (13). Previous studies and reviews have documented its antiinflammatory, antioxidant, antimicrobial, anticancer, and hepatoprotective properties (14,15). Compounds such as costunolide, dehydrocostus lactone, naringenin, kaempferol, caffeic acid, and ferulic acid are particularly noteworthy because they participate in molecular pathways central to inflammation and redox homeostasis (16,17).

Despite these pharmacological indications, the molecular basis through which *S. costus* may influence NAFLD—particularly mechanisms related to inflammatory signaling and intestinal barrier–associated pathways—remains insufficiently clarified. Herbal medicines typically exert their biological effects through synergistic combinations of compounds acting on multiple targets and interconnected pathways, rather than through isolated ligand–receptor interactions. Network pharmacology is therefore an appropriate approach for studying medicinal plants, as it integrates phytochemical profiles, target prediction, disease-associated genes, protein–protein interaction networks, and functional enrichment analyses (18,19). Accordingly, this study is well suited to exploring the potential molecular mechanisms of *S. costus* in NAFLD, with particular emphasis on inflammation and intestinal barrier dysfunction.

MATERIALS AND METHODS

Study design

This study was designed as an *in silico* network pharmacology analysis to explore the potential molecular mechanisms of *Saussurea costus* in nonalcoholic fatty liver disease, with particular emphasis on inflammatory responses and tight-junction-related mechanisms. The workflow consisted of literature-based phytochemical identification, retrieval of molecular data, drug-likeness and ADMET screening, target prediction of *S. costus* phytochemicals, collection of NAFLD-associated targets, identification of overlapping targets, functional enrichment analysis, protein–protein interaction analysis, and network visualization (20).

Collection of *Saussurea costus* phytochemicals

Candidate phytochemicals from *Saussurea costus* were identified from the published literature. Because the phytochemical profile of the ethanolic root extract used in the subsequent *in vivo* study has not yet been experimentally

characterized, the compounds included in this analysis were considered literature-derived candidate phytochemicals of *S. costus* (21,22).

Retrieval of molecular data from PubChem

The PubChem compound identifier, canonical SMILES, and three-dimensional molecular structures of each selected compound were retrieved from PubChem. PubChem was used as the primary source of molecular information because it provides chemical structures, identifiers, bioactivity data, and chemical–gene/protein/disease-related information on small molecules (23). Canonical SMILES obtained from PubChem were used as input to the SEA Target server for subsequent target prediction.

Drug-likeness and ADMET screening

The drug-likeness and ADMET-related properties of the selected compounds were evaluated using ADMETlab. ADMETlab is a web-based platform that is used to predict physicochemical, pharmacokinetic, toxicity, and medicinal chemistry-related properties of chemical compounds (24).

The evaluated drug-likeness parameters included molecular weight, LogP, hydrogen-bond acceptors, hydrogen-bond donors, and Lipinski's rule of five. Based on Lipinski's rule of five, compounds are generally considered to have less favorable oral absorption or permeability if they violate the following criteria: molecular weight $>$ 500 Da, $>$ 5 hydrogen-bond donors, $>$ 10 hydrogen-bond acceptors, and LogP $>$ 5. Compounds were classified as accepted or rejected according to these criteria (20).

Prediction of *Saussurea costus*-related targets using SEA Target

Putative protein targets of *S. costus* phytochemicals were predicted using the Similarity Ensemble Approach Target server. SEA predicts potential protein targets of small molecules based on chemical similarity between query compounds and known ligands associated with protein targets. The SEA approach has been widely applied in target prediction and drug repurposing studies based on ligand set similarity (25).

The canonical SMILES of each selected compound was submitted to the SEA Target server. The predicted targets were filtered using a P-value $<$ 0.01 and a maximum Tanimoto coefficient $>$ 0.4. The Tanimoto coefficient ranges from 0 to 1, with higher values indicating greater chemical similarity between the query compound and known ligands of the predicted target. A cut-off value of $>$ 0.4 was applied to retain target predictions with stronger similarity-based evidence (26). The predicted targets were standardized to official gene symbols, duplicate entries were removed, and only human-related targets were retained for further analysis.

Collection of NAFLD-associated targets

NAFLD-associated targets were collected from three public disease-target databases: DisGeNET, Open Targets Platform, and GeneCards. The search was performed using the keywords "non-alcoholic fatty liver disease" and "NAFLD."

DisGeNET was used because it integrates gene–disease and variant–disease associations from curated repositories, GWAS catalogs, animal models, and the scientific literature. The Open Targets Platform was used because it integrates target–disease evidence from multiple sources, including genetic associations, known drugs, differential expression, animal models, pathways, and systems biology evidence. GeneCards was used as an integrative human gene database that provides gene-centric information related to genomic, proteomic, functional, clinical, and disease-associated data (27).

All retrieved NAFLD-associated targets were standardized to official gene symbols, and duplicate entries were removed prior to further analysis.

Identification of overlapping targets

The intersection between *S. costus*-related and NAFLD-associated targets was identified using Venny. Venny is an interactive online tool for comparing lists using Venn diagrams. The overlapping targets were used for subsequent functional enrichment analysis, protein–protein interaction analysis, and network visualization.

Functional enrichment analysis

Functional enrichment analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). DAVID is a bioinformatics resource used for functional annotation and enrichment analysis of gene lists (28).

Gene Ontology Biological Process and Kyoto Encyclopedia of Genes and Genomes pathway analyses were conducted to identify the biological processes and signaling pathways associated with the overlapping targets. Enrichment results with P values < 0.05 were considered statistically significant.

Protein–protein interaction analysis

Protein–protein interaction analysis of the overlapping targets was performed using the STRING database (version 12.0). STRING provides protein–protein association networks and functional enrichment analyses for organisms of interest (29).

The organism was restricted to Homo sapiens, and the minimum required interaction score was set to a high-confidence threshold of 0.9. The resulting interaction data were exported in the TSV format for subsequent network analyses.

Network visualization using Cytoscape

The TSV file obtained from STRING was imported into Cytoscape version 3.10 for network visualization and further analyses. Cytoscape is an open-source software platform for visualizing biological networks and integrating them with attribute data (29).

The network was analyzed to explore the relationship between overlapping targets and biological mechanisms relevant to NAFLD, including inflammatory response, oxidative stress, NF-κB-related signaling, TNF signaling, MAPK signaling, extracellular matrix remodeling, and tight-junction-related mechanisms.

Scope of mechanistic interpretation

This study focused on target- and pathway-level network pharmacology analyses. Although target prediction was performed for individual *S. costus* phytochemicals using the SEA Target server, a direct compound–target network was not constructed in the present analysis. Therefore, the contribution of individual phytochemicals to specific protein targets was not determined at this stage. This limitation was considered in interpreting the findings, and further compound–target network analysis and molecular docking are required to identify the key phytochemicals responsible for modulating NAFLD- and TJ-related mechanisms.

RESULTS

This study applied a network pharmacology workflow to predict how *S. costus* may regulate biological processes relevant to NAFLD-associated inflammation and intestinal barrier impairment. Candidate compounds were first assessed for drug-likeness, followed by target prediction and integration with NAFLD-related disease targets. The analysis included 23 candidate phytochemicals, 217 predicted *S. costus*-related targets, and 827 NAFLD-associated targets, yielding 49 overlapping targets. These shared targets were then subjected to functional enrichment and protein-protein interaction analyses to identify biological processes, signaling pathways, and key

molecular nodes potentially involved in *S. costus* activity.

Drug-likeness profile of *Saussurea costus* candidate phytochemicals

A total of 23 literature-derived candidate phytochemicals of *Saussurea costus* were evaluated for drug-likeness properties using ADMETlab, based on Lipinski's rule of five. The analyzed compounds included coumarin, phenol, quinone, costunolide, dehydrocostunolide lactone, betulinic acid, reynosin, santamarine, gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, syringic acid, rutin, ellagic acid, p-coumaric acid, vanillin, ferulic acid, naringenin, taxifolin, cinnamic acid, and kaempferol (Table 1).

Of the 23 candidate phytochemicals, 22 compounds fulfilled Lipinski's rule of five and were classified as acceptable, whereas rutin was classified as unacceptable. Most of the acceptable compounds had molecular weights below 500 Da, acceptable logP values, and hydrogen bond donor and acceptor counts within the recommended

Table 1. Drug-likeness properties of candidate phytochemicals from *Saussurea costus* based on Lipinski's rule of five

Compound Name	CID	LogP	MW	nHA	nHD	Lipinski
coumarin	323	1.672	146.04	2	0	Accepted
phenol	996	1.447	94.04	1	1	Accepted
quinone	4650	0.281	108.02	2	0	Accepted
costunolide	5281437	3.436	232.15	2	0	Accepted
dehydrocostunolide lactone	73174	2.759	230.13	2	0	Accepted
betulinic acid	64971	**5.867**	456.36	3	2	Accepted
reynosin	482788	2.16	248.14	3	1	Accepted
santamarine	188297	2.66	248.14	3	1	Accepted
gallic acid	370	0.645	170.02	5	4	Accepted
chlorogenic acid	1794427	0.331	354.1	9	**6**	Accepted
catechin	9064	1.343	290.08	6	5	Accepted
methyl gallate	7428	1.033	184.04	5	3	Accepted
caffeic acid	689043	1.43	180.04	4	3	Accepted
syringic acid	10742	1.212	198.05	5	2	Accepted
rutin	5280805	-0.038	**610.15**	**16**	**10**	**Rejected**
ellagic acid	5281855	1.117	302.01	8	4	Accepted
p-coumaric acid	637542	1.923	164.05	3	2	Accepted
vanillin	1183	1.096	152.05	3	1	Accepted
ferulic acid	445858	1.803	194.06	4	2	Accepted
naringenin	439246	2.596	272.07	5	3	Accepted
taxifolin	439533	0.449	304.06	7	5	Accepted
cinnamic acid	444539	1.97	148.05	2	1	Accepted
kaempferol	5280863	2.656	286.05	6	4	Accepted

Table 1. Drug-likeness prediction of 23 literature-derived candidate phytochemicals from *Saussurea costus* was performed using ADMETlab according to Lipinski's rule of five. The table presents the PubChem CID, LogP, molecular weight, number of hydrogen-bond acceptors, number of hydrogen-bond donors, and Lipinski classification. Compounds classified as "Accepted" were considered to have favorable physicochemical properties for subsequent target prediction, whereas rutin was classified as "Rejected" due to its high molecular weight and excessive hydrogen bond donor and acceptor counts

range. Rutin was rejected due to its relatively high molecular weight and a high number of hydrogen-bond donors and acceptors, which may reduce its predicted oral drug-likeness profile. ADMETlab is widely used as an online platform for the computational prediction of pharmacokinetic and drug-likeness properties, including physicochemical and ADMET-related parameters (20).

Several compounds, including costunolide, dehydrocostunolide lactone, reynosin, santamarine, naringenin, kaempferol, ferulic acid, caffeic acid, and p-coumaric acid, exhibited favorable physicochemical profiles and were therefore considered suitable for subsequent target prediction and network pharmacology analysis. These findings suggest that most of the selected *S. costus* phytochemicals exhibit properties suitable for further computational exploration as bioactive candidates.

Figure 1. Venn diagram of overlapping targets between *Saussurea costus* and NAFLD

Venn diagram showing the intersection between putative *Saussurea costus*-related targets and NAFLD-associated targets. A total of 217 putative targets were identified for *S. costus*, while 827 NAFLD-associated targets were retrieved from disease-related databases. The intersection yielded 49 overlapping targets, which were considered potential molecular targets through which *S. costus* may modulate NAFLD-related pathological mechanisms

Identification of *S. costus*-related and NAFLD-associated targets

Target prediction of candidate *S. costus* phytochemicals was performed using the SEA Target server. After filtering, standardization, and duplicate removal, 217 putative *S. costus*-related targets were identified in the present study. NAFLD-associated targets were collected from DisGeNET, Open Targets, and GeneCards using the search terms "non-alcoholic fatty liver disease" and "NAFLD." Following standardization and deduplication, 827 disease-related targets were obtained (Figure 1).

Intersection analysis was performed to determine the shared targets between the *S. costus* target set and the NAFLD target set. This analysis yielded 49 overlapping targets, which were considered potential molecular targets through which *S. costus* may modulate NAFLD-related mechanisms. The overlapping targets included APP, ABCG2, AHR, ALDH2, GFER, AR, HSPD1, CYP1A1, EP300, EGFR, ERN1, ESR1, CES1, SLC2A1, HDAC1, HDAC8, HSPA1A, HSP90AA1, JUN, CAMKK2, ALOX12, LOXL2, MAP3K8, ABCB1, MET, MIF, MMP1, MMP2, MMP9, NFE2L2, NFKB1, SERPINE1, P4HB, PTGS2, ALPL, PTPN11, PTPN1, SIK2, SMAD3, STAT1, TAOK1, RELA, F3, TLR9, TNFRSF1A, TTR, VEGFA, and XDH.

These overlapping targets suggest that the projected biological activity of *S. costus* in NAFLD is likely mediated by multiple molecular mechanisms rather than a single mechanism. This is consistent with the principles of network pharmacology, which are particularly relevant to herbal medications, which typically contain multiple chemicals that act at multiple sites and along multiple pathways (19).

Functional enrichment study of co-targets

Functional enrichment analysis of the 49 overlapping targets was performed using DAVID with a $P < 0.05$ threshold. DAVID is a popular bioinformatics tool for gene list functional

annotation and enrichment analysis (28). The enrichment data were clustered into the Gene Ontology Biological Process, Cellular Component, and Molecular Function categories, as well as the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway category.

In the Gene Ontology Biological Process category, the overlapping targets were enriched in inflammatory response, positive regulation of angiogenesis, response to oxidative stress, positive regulation of interleukin-8 production, and positive regulation of canonical and non-canonical NF-κB signaling pathways. The most frequent term was inflammatory response, which involved 21 genes. These data suggest that inflammation is a significant biological motif of the candidate targets of *S. costus* in NAFLD, Figure 2.

The enriched terms in the Gene Ontology (GO) Cellular Component category were protein-containing complex, extracellular exosome, and transcription regulator complex. The large number of extracellular exosome targets suggests multiple overlapping targets related to intercellular communication compartments. In the Molecular Function category, enriched terms included protein serine kinase activity, enzyme binding, protein kinase activity, and NF-kappaB binding. These results indicate that the overlapping targets are closely connected with kinase-mediated signaling and transcriptional regulation.

KEGG enrichment identified several relevant pathways, including lipid and atherosclerosis, the MAPK signaling pathway, the TNF-α signaling pathway, and the adherens junction. These

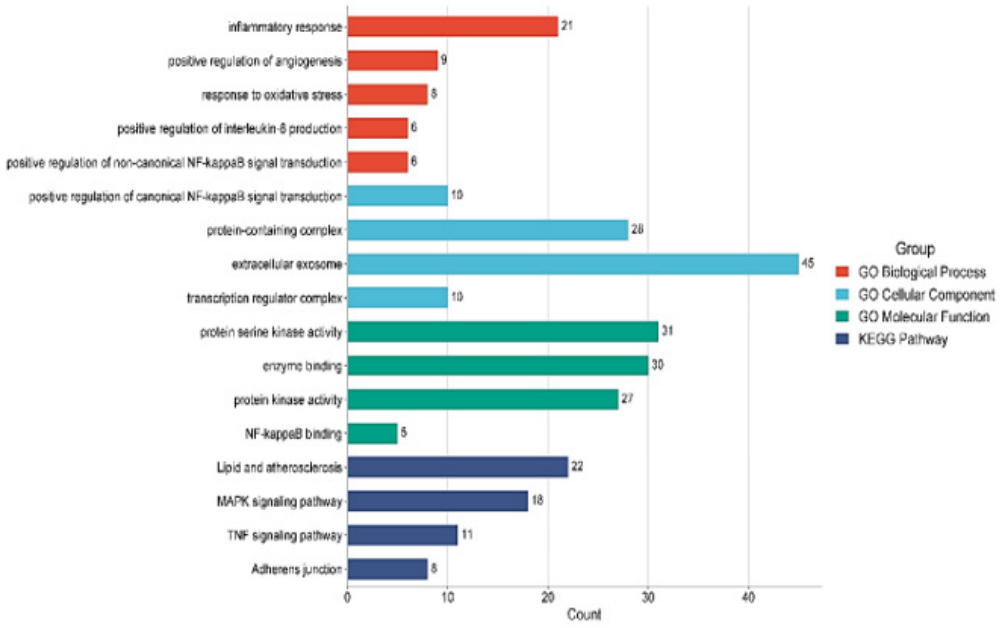

Figure 2. Functional enrichment analysis of 49 overlapping targets between *Saussurea costus* and NAFLD

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the 49 overlapping targets was performed using DAVID with a significance threshold of $P < 0.05$. The enriched terms were grouped into Gene Ontology Biological Process, Cellular Component, and Molecular Function categories, as well as KEGG pathway categories. Major enriched biological themes included inflammatory response, oxidative stress response, NF-κB signaling, kinase-mediated signaling, lipid-associated inflammation, TNF signaling, MAPK signaling, and adherens junction regulation

pathways correspond to biological processes involved in lipid-associated inflammation, cellular stress response, inflammatory amplification, and epithelial junction regulation. The term "adherens junction" is especially relevant to gut-liver axis biology because epithelial junctional organization supports intestinal barrier function, which may influence hepatic inflammation via gut-derived signals (12), Table 2.

Protein–protein interaction network analysis

Protein-protein interaction analysis was performed using STRING and visualized in Cytoscape. STRING provides information on known and predicted protein associations, whereas Cytoscape is commonly used to visualize and analyze biomolecular networks (29,30). The 49 overlapping targets formed an interconnected

Table 2. Key enriched GO terms and KEGG pathways associated with the 49 overlapping targets

Category	Enriched term	Count	Representative genes	Relevance to study
GO-BP	Inflammatory response	21	RELA, NFKB1, TNFRSF1A, MIF, TLR9, NFE2L2	NAFLD inflammation, gut–liver axis
GO-BP	Response to oxidative stress	8	NFE2L2, PTGS2, EGFR, APP	ROS-mediated liver and barrier injury
GO-BP	Canonical/non-canonical NF-κB signaling	10/6	RELA, NFKB1, TNFRSF1A, TLR9, NOD2, EGFR	inflammatory transcriptional regulation
GO-CC	Extracellular exosome	45	MMPs, HSPs, RELA-related targets	intercellular communication
GO-CC	Transcription regulator complex	10	JUN, SMAD3, EP300, AHR, RELA, NFKB1	gene expression regulation
GO-MF	Protein kinase activity	27	MAP3K8, ERN1, EGFR-related targets	kinase-mediated signaling
GO-MF	NF-κB binding	5	HDAC1, HDAC2, HDAC3, EP300, RELA	inflammatory gene regulation
KEGG	Lipid and atherosclerosis	22	RELA, NFKB1, TNFRSF1A, MMP9, NFE2L2	lipid-inflammation axis
KEGG	MAPK signaling pathway	18	JUN, EGFR, MAP3K8, RELA, TNFRSF1A	stress/inflammatory signaling
KEGG	TNF signaling pathway	11	JUN, NFKB1, RELA, PTGS2, TNFRSF1A	inflammatory amplification
KEGG	Adherens junction	8	SMAD3, EP300, MET, RHOA, EGFR	epithelial junction regulation

Summary of selected Gene Ontology and KEGG enrichment terms derived from the 49 overlapping targets between *Saussurea costus* and NAFLD. The table highlights enriched categories, representative genes, and their relevance to NAFLD-related inflammation, oxidative stress, gut–liver axis regulation, kinase-mediated signaling, and epithelial barrier-associated pathways. These enriched terms support the potential role of S. costus in modulating inflammatory and intestinal barrier-related mechanisms in NAFLD

network, indicating that these targets were not isolated entities but were functionally linked within biological processes relevant to NAFLD (Figure 3).

Several proteins are biologically important in the network, including RELA, NFKB1, TNFRSF1A, PTGS2, JUN, MAP3K8, NFE2L2, EGFR, MMP2, MMP9, SMAD3, VEGFA, EP300, HSP90AA1, and STAT1. These proteins are involved in inflammatory signaling, oxidative stress regulation, kinase-mediated pathways, extracellular matrix remodeling, angiogenesis, and transcriptional regulation. The presence of RELA and NFKB1 supports NF-κB-mediated inflammatory transcription, whereas TNFRSF1A, PTGS2, and JUN indicate TNF- and MAPK-related inflammatory pathways.

The target–pathway relationship also suggested a functional link between inflammatory response and junction-related mechanisms. Although classical tight junction structural proteins such as TJP1, TJP2, TJP3, and OCLN were not interpreted as direct compound-specific targets in the present analysis, the enrichment of adherens junction and the presence of upstream regulators such as RELA, NFKB1, TNFRSF1A, JUN, MAP3K8, EGFR, MMP2, MMP9, and NFE2L2 support the possibility that *S. costus* may influence intestinal

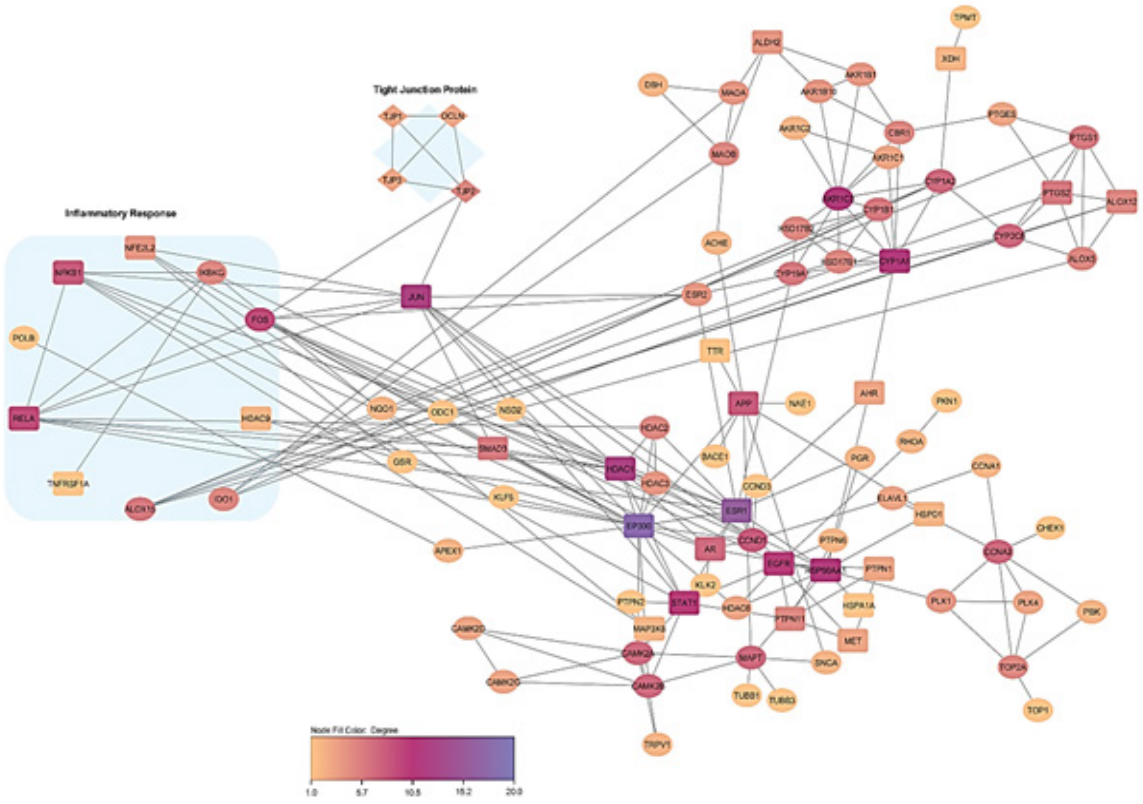

Figure 3. Protein–protein interaction network between *Saussurea costus* and NAFLD

Protein–protein interaction network of the 49 overlapping targets constructed using STRING and visualized in Cytoscape. Nodes represent target proteins, while edges indicate predicted or known protein–protein interactions. Node color intensity indicates degree centrality, with darker purple nodes representing proteins with a higher number of interactions. Highly connected proteins, including RELA, NFKB1, TNFRSF1A, PTGS2, JUN, MAP3K8, NFE2L2, EGFR, MMP2, MMP9, SMAD3, VEGFA, EP300, HSP90AA1, and STAT1, may represent biologically relevant targets involved in inflammation, oxidative stress regulation, kinase signaling, extracellular matrix remodeling, angiogenesis, and transcriptional regulation

barrier integrity indirectly through regulation of inflammation, oxidative stress, kinase signaling, and epithelial remodeling.

DISCUSSION

This network pharmacology study provides a system-level prediction of the molecular mechanisms through which *Saussurea costus* may affect NAFLD-associated inflammation and intestinal barrier dysfunction. The identification of 49 shared targets between *S. costus*-related proteins and NAFLD-associated proteins suggests that the potential activity of this medicinal plant is likely to arise from multi-target regulation. This interpretation is consistent with the pharmacological nature of medicinal plants, which typically contain diverse bioactive molecules that can act on multiple molecular pathways simultaneously (14,18,31).

Lipinski's rule of five (RO5) was satisfied by 22 of 23 candidate phytochemicals, except rutin, which was excluded due to its unfavorable physicochemical properties. This result implies that most selected compounds from *S. costus* exhibit acceptable predicted oral drug-similarity profiles and can be used for future computational target selection. Costunolide, dehydrocostunolide lactone, naringenin, kaempferol, ferulic acid, caffeic acid, and p-coumaric acid are particularly important because they have been frequently associated with anti-inflammatory and antioxidant effects (32–35). Previous reviews and experimental studies have described *S. costus* as having anti-inflammatory, antioxidant, antimicrobial, anticancer, and potential hepatoprotective activities (13–15,36). These features justify the biological plausibility of *S. costus* as a natural product candidate for NAFLD-related processes.

The most significant biological theme in the enrichment analysis was inflammation. Enrichment was observed for the GO Biological Process categories of inflammatory response, interleukin-8 production, and canonical and non-canonical NF-κB signaling. RELA and NFKB1 were identified as biologically meaningful targets within the PPI network, indicating NF-κB-mediated transcriptional regulation. NF-κB is a major inflammatory signaling system that controls the expression of cytokines, chemokines, adhesion molecules, and other mediators involved in immune activation (37). In NAFLD, inflammatory signaling contributes to the transition from steatosis to steatohepatitis, fibrosis, and advanced liver disease. The AASLD Practice Guidance states that NAFLD is a clinically relevant chronic liver disease with increasing inflammatory and fibrotic outcomes in susceptible individuals (5). Thus, the enrichment of NF-κB-related targets supports the notion that *S. costus* might influence NAFLD progression by suppressing inflammatory signaling (38).

TNF signaling was also identified as a significant KEGG pathway. TNF-associated pathways are closely linked to hepatic inflammation, insulin resistance, oxidative stress, and hepatocellular damage in NAFLD (8,39). In the present analysis, TNFRSF1A, RELA, NFKB1, JUN, and PTGS2 were among the targets involved in inflammatory amplification. TNFRSF1A encodes a receptor that mediates TNF signaling, while PTGS2 contributes to the synthesis of inflammatory prostaglandins (7,40,41). JUN, as part of the AP-1 transcriptional complex, participates in stress-responsive inflammatory regulation (42). Together, these targets suggest that the predicted effect of *S. costus* may include regulation of TNF-driven inflammatory cascades relevant to NAFLD pathogenesis (43).

The enrichment of MAPK signaling further supports the interpretation that *S. costus* may act through stress-response and inflammatory pathways. MAPK signaling is activated by cytokines, oxidative stress, metabolic stress, and tissue injury (41,44). In this analysis, JUN, EGFR, MAP3K8, RELA, and TNFRSF1A were linked to this pathway. MAPK signaling can interact with NF-kappaB-dependent mechanisms to contribute to cytokine production, hepatocyte injury, and immune cell activation (44,45). The fact that pathways related to NF-κB, TNF, and MAPK are all enriched at the same time suggests that *S. costus* may exert its effects through the coordinated regulation of inflammatory signaling

networks rather than a single isolated pathway (37,45).

Response to oxidative stress was another significantly enhanced biological function. One of the overlapping targets was NFE2L2, which encodes NRF2 and was deemed biologically relevant in the network. NRF2 is a master transcriptional regulator of antioxidant defense and cellular redox homeostasis (7,46). Whereas NF-κB is a central regulator of inflammatory signaling. These two pathways are functionally interconnected: reduced NRF2 activity may enhance NF-κB activation and cytokine production, whereas NRF2-mediated antioxidant signaling can attenuate NF-κB-mediated inflammatory responses (47). Oxidative stress dysregulation results in lipid peroxidation, mitochondrial dysfunction, hepatocellular damage, and inflammatory activation in NAFLD. A review on oxidative stress in NAFLD indicates that oxidative injury is crucial to disease progression and an important therapeutic target (6). Moreover, NRF2 and NF-κB signaling may crosstalk in regulating oxidative stress and inflammation, suggesting that inhibition of inflammatory injury can be achieved indirectly by modulating NFE2L2/NRF2 (7,46,47). Therefore, NFE2L2 was identified among the overlapping targets, supporting the possible involvement of *S. costus* in modulating the oxidative stress–inflammation axis in NAFLD (7,46).

Another noteworthy discovery was the enrichment of lipid- and atherosclerosis-related pathways. This KEGG term is not liver-specific but reflects biological processes associated with lipid metabolism, vascular inflammation, oxidative stress, immunological activation, and inflammatory remodeling. NAFLD is strongly associated with systemic metabolic dysfunction, including obesity, insulin resistance, dyslipidemia, and increased cardiovascular risk (5,42,48). Thus, enrichment of lipid-related inflammatory pathways may suggest that the *S. costus* target proteins are associated not only with hepatic inflammation but also with broader metabolic-inflammatory pathways important in NAFLD (14,49,50).

The enrichment of extracellular exosomes in the cellular component category is notable. Exosomes are involved in intercellular communication and can transport proteins, lipids, nucleic acids, and inflammatory signals between cells. In metabolic liver disease, extracellular vesicles have been implicated in communication among hepatocytes, immune cells, stellate cells, endothelial cells, and intestinal-derived signaling systems. Although the present study did not directly measure exosomal activity, the enrichment of extracellular exosomes suggests that several overlapping targets may participate in cell-to-cell communication processes relevant to inflammation and tissue remodeling.

An important aspect of this study is the attempt to connect inflammatory mechanisms in NAFLD with intestinal barrier-associated pathways. NAFLD is increasingly interpreted through the gut-liver axis, in which gut microbiota, intestinal permeability, microbial metabolites, and endotoxin translocation contribute to hepatic inflammation (11,12). Impaired intestinal barrier function may increase portal delivery of microbial products and activate innate immune responses in the liver, thereby promoting inflammatory injury (11,51,52). In this regard, the biological significance of adherens junction enrichment is apparent. Although classical tight junction proteins, such as TJP1, TJP2, TJP3, and OCLN, were not identified as direct compound-specific targets, the regulation of adherens junctions may still influence epithelial barrier stability and crosstalk with tight junction organization.

The presence of EGFR, SMAD3, MMP2, MMP9, VEGFA, RELA, and NFKB1 indicated a probable indirect mechanism of *S. costus* in intestinal barrier remodeling. EGFR signaling in epithelial repair and barrier homeostasis. MMP2 and MMP9 are involved in extracellular matrix remodeling and responses to tissue injury; SMAD3 in TGF-β signaling and tissue remodeling; VEGFA in angiogenesis and vascular permeability; and RELA and NFKB1 may regulate inflammatory responses that indirectly alter epithelial junction integrity (53). *S. costus* may modulate intestinal barrier function by regulating upstream pathways,

such as inflammation, oxidative stress, epithelial remodeling, and adherens junction-related processes, rather than directly targeting structural tight junction proteins (54,55).

This is vital for scientific accuracy. The current data do not indicate that *S. costus* directly modulates tight-junction proteins. Instead, the data show a plausible indirect link between *S. costus* targets and intestinal barrier-associated processes. This distinction adds substance to the topic, as it does not overclaim beyond the current computational proof. Based on the enrichment profile and protein-protein interaction network, *S. costus* may ameliorate gut-liver axis-related injury by inhibiting inflammatory signaling, reducing oxidative stress, and modulating epithelial remodeling processes relevant to barrier integrity (34,55).

Several limitations should be considered. First, the study is based on computational predictions and database-derived information; therefore, the findings should be interpreted as hypothesis-generating rather than confirmatory. Second, target prediction is influenced by the completeness and quality of available phytochemical and disease-target databases. Third, direct compound-target interactions were not confirmed by molecular docking, molecular dynamics simulation, or experimental assays. Fourth, intestinal barrier dysfunction was inferred from adherens junction enrichment and upstream inflammatory regulators, rather than from direct identification of classical tight junction proteins. Finally, *in vitro* and *in vivo* studies are required to confirm whether *S. costus* or its major compounds can modulate NAFLD-related inflammation, oxidative stress, and intestinal barrier integrity.

CONCLUSION

This network pharmacology analysis suggests that *S. costus* may have potential modulatory effects on NAFLD through multi-target regulation of inflammatory response, oxidative stress, NF-kappaB signaling, TNF signaling, MAPK signaling, lipid-associated inflammation, and adherens junction-related pathways. Key molecular nodes, including RELA, NFKB1, TNFRSF1A, PTGS2, JUN, MAP3K8, NFE2L2, EGFR, MMP2, MMP9, SMAD3, VEGFA, EP300, HSP90AA1, and STAT1, may link *S. costus* phytochemicals with NAFLD-relevant pathophysiological mechanisms. These results provide a mechanistic basis for further experimental validation of *S. costus* as a natural product candidate for regulating inflammation and intestinal barrier dysfunction in NAFLD.

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